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STUDIES ON LONGIDORIDAE (NEMATODA, DORYLAIMIDA)  
AND RASPBERRY RINGSPOT VIRUS SPREAD  
IN SOME ARTICHOKE FIELDS IN GREECE

by

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Artichoke plants infected by raspberry ringspot virus (RRV) were found in Greece some years ago (Kyriakopoulou and Bem, 1982). RRV was isolated from plants showing yellow blotches on their leaves and from some without symptoms. Two artichoke strains of the virus, one from Greece and the other from Turkey and serologically different from the English and Scottish strains, were recently studied with regard to biological, physico-chemical and serological properties (Rana *et al.*, 1985). RRV is transmitted in nature by two *Longidorus* species: *L. elongatus* (de Man) Thorne *et* Swanger, (Taylor, 1962), and *L. macrosoma* Hooper (Harrison, 1964).

Investigations on the distribution of RRV and its nematode vectors in artichoke fields in Greece were carried out during spring 1984 and the results from these are reported here.

*Materials and Methods*

Leaf and soil samples were collected from seven different artichoke fields in the Marathon region, near Athens, and from one field in Iria area (Argolis). In all there were 79 leaf samples each consisting of two leaves from randomly selected symptomless artichoke plants. Eight soil samples (10 kg each) were collected from the rhizosphere of selected infected plants. Samples were taken to a depth of 30 cm and were used for transmission tests one week later. RRV infection of the leaf samples was checked by sap inoculation to the following herbaceous species: *Chenopodium quinoa*

Willd, *Nicotiana tabacum* L. cv. White Burley, *Phaseolus vulgaris* L. cv. La Victoire and *Petunia hybrida* Vilm. After inoculation the plants were kept in a temperature controlled glasshouse at  $22 \pm 2^\circ\text{C}$  to allow symptoms to develop.

Nematodes were extracted from soil samples using Cobb's wet sieving technique. Specimens for morphometric studies were fixed with 5% hot formalin and mounted in glycerol on nematology slides.

Nematode transmission tests were done either using field collected soil from the rhizosphere of artichoke plants, positively tested for RRV infection, or using groups of 20 hand picked adults of the two *Longidorus* species: *L. euonymus* Mali et Hooper and *L. proximus* Sturhan et Argo extracted from the bulk soil samples. The hand-picked nematodes were added to 25 ml clay containers filled with steam-sterilized sandy loam. Single seedlings of *C. quinoa*, *Cucumis sativus* L., *N. tabacum* cv. White Burley and *P. hybrida* were planted in each. There were 10 pots for each test plant and 10 pots without nematodes for the control. The pots were kept in a temperature controlled cabinet at  $16 \pm 1^\circ\text{C}$  for 4-5 weeks before testing leaves and roots separately for virus infection by sap inoculation to *C. quinoa*, *P. vulgaris* or *P. hybrida* indicator plants. Nematodes were recovered from each pot at the end of the experiment and the roots of the bait seedlings were checked for the presence of feeding galls.

Antisera against Greek and Turkish RRV strains (RRV-G and RRV-T) (Rana *et al.*, 1985) with titer of 1:128 were employed in Outcherlony's double diffusion test in agar for checking the presence of the virus in herbaceous species infected by sap inoculation from artichoke leaf samples. Serological differences of some of the new artichoke RRV isolates from RRV-G and/or RRV-T were evident in the formation of spurs in the agar gel. In these tests, sap extracted from systemically infected leaves of *C. quinoa* and the above antisera were used as virus and antibody source, respectively.

## Results

As shown in Table I, many of the leaf samples were infected by RRV (ranging from 33.3 to 83.3%). Some of the new RRV isolates, when serologically compared to RRV-G and RRV-T, appeared serologically different from them (Fig. 1).

Two species of *Longidorus*: *L. euonymus* and *L. proximus* and one of *Xiphinema*: *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 were found.

Table 1 - RRV diffusion and nematodes (Longidoridae) found in artichoke fields in Marathon and Iria areas (Greece).

Area and field number	Number of tested/ Number of infected samples	Number of RRV isolates serologically different from RRV-G	Nematode species found
Marathon			
1	25/14	2	<i>X. pachtaicum</i>
2	12/10	1	—
3	13/10	1	—
4	6/5	0	<i>L. proximus</i> , <i>L. euonymus</i>
5	6/2	0	<i>L. proximus</i> , <i>X. pachtaicum</i>
6	6/5	1	<i>L. euonymus</i> , <i>X. pachtaicum</i>
7	12/7	0	<i>L. proximus</i>
Iria			
1	6/5	1	—

The morphometrics of these species will be described in detail elsewhere (Roca, 1987).

The morphometric characters of the Greek population of *L. euonymus* collected at Kato Souli village, Marathon area, are identical with those of the original description of *L. euonymus* (Mali and Hooper, 1974) except that the Greek specimens are longer (L=8.5 vs 6.91) and more slender (ratio «a» 201 vs 153).

The morphometric characters of a population of *L. proximus* collected from artichokes at Kato Souli village, Marathon area, fit the original description of the species (Sturhan and Argo, 1983).

The population of *X. pachtaicum* collected from Kambos, Marathon area, is identical with the Italian and Mediterranean populations and with the species description of Lamberti and Martelli, 1971.

None of the seedlings grown in field soil, or exposed to hand picked nematodes, nor the controls, showed symptoms indicative of RRV infection and none of the sap-inoculated indicator plants was infected.

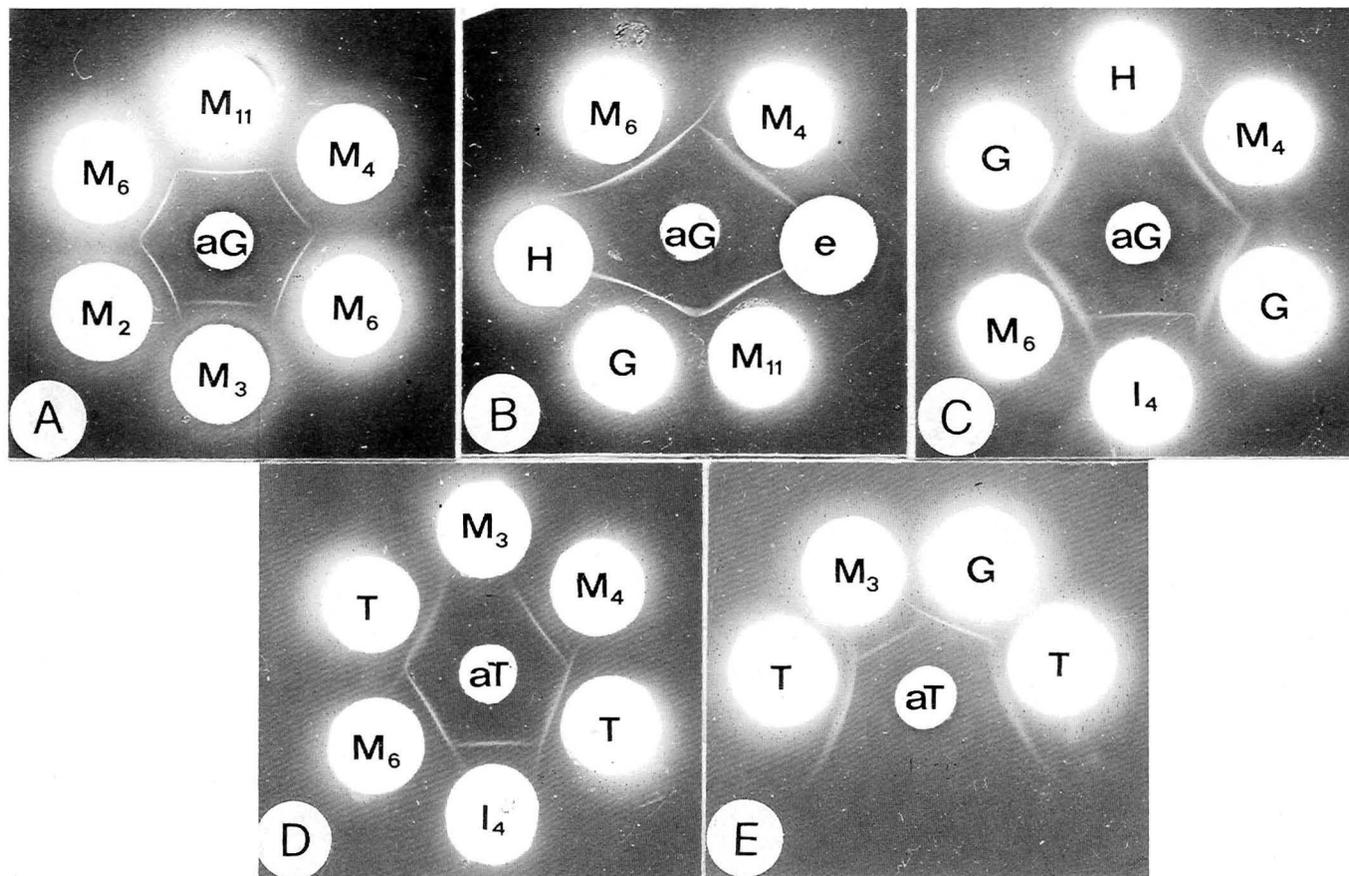


Fig. 1 - Gel diffusion tests between various Greek isolates of RRV, from Marathon ( $M_2$ ,  $M_3$ ,  $M_4$ ,  $M_6$ ,  $M_{11}$ ), and Iria ( $I_4$ ) and two RRV antisera prepared against a Greek (aG) and a Turkish (aT) isolate of the virus (RRV-G or G and RRV-T or T, respectively). H=healthy *C. quinoa* sap; e=empty well. The results show serological differences (spur formation) or similarity (no spur formation) between various RRV strains; in the tests with RRV-G antiserum (aG central well) (A, B, C)  $M_6$  and  $M_{11}$  are identical to RRV-G (well G),  $I_4$  differs from RRV-G, and the members of each of the pairs  $M_2$ - $M_3$ ,  $M_3$ - $M_6$ ,  $M_4$ - $M_6$ ,  $M_6$ - $I_4$  from each other; in the tests with RRV-T antiserum (aT central well) (D, E)  $M_3$ ,  $M_4$  and  $I_4$  differ from RRV-T.

Numerous galls were found on the roots of a *P. hybrida* bait plants exposed to *L. proximus* feeding but not on any other bait plants. The percentage of nematodes recovered from the pots at the end of the experiment ranged from 60-70% of the original inoculum.

### Discussion

On the basis of the results of the present study it can be concluded that RRV is widespread in artichoke fields of the Marathon area of Greece and that it is also present in the Iria area of the country. Several serological strains of the virus seem to exist, differing from each other or from the original artichoke isolates of RRV, RRV-G and RRV-T.

Three nematode species were isolated from the rhizosphere of RRV-infected plants. Transmission tests with *L. euonymus* and *L. proximus* gave negative results and this is in line with knowledge of transmission of RRV (Harrison, 1964; Taylor, 1962); so, the presence of the two species would seem to be occasional. The presence of the third species, *X. pachticum*, not employed in RRV transmission tests, may be regarded as a normal finding, since it is a common species in the Mediterranean region (Lamberti *et* Bleve-Zacheo, 1979; Lamberti, 1981). None of the two *Longidorus* species, *L. elongatus* and *L. macrosoma*, well known as vectors of RRV, were found in the rhizosphere of infected plants. These negative results constitute a stimulus to further investigate on presence of other *Longidorus* species, as possible vectors of RRV-strains occurring in Greece.

### S U M M A R Y

The presence and spread of RRV in artichoke fields in Greece was investigated. The virus was widespread in the areas investigated and occurred as strains serologically different from those originally found in artichoke fields in Greece and Turkey. Negative results were obtained in transmission tests undertaken with two different species of *Longidorus*, *L. euonymus* Mali *et* Hooper, 1974 and *L. proximus* Sturhan *et* Argo, 1983, which were found in the rhizosphere of infected artichokes. The morphometric characters of the Greek populations of the nematode are similar to the original descriptions of the species.

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