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DISTRIBUTION OF *LONGIDORUS ATTENUATUS* HOOPER IN APULIAN ARTICHOKE FIELDS AND ITS RELATIONSHIP WITH ARTICHOKE ITALIAN LATENT VIRUS (1)

by

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The crop

Artichoke (*Cynara scolymus* L.) is grown in Apulia on about 15,000 ha (i.e. more that 25% of the total acreage in Italy), thus constituting a crop of major economic importance. Traditionally, it used to be cultivated on the coastal vegetable gardens in the vicinity of Bari from where it spread to the provinces of Brindisi and Foggia (Fig. 1). Favourable environmental conditions and availability of irrigation have greatly contributed to the considerable development of the crop in these areas, which are now the leading Apulian districtis in artichoke production (Marzi, 1967).

Although artichoke plants, apart from a few remarkable exceptions (for a review see: Martelli and Rana, 1973), do not seem to react with clear-cut symptomatological responses to infection by viruses, it is evident that, in Apulia, the health of crops is far from satisfactory.

There is little doubt that local artichoke crops, mostly of the cv. « Precoce di Mola », are extensively affected by a disorder resembling the ill-defined « degeneration », reported from several Mediter-

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ranean countries (Martelli and Rana, 1973), whose etiology is still imperfectly known. In Apulia, several viruses have been isolated from plants in various stages of « degeneration » as well as from apparently healthy ones. Thus, their role as possible etiological agents of the disorder remains to be ascertained.

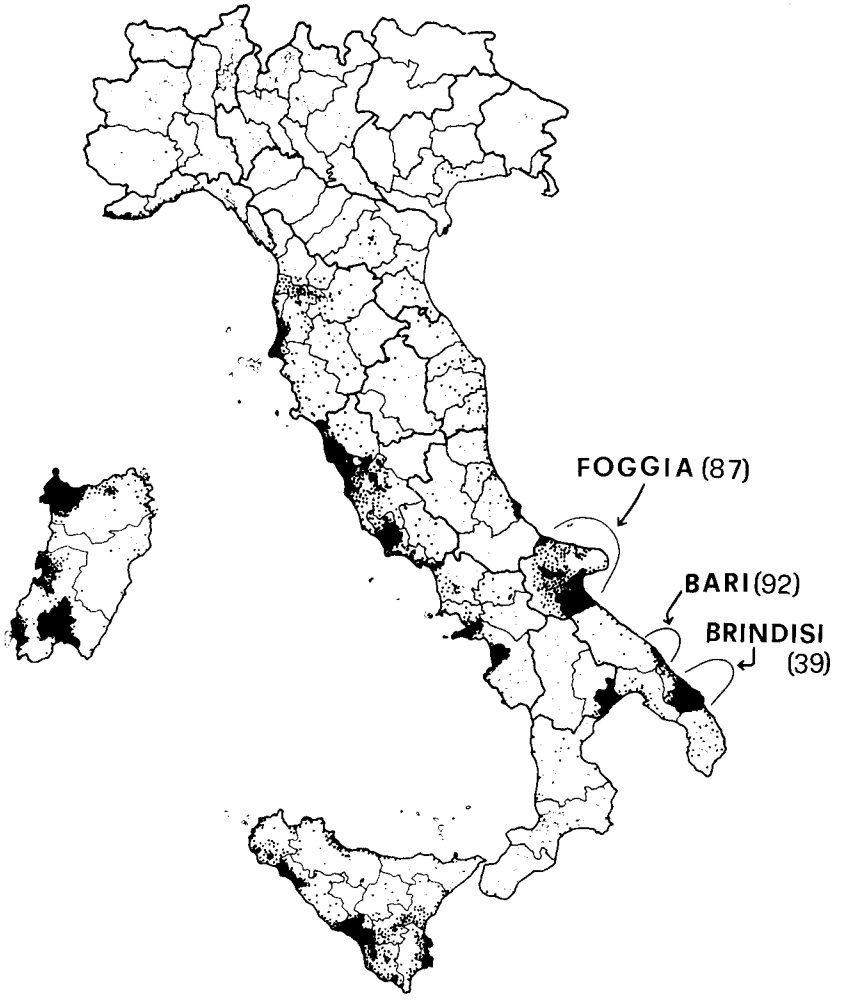

Fig. 1 - Artichoke distribution in Italy (dotted and black areas) and areas sampled in the present work. Figures in brackets are the number of leaf ana soil samples examined in each district for presence of virus and nematodes. (Photograph, courtesy of Prof. V. Marzi).

The virus

Artichoke Italian latent (AILV) is one of the above viruses. It was recovered from symptomless plants by Majorana and Rana (1970) who named it accordingly. Originally, AILV was isolated from artichoke fields of the Bari area but the results of recent random sampling in other districts indicate that it has a much wider distribution.

AILV is a split-genome, multi-component entity transmitted by *Longidorus attenuatus* Hooper, though at a relatively low rate (Rana and Roca, 1973), and having morphological and physico-chemical characteristics of nepoviruses (Rana and Quacquarelli, unpublished results). It is similar but not serologically related to tomato black ring virus (Vovlas *et al.*, 1971; Martelli, 1975). Its pathogenicity is difficult to assess owing to the low reactivity of the host to infection, but in nature the virus has been also isolated from stunted plants with mild yellowing of the leaves (Martelli and Rana, 1973).

The nematode

The association of *L. attenuatus* with artichoke became evident during the field surveys preliminary to the nematode transmission experiments of 1973 (Rana and Roca, 1973). At that time, soil samples were primarily taken from crop growing near Bari, in some of which patches of stunted plants were seen occasionally (Fig. 2 A). In these patches, artichokes were smaller than the surrounding ones and exhibited reduced foliage with small-sized leaves which, however, were neither missphagen nor chlorotic.

Conversely injuries to the roots system, consisting of necrotic lesions, mostly in the proximity of or at the growing tip, distortions, swellings and rare coralloid growth were of common occurrence (Fig. 2, B and C). Interestingly, in these areas very high populations of *L. attenuatus* were always present, reaching 500-600 individuals per 500 g of soil, i.e. levels far above the average counts (30 to 150 nematodes per 500 g of soil) recorded elsewhere in the same field or in plots where no manifestly stunted plants occurred.

On the basis of the above observations, it appeared that, at least in one of its major growing areas of Apulia, artichoke was

exposed to two potential pathogens (a virus and a nematode) liable to interact with the crop and with one another by virtue of their parasite-vector relationship.

Such a complex situation prompted us to further studies on the interrelationships between artichoke, AILV and *L. attenuatus* with the aim of elucidating some aspects of the ecology of the virus and the nematode.

The results of the investigations carried out so far are reported in this paper.

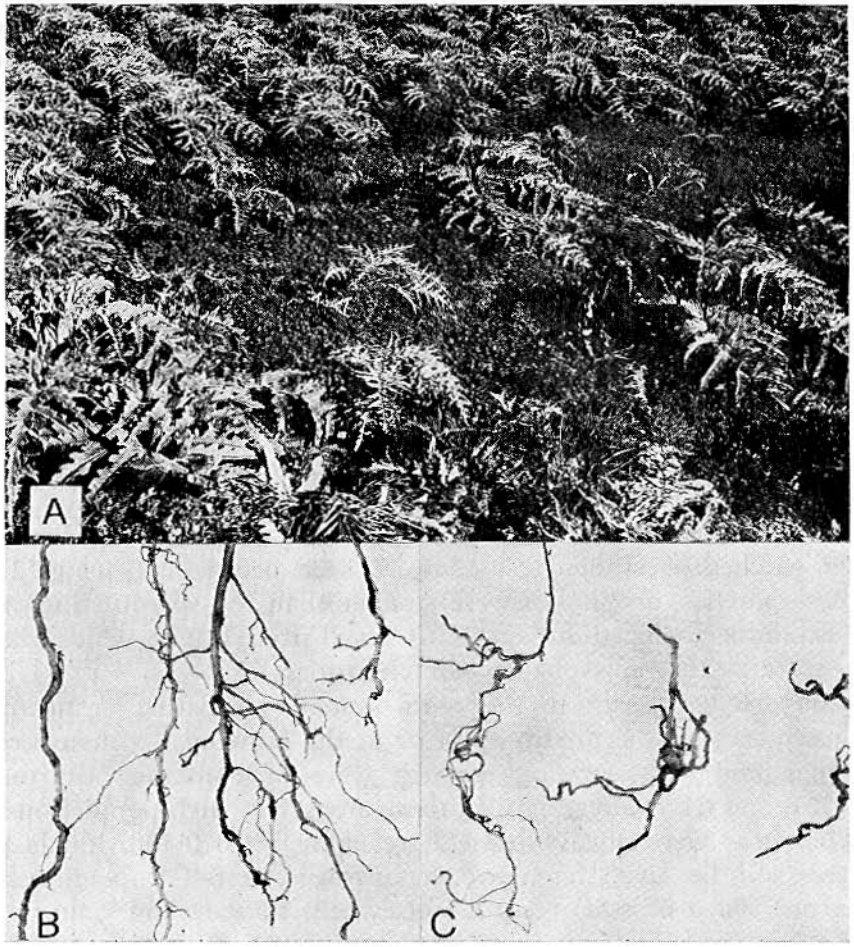

Fig. 2 - A: a patch of severely stunted plants in an artichoke field near Bari where a very high population of *L. attenuatus* was present. B and C: damage to roots of artichoke plants heavily infested by *L. attenuatus*.

Pathogenicity trials with L. ATTENUATUS

Pathogenicity trials were carried out from January through April 1974 in an outdoor shelter exposed to ambient temperatures, although affording protection from meteorological adversities.

Plastic containers 10 cm in diameter were filled with autoclaved sandy loam and each planted with two artichoke seedlings cv. « Precoce di Mola » at the cotyledon stage. The experiments comprised six series of ten containers. *L. attenuatus* were extracted by Cobb's sieving technique from infested field soil and batches of 10, 20, 50 or 100 handpicked females respectively were added to four of the series.

Controls consisted of two series of ten containers which received either plain water or a washing of field soil through a 325 mesh sieve (45 μ) not containing active *L. attenuatus* nor eggs of the same.

The growth of the seedlings was recorded monthly by measuring their height and the total leaf surface area. After four months, when the experiment was discontinued, the roots were carefully inspected for possible injuries and the weights of the tops and of the root system were recorded separately. Surviving nematodes were extracted from each pot and counted.

During the trials no appreciable differences were observed in the growth rate of the seedlings of any of the series, including the controls. Similary, the small variations (Tab. I) in the weight of the root system of plants inoculated with 20, 50 or 100 nematodes were not statistically significant with respect to the weight of the control.

Nevertheless, the roots of all series containing *L. attenuatus* were variously injured the exhibited necrotic lesions, distortion of the tips or occasional swelling which were most frequent and severe in pots inoculated with 50 or 100 nematodes. Both control series had perfectly normal roots.

At the end of the experiment the number of surviving nematodes was drastically reduced (Tab. I) but, in several cases, first stage larvae were found together with adult females showing that egg laying had occurred.

Table I - *Pathogenicity trials of* L. attenuatus *on artichoke seedlings.*

Number of inoculated nematodes	Mean weight of roots (g)	Mean weight of tops (g)	Mean number of nematodes recovered from each pot 4 months after inoculation
10	9.9	6.5	3
20	8.1	4.7	5
50	7.3	4.8	6
100	8.6	4.6	10
Control 1 [a)]	10.3	4.8	0
Control 2 [a)]	10.5	4.2	0

[a)] Control 1 = addition of plain water.
Control 2 = addition of soil washing through a 325 (45 μ) mesh sieve.

Field distribution of L. ATTENUATUS *and AILV*

The distribution of AILV and its vector was studied in the three major artichoke-growing districts of Apulia (Bari, Brindisi and Foggia) (Fig. 1).

In these areas, a total of 218 fields were inspected during autumn and winter 1973-74. Soil samples from the rhizosphere of plants chosen at random (4 to 6 in each field) were brought to the laboratory and checked by the Cobb wet sieving technique for the presence of *L. attenuatus*. The leaves of the same plants were used for sap-inoculating French bean, a suitable host for assaying AILV (Rana and Roca, 1973).

The results of this survey (Tab. II) show that AILV prevails in the Bari area, where it was found in more than 70% of the 92 sampled fields. The virus was less widespread in the artichoke cultivations of Brindisi and Foggia where it was recovered from 24 and 8.5% of the samples respectively (39 and 87 fields sampled).

Table II - *Distribution of AILV and* L. attenuatus *in three artichoke growing districts of Apulia.*

L. attenuatus	AILV	Bari (92 samples)	Brindisi (39 samples)	Foggia (87 samples)
+	+	40 %	3 %	1.5 %
+	—	2 %	13 %	1.5 %
—	+	33 %	21 %	7 %
—	—	25 %	64 %	90 %

+ presence of virus or nematode.
— absence of virus or nematode.

L. attenuatus had a comparable distribution pattern although it was encountered less frequently than AILV. In fact, only 42 (Bari), 16 (Brindisi) and 3% (Foggia) of the soil samples contained the nematode. In general, the highest populations of the nematode were found in the plantings located near the sea shore, where soils had a looser texture than those of the inland fields.

Occurrence of AILV in wild plants

In a limited survey, carried out in some artichoke fields near Bari harbouring viruliferous nematodes, AILV was isolated by mechanical inoculation from the following weeds of the family *Compositae*: *Crepis neglecta* L., *Urospermum dalechampii* Schm. and *Hypochoeris aetnensis* Ball. None of these plants showed obvious signs of infection.

Virus transmission trials with L. ATTENUATUS

The low rate of transmission (infection of 0.5 to no more than 10% of the bait plants) obtained in previous tests in which field soil or hand-picked nematodes from the rhizosphere of infected artichokes were used (Rana and Roca, 1973), had cast some doubt on the efficiency of *L. attenuatus* as vector of AILV. Therefore, further experiments were undertaken to make a better assessment of the vectoring ability of this nematode. The tests were carried out under glasshouse conditions at a mean temperature of 20°C. Different numbers (5, 10, 20 and 30) of hand-picked females of *L. attenuatus* were added to four series of 50 ml plastic containers (six per series) filled with steam-sterilized sandy loam, in which two « Supermarmande » tomato seedlings were growing. A series of six comparable containers without nematodes served as a control.

About four weeks after nematode addition, two seedlings of the 30 nematode series showed a mild chlorotic ring-spotting and line pattern of the leaves. Mechanical transmission from these plants yielded a virus indistinguishable from AILV. All the seedlings under trial were then assayed for presence of the virus and inoculations were repeated from the roots of all plants after four more weeks.

The results (Tab. III) clearly indicate that although after two months the nematode populations were reduced on average by more than 50%, the rate of transmission was always high and with a tendency to increase with the level of inoculum. No transmission ever occurred in the absence of *L. attenuatus*.

Table III - *Transmission of AILV with hand-picked* L. attenuatus.

Number of nematodes inoculated	Number (minimum and maximum) of nematodes recovered after 2 months	Percentage trasmission to tomato seedlings
5	1 — 3	42
10	2 — 4	25
20	5 — 9	67
30	0 — 15	58
0 (Control)	0	0

Discussion

Although the results of the pathogenicity trials were inconclusive because no statistically significant reduction in the growth of artichoke seedlings exposed to different levels of nematode inoculum was observed, nonetheless there are indications that *L. attenuatus* might indeed be an artichoke pathogen. This possibility is sustained by the following observations: a) the roots of all plants grown in presence of the nematode exhibit injuries, though of varying intensity, whereas no such reactions were recorded in the controls; b) artichoke seems able to support the multiplication of *L. attenuatus* as indicated by the presence of first stage larvae in several pots where only adult females had been added originally; c) depressed growth of artichoke plants in the field is always associated with exceedingly high nematode populations. A new set of trials, now under way, should provide final evidence of this.

As to the relationship of AILV with *L. attenuatus*, it is evident that a very close association between them exists in the artichoke

cultivations of the Bari area, where nearly all nematode populations encountered were presumably viruliferous. Although in Brindisi and Foggia nematode populations were also found in virus-infected crops, it was more normal to find AILV infections without the vector being present. Hence, it is conceivable that AILV has spread around primarily with infected propagating material.

Based on the available information, it is difficult to establish whether or not *L. attenuatus* existed in the provinces of Brindisi and Foggia before the introduction of artichoke but the likelihood exists that, at least in Foggia, the nematode was brought in with rooted artichoke sprouts used for propagation.

Finally, under experimental conditions, *L. attenuatus* proved to be an efficient vector of AILV, in accordance with the expectations based on circumstantial field evidence. In this connection, it is worth mentioning that this finding was further confirmed by recent transmission tests to Chicory (*Cichorium intybus* L.), another natural host of AILV (Vovlas and Roca, 1975).

In conclusion, although the studies are far from being completed, it seems that for AILV too, an ecological behaviour similar to that of most nepoviruses (for review see: Murant, 1970; Taylor, 1971) can be envisaged. In fact, virus survival is guaranteed by two major crops (i.e. artichoke and chicory) that are often grown in succession in the same plots and, by several weeds, presumably many more than those identified so far. Virus dissemination over short distances rely on nematode activity and, perhaps, on seed-transmission in wild plants whereas distribution of virus-infected propagating material plays a major role in long-range spread.

SUMMARY

The dorylaimoid nematode *Longidorus attenuatus* Hooper is shown under experimental conditions, to be an efficient vector of the artichoke Italian latent virus (AILV). The results of a survey indicate that AILV prevails in the Bari area (70% of the sampled fields) and it is less widespread in the provinces of Brindisi and Foggia (24 and 8.5% of the sampled fields, respectively). *L. attenuatus* had a comparable distribution pattern although it was encountered less frequently than AILV. The potential pathogenicity of the nematode to artichoke is discussed.

RIASSUNTO

Distribuzione di Longidorus attenuatus *Hooper in carciofaie Pugliesi e relazione con il virus latente italiano del Carciofo.*

Viene dimostrato sperimentalmente che il nematode dorilaimoideo *Longidorus attenuatus* Hooper è un efficiente vettore del virus latente italiano del Carciofo (AILV). I risultati di un'indagine indicano che l'AILV è molto più diffuso in provincia di Bari (70% dei campi campionati) che non nelle province di Brindisi e Foggia (24 e 8,5% rispettivamente, dei campi campionati). *L. attenuatus*, ha mostrato, grosso modo, una distribuzione paragonabile a quella del virus anche se è stato incontrato con meno frequenza che non l'AILV. È discussa la patogenicità potenziale del nematode alla coltura del Carciofo.

RESUMÉ

Distribution de Longidorus attenuatus *Hooper dans les artichautières pouillaises et rapport avec le virus latent italien de l'artichaut.*

Le nématode dorylaimoide *Longidorus attenuatus* Hooper s'est montré, en conditions contrôlées, un efficient vecteur du virus latent italien de l'artichaut (AILV). Les résultats d'une recherche indiquent que l'AILV est plus diffus dans la province de Bari (70% des champs échantillonnés) que dans les provinces de Brindisi et Foggia (24 et 8.5% respectivement des champs échantillonnés). *L. attenuatus* a une distribution comparable avec la présence du virus, même s'il était rencontré moins fréquemment que l'AILV. La pathogénie potentielle du nématode à la colture de l'artichaut est aussi discutée.

LITERATURE CITED

MAJORANA G. and RANA G. L., 1970 - Un nuovo virus latente isolato da Carciofo in Puglia. *Phytopath. medit., 9*: 193-196.

MARTELLI G. P., 1975 - Some features of nematode-borne viruses and their relationship with the host tissues. Nematode vectors of plant viruses (F. Lamberti, C. E. Taylor and J. W. Seinhorst, Eds.) Plenum Publishing Co. London, 233-252.

MARTELLI G. P. and RANA G. L., 1973 - Virus and virus diseases of globe artichoke and cardoon. Atti II Congr. Internaz. Studi Carciofo, Bari, Novembre 1973 (in press).

MARZI V., 1967 - La coltura del carciofo in Italia: distribuzione, varietà e tecniche colturali per regioni. Atti I Congr. Internaz. Studi Carciofo, Bari, Novembre 1967: 343-369.

MURANT A. F.. 1970 - The importance of wild plants in the ecology of nematode-transmitted plant viruses. *Outlook Agric., 6*: 114-121.

RANA G. L. and ROCA F., 1973 - Trasmissione con nematodi del virus italiano latente del Carciofo (AILV). Atti II Congr. Internaz. Studi Carciofo, Bari, Novembre 1973 (in press).

TAYLOR C. E., 1971 - Nematodes as vectors of plant viruses. In: Plant parasitic nematodes (B. M. Zuckerman, W. F. Mai and R. A. Rohde, Eds.) Acad. Press, London, *2*: 185-211.

VOVLAS C., MARTELLI G. P. and QUACQUARELLI A., 1971 - Le virosi delle piante ortensi in Puglia. VI. Il complesso delle maculature anulari della cicoria. *Phytopath. medit., 10*: 244-254.

VOVLAS C. and ROCA F., 1975 - Trasmissione con nematodi dell'agente della maculatura anulare clorotica della cicoria, un ceppo del virus latente italiano del Carciofo. *Nematol. medit., 3*: 83-90.

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