

A RE-EVALUATION OF THE GEOGRAPHICAL DISTRIBUTION OF QUARANTINE NEMATODES REPORTED IN SPAIN

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Summary. The lists of plant parasitic nematodes which are regulated by the European Union (EU Directive 2000 and/or the EPPO quarantine lists) contain 16 species. Seven of these species, *Ditylenchus dipsaci*, *Globodera pallida*, *G. rostochiensis*, *Xiphinema rivesi* (*Xiphinema americanum* group), *Ditylenchus destructor*, *Nacobbus* sp. and *Xiphinema americanum* have been reported in Spain. A re-examination of the voucher specimens concerning these reports confirmed that only *Ditylenchus dipsaci*, *Globodera pallida*, *G. rostochiensis* and *Xiphinema rivesi* occur in Spain. The reports of *Ditylenchus destructor*, *Nacobbus* sp. and *Xiphinema americanum* were not validated and are to be considered as erroneous. The remaining quarantine nematodes on the lists, *Aphelenchoides besseyi*, *Bursaphelenchus xylophilus*, *Heterodera glycines*, *Hirschmanniella* spp. (other than *H. gracilis*), *Longidorus diadecturus*, *Meloidogyne chitwoodi*, *M. fallax*, *Radopholus similis* (syn. *R. citrophillus*), *Xiphinema bricolense*, and *X. californicum* have not so far been detected in Spain. The necessity of extensive surveys to delimit the spread of the regulated nematodes occurring in the country and to detect the introduction of exotic quarantine nematodes from abroad is highlighted.

In Spain, the increasing interest in nematology that began during the 1960s led to many surveys being carried out and many species of nematodes have been described or reported, some of which are currently included in the EU Directive 2000/29/EC and/or the EPPO quarantine lists (Table I). Many of the reports are probably invalid as some of them relied on juvenile stages, improperly fixed material, or were of species that have since been included in species complexes, as for *X. americanum sensu lato*. In other cases, introduced nematodes such as *Globodera pallida* and *G. rostochiensis* have not been encountered from some areas in the last twenty years. Recently, two monographs have been published dealing with dorylaimids and tylenchids found in the Iberian peninsula and the Balearic Islands (Peña *et al.*, 2003, 2004). These monographs contain lists of the species belonging to these orders based on information reported by other authors, including the records of quarantine nematodes: *Ditylenchus destructor* Thorne, 1945; *D. dipsaci* (Kühn, 1857) Filipjev, 1936; *Globodera pallida* (Stone, 1973) Behrens, 1975; *G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959; *Xiphinema americanum* Cobb, 1913; and *X. rivesi* Dalmaso, 1969. Another quarantine nematode reported from Spain is *Nacobbus* sp., which was listed as a new record by Jiménez-Millán (1964). Some of these published records are used by CAB International and the Fauna Europea Database for the preparation of Distribution Maps of Nematodes and Plant Pests. These maps are used by in-

ternational quarantine agencies for the preparation of pest risk assessments and the implementation of regulatory measures.

Taking into consideration the potential adverse impact that these reports of quarantine nematodes have on the trade of agricultural commodities between Spain and other countries, a study was conducted by the National Reference Laboratory of Nematology in order to verify the true status of these reports, which often lead to confusion.

MATERIALS AND METHODS

All published material dealing with quarantine nematodes from Spain were analysed and this included more than 350 papers. The original material, consisting of voucher specimens deposited in the Nematode Collection at the Department of Agroecology, CCMA, CSIC (Madrid, Spain), that was used in these reports was studied and the nematode identification verified.

RESULTS AND DISCUSSION

The quarantine nematodes regulated by the European Union (EU Directive 2000/29/EC (01-05-2004) and/or the EPPO quarantine lists) reported in Spain include: *Ditylenchus destructor*, *D. dipsaci*, *Globodera pallida*, *G. rostochiensis*, *Nacobbus* sp.; *Xiphinema americanum*, and *Xiphinema rivesi* (Tables I and II). The situation for each of these nematodes is analysed below.

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***Ditylenchus destructor* Thorne, 1945**

Ditylenchus destructor was reported by Bello (1969) in the Canary Islands without mentioning the locality or the host plant. Romero and Arias (1969) found this species on eggplant in the coastal area of Granada-Málaga, in the locality of “La Mayora” (Algarrobo, Málaga) (Romero *et al.*, 1970). In their revision of tylenchid nematodes in Spain, Gómez Barcina *et al.* (1989) included these records and another from uncultivated natural areas of Navarra recovered from the unpublished doctoral thesis by Mateo-Cervera (1986). However, Escuer (1998) and Peña *et al.* (2004) consider the report by Romero and Arias (1969) doubtful. There are no further reports of this species from Spain. The study of the relevant voucher specimens deposited in the Nematode Collection of the Dept. of Agroecology, CCMA, CSIC (Spain), failed to confirm the previous identification of this species. The deposited voucher material has therefore been re-classified as *Ditylenchus* spp. until new studies can verify the correct identity of the species. Thus, these findings indicate that records of *D. destructor* in Spain must be considered invalid and should be included in an epidemiological study.

***Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936**

Ditylenchus dipsaci was first reported in Spain by Navarro (1902) from wheat without specifying the locality, and later by Navarro (1911) from hemp (*Cannabis sativa* L.) in Murcia and from garlic in Palma de Mallorca as *Tylenchus devastatrix* (Kühn) Örley 1880. Since this species has not been reported again from these localities, we

are in agreement with Escuer *et al.* (2004) who consider the record from Murcia as doubtful. Other authors have reported this species as being widespread in Spain and associated with a range of economically important crops, such as cereals, garlic, legumes, onion, and strawberry (Martínez-Beringola and Alfaro, 1979; Nombela *et al.*, 1985; Gómez-Barcina *et al.*, 1989; Escuer, 1998; Peña *et al.* 2004). Our research confirms the presence of *D. dipsaci* in Spain, but a deeper study of the distribution and epidemiology of *D. dipsaci* should be carried out due to the economic importance of this nematode.

***Globodera* (= *Heterodera*) (Wollenweber, 1923)**

Skarbilovich, 1959

***Heterodera rostochiensis* Wollenweber, 1923**

Globodera rostochiensis Woll., the golden nematode listed as *Heterodera rostochiensis* (*Globodera rostochiensis sensu lato*), was first reported as having a wide distribution by Domínguez García Tejero (1957, 1961), who also outlined the distribution of this species based on the number of specimens deposited in the “Estación Central de Fitopatología Agrícola de Madrid”. This species was later reported from the Island of Tenerife by Chamberlain (1961) and from 23 localities in the Canary Islands by Bello (1968). Martínez-Beringola *et al.* (1976, 1987a,b) reported the distribution and pathotypes of *G.* (= *H.*) *rostochiensis* Woll. *sensu lato*. Following the description of *Globodera* (Skarbilovich, 1959) Behrens, 1975, separate studies of *Globodera rostochiensis* and *G. pallida* have been carried out in Spain.

Pathotypes Ro1/Ro4 of *G. rostochiensis* have been reported in Spain. Most of these records date back to mo-

Table I. EPPO Quarantine list of nematodes in Europe (October 2004).

A1 list:

- *Bursaphelenchus xylophilus* (Steiner *et* Bühner, 1934) Nickle *et al.*, 1970
- *Nacobbus aberrans* Thorne *et* Allen, 1944
- *Radopholus citrophilus* Huettel, Dickson *et* Kaplan, 1984
- *Xiphinema americanum* Cobb, 1913 *sensu stricto*
- *X. bricolense* Ebary, Vrain *et* Graham, 1989
- *X. californicum* Lamberti *et* Bleve-Zacheo, 1979

A2 list:

- *Aphelenchoides besseyi* Christie, 1942
 - *Ditylenchus destructor* Thorne, 1945
 - *D. dipsaci* (Kühn, 1857) Filipjev, 1936 (*)
 - *Globodera pallida* (Stone, 1973) Behrens, 1975 (*)
 - *G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (*)
 - *Heterodera glycines* Ichinohe, 1952
 - *Meloidogyne chitwoodi* Golden *et al.*, 1980
 - *M. fallax* Karsen, 1996
 - *Radopholus similis* (Cobb 1983) Thorne, 1949
 - *Xiphinema rivesi* Dalmaso, 1969 (*)
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(*) Nematodes present in Spain. A1 list: quarantine pests not present in that area. A2 list: quarantine pests present in that area but not widely distributed but that are being officially controlled.

re than 20 years ago and need to be confirmed. Paz-Vivas *et al.* (1985) used electrophoresis to confirm records of *G. rostochiensis* from El Barco de Ávila (Ávila), Caba (Cordoba), Lajosa (Lugo) and Ciudad Rodrigo (Salamanca). These populations were from a laboratory collection previously reported as *G. (= H.) rostochiensis* by Martínez-Beringola *et al.* (1976). Later, Martínez-Beringola *et al.* (1987a,b, 1988) identified *G. rostochiensis* from Ávila (Ro1), Burgos (Ro1/Ro4), Cordoba (Ro1/Ro4), La Rioja (Ro1/Ro4), Gran Canaria (mixed with *G. pallida*), León, Lugo (Ro1), Orense (Ro1/Ro4), and Salamanca (Ro1/Ro4). Fullaondo *et al.* (1999) reported *G. rostochiensis* Ro1 in Angostina and Lagran (Álava), El Barco de Ávila (Ávila), Valencia, Sandias and Xinzo de Limia (Orense), and Prades (Tarragona). All of the populations used in this study belonged to different collections but the date of sampling is not given and should therefore be confirmed.

González *et al.* (1991, 1992, 1996, 1997) and Bello and González (1994) reported Ro1/Ro4 to be widely distributed in Tenerife. Salazar (1992) and Salazar and Ritter (1992) reported Ro1 at Santo Domingo de la Calzada (La Rioja) and Urturi (Álava). Roa *et al.* (2002) determined the presence of *G. rostochiensis* in Navia and Valdés (Asturias). Rosende *et al.* (2003) detected the presence of *G. rostochiensis* in Bergantiños (A Coruña), Villalba (Lugo) and Xinzo de Limia (Orense); Bello *et al.* (1994) in the Balearic Islands; Alonso *et al.* (2004) in Sa Pobla (Mallorca) and García-Álvarez *et al.* (2005) in the upper region of La Rioja.

The reports by Martínez-Beringola *et al.* (1987a,b, 1988) of *G. rostochiensis* in Ávila (Ro1), Burgos (Ro1/Ro4), Cordoba (Ro1/Ro4), Las Palmas (mixed with *G. pallida*) and Salamanca (Ro1/Ro4), and by Fullaondo *et al.* (1999) of *G. rostochiensis* Ro1 in Ávila, Tarragona and Valencia, should be confirmed, as most of

Table II. EU Directive 2000/29/EC (01-05-2004)

ANNEX I- Part A: Harmful Organisms Whose Introduction Into, And Spread Within, All Member States Shall Be Banned.

Section I. Harmful organisms not known to occur in any part of the community and relevant for the entire community:

- *Hirschmanniella* spp. other than *H. gracilis* Luc *et* Goodey
- *Longidorus diadecturus* Eveligh *et* Allen
- *Nacobbus aberrans* (Thorne) Thorne *et* Allen
- *Xiphinema americanum* Cobb *sensu lato* (non-European populations)
- *X. californicum* Lamberti *et* Bleve-Zacheo

Section II. Harmful organisms known to occur in the Community and relevant for the entire community:

- *Globodera pallida* (Stone) Behrens (*)
- *G. rostochiensis* (Wollemweber) Skarbilovich (*)
- *Meloidogyne chitwoodi* Golden *et al.* (all populations)
- *M. fallax* Karssen

ANNEX I- Part B: ORGANISMS WHOSE INTRODUCTION INTO, AND WHOSE SPREAD WITHIN, CERTAIN PROTECTED ZONES SHALL BE BANNED:

- *Globodera pallida* (Stone) Behrens (*)

ANEX II- Part A: HARMFUL ORGANISMS WHOSE INTRODUCTION INTO, AND SPREAD WITHIN, ALL MEMBER STATES SHALL BE BANNED IF THEY ARE PRESENT ON CERTAIN PLANTS OR PLANT PRODUCTS.

Section I. Harmful organisms not known to occur in the community and relevant for the entire community:

- *Aphelenchoides besseyi* Christie (seeds of *Oryza* spp.)
- *Bursaphelenchus xylophilus* (Steiner *et* Buhere) Nickle
- *Radopholus citrophilus* Huettel, Dickson *et* Kaplan

Section II. Harmful organisms known to occur in the community and relevant for the entire community:

- *Aphelenchoides besseyi* Christie (strawberry runners)
- *Ditylenchus destructor* Thorne (bulbs and tubers)
- *D. dipsaci* (Kühn) Filipiev (seeds and bulbs) (*)
- *Radopholus similis* (Cobb) Thorne

(*) Nematodes present in Spain.

the populations used belonged to the laboratory collection of the “Estación de Fitopatología Agrícola de Madrid” with unknown dates of collection as reported by Martínez-Beringola *et al.* (1976, 1987a,b).

In conclusion, we are certain that the pathotypes

Ro1/Ro4 of *G. rostochiensis* occur throughout Álava, La Rioja and Tenerife (Canary Islands) and *G. rostochiensis* in Asturias, Galicia and Mallorca (Fig. 1). Further studies are needed to confirm the current distribution and economic importance of the golden nematode pathotypes in Spain.

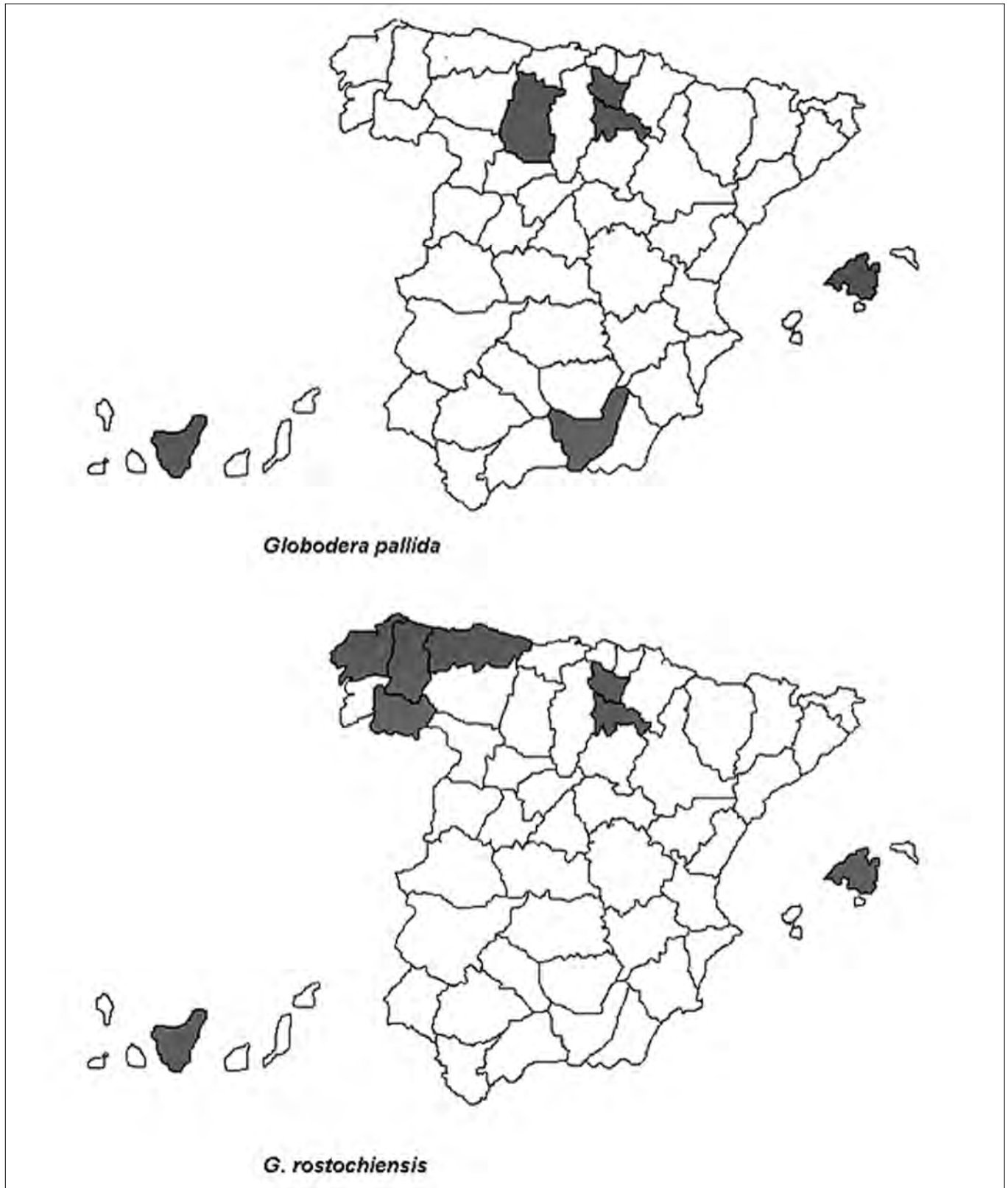


Fig. 1. Distribution of *Globodera pallida* and *G. rostochiensis* in Spain by province and Islands.

***Globodera pallida* (Stone, 1973) Behrens, 1975**

Pathotypes Pa2/3 of the pale potato cyst nematode, *G. pallida*, are reported to be widely distributed in continental Spain and Pa1 in Tenerife (Canary Islands). Most of these records were published approximately 20 years ago, or based on laboratory collections of potato cyst nematodes (PCN). The distribution of these pathotypes in Spain should be re-examined.

Paz-Vivas *et al.* (1985) used electrophoresis to identify *G. pallida* from Mataró (Barcelona), La Puebla (Mallorca) and La Orotava (Tenerife) in a specimen collection previously reported as *Heterodera rostochiensis* by Martínez-Beringola *et al.* (1976). Later, Martínez-Beringola *et al.* (1987a,b, 1988) identified *G. pallida* in the Balearic Islands, Barcelona (Pa2), La Rioja (Pa2), León, Palencia (Pa2), Tenerife (Pa2) and Zamora, but indicated that in some cases the populations were a mixture of both *G. pallida* and *G. rostochiensis*, complicating the correct identification. Fullaondo *et al.* (1999) using the same collection of the Estación Central de Fitopatología Agrícola de Madrid, reported *G. pallida* Pa 2/3 from Mataró (Barcelona), Cabra (Cordoba), Valle Seco (Gran Canaria), Caleta de Vélez and Torre del Mar (Malaga), Son Ferrant (Sa Pobla-Muro, Mallorca), Prades (Tarragona), Palencia, and La Orotava (Tenerife).

González *et al.* (1991, 1992, 1997), Bello and González (1994) and González and Phillips (1996) reported that *G. pallida* Pa 2/3 is widely distributed in Tenerife. González *et al.* (1996) also confirmed the presence of pathotype Pa1. Salazar (1992) found Pa2 at Santo Domingo de la Calzada (La Rioja) and Urturi (Álava). Talavera *et al.* (1998) reported *G. pallida* in Motril and Salobreña (Granada). The records from the province of Palencia (Castilla y León) were confirmed later by Díez-Rojo *et al.* (2002). Alonso *et al.* (2004) confirmed the presence of *G. pallida* in Sa Pobla (Mallorca) and García-Álvarez *et al.* (2005) in the upper region of La Rioja.

Rosende *et al.* (2003) detected the presence of *G. pallida* in Arguidello (Xinzo de Limia, Orense) by serological tests, but this record could not be confirmed by morphological studies because in subsequent samplings no material could be found.

In conclusion, *G. pallida* is present in the country and is represented by the pathotypes Pa2/Pa3 and Pa1 that occur in Tenerife. The pathotype Pa2 was also found in the locality of San Domingo de la Calzada (La Rioja), Palencia province, and Urturi (Álava); *G. pallida* is also present in Motril and Salobreña (Granada) and Sa Pobla (Mallorca) (Fig. 1). The reports on the distribution of these pathotypes in other parts of the country are questionable and therefore, further, studies are needed to confirm the current distribution and damage caused by the pathotypes of this quarantine species.

***Nacobbus* Thorne *et Allen*, 1944**

Jiménez-Millán (1964) reported *Nacobbus* sp. “in irrigated crops which were heavily infested in the Central

Region” in the abstracts of the Proceedings of the VIIth International Symposium of the European Society of Nematology, without determining the species or specifying the host plant or locality. A later examination of the material deposited in the Nematode Collection within the Dept. of Agroecology, CCMA, CSIC (Spain), revealed that the voucher specimens were of *Meloidogyne incognita*, a common pest of tomato in Arcicóllar (Toledo) in the Central Region (Jiménez-Millán *et al.*, 1964). Therefore the record of *Nacobbus* sp. in Spain must be considered erroneous.

***Xiphinema americanum* Cobb, 1913**

Xiphinema americanum is a dagger nematode regulated by the EU because of its role as a vector of quarantine viruses, such as Tobacco ring spot virus (TRSV), Tomato ring spot virus (ToRSV) and Peach rosette mosaic virus (PRMV) in American vineyards and peach orchards. Due to the variability within *X. americanum*, Lima (1965, 1968) and Tarjan (1969) hypothesized that this species was a complex of different species. Lamberti and Bleve-Zacheo (1979) studied hundreds of populations from different countries and distinguished 25 species within the *X. americanum* group. These authors consider *X. americanum sensu stricto* as a dagger nematode species native to the eastern areas of the United States. Currently, more than 40 species have been described and assigned to the *X. americanum* group (Luc *et al.*, 1998; Lamberti *et al.*, 2000). These dagger nematode species are also regulated by the EU.

Xiphinema americanum was first reported from Spain by S'Jacob *et al.* (1959) on olives in Camino de la Fargue (Granada), and later by López-Abella *et al.* (1966) on *Calendula* sp. and *Petunia* sp. from Madrid. Palomo (1975, 1979) found it in natural areas from Sierra de Gata (Salamanca), and Ripoll (1980) found it in vineyards from Cerdanyola del Vallés (Barcelona). As a consequence of the revision of this group by Lamberti and Bleve-Zacheo (1979), the records by S'Jacob *et al.* (1959), López-Abella *et al.* (1966), Palomo (1975, 1979) and Ripoll (1980) were re-examined by Arias *et al.*, (1985) who concluded that the species listed in these reports consisted only of *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951, which is a dagger nematode species belonging to the *X. americanum* group. More recently, Werland-Ardaiz and Pérez-Camacho (1995) reported *X. americanum* in vineyards from El Condado de Huelva (Huelva) and Talavera *et al.* (1999) in a forest tree nursery at Andújar (Jaén). However, these records were disproved by the results of recent surveys, which indicated that *X. pachtaicum* was the only dagger nematode species occurring in the surveyed area.

So far we have no evidence that *X. americanum* occurs in Spain and previous reports of the presence of this species in Spain must be considered erroneous. The only representatives of the *Xiphinema americanum* group occurring in Spain are *X. brevicollum* Lordello *et Da Costa*, 1961, *X. pachtaicum* and *X. rivesi* Dalmasso, 1969.

Xiphinema rivesi Dalmasso, 1969

This species, belonging to the *X. americanum* group, was first described from a population from vineyards in France and later was also reported from vineyards in Madrid (Arias and Navacerrada, 1973). Arias *et al.* (1991) reported it as widespread in the southern half of the Spanish peninsula and in Tenerife, without further specifying the localities. It was found associated mainly with grapevines and fruit trees but also with vegetable and flowers causing damage to rose plants, and uncultivated areas. This species has also been reported in vineyards from Valdepeñas and Villarrubia de los Ojos (Ciudad Real) and Erustes (Toledo) (Arias *et al.*, 1997), in Jumilla (Murcia) (Bello *et al.*, 2004) and from natural environments in the Montseny Mountains (Barcelona) (Escuer, 1995). Peña *et al.* (2004), in a revision of the Dorylaimida in the Iberia peninsula mention only reports from Portugal and do not cover any of the records from Spain. Our records indicate that *X. rivesi* is widely distributed in the southern half of the Spanish peninsula, Cataluña and the Canary Islands.

This nematode is a potential vector of Tomato ring spot virus (TomRSV) and Tobacco ring spot virus (TRSV) infecting grapes in the USA and, for this reason, it is included in the EPPO quarantine list. Further studies of the distribution and epidemiology of this species should be carried out.

Other quarantine nematodes regulated by the EU

The remaining quarantine nematodes regulated by the EU - *Aphelenchoides besseyi* Christie, 1942, *Bursaphelenchus xylophilus* (Steiner *et* Buhner, 1934) Nickle *et al.*, 1970, *Heterodera glycines* Ichinohe, 1952, *Hirschmanniella* Luc *et* Goodey, 1964 (other than *H. gracilis*), *Longidorus diadecturus* Eveliegh *et* Allen, 1982, *Meloidogyne chitwoodi* Golden *et al.*, 1980, *M. fallax* Karssen, 1996, *Nacobbus aberrans* (Thorne, 1935) Thorne *et* Allen, 1944, *Radopholus similis* (Cobb, 1893) Thorne 1949 (syn.: *R. citrophillus* Huettel *et al.*, 1984), *Xiphinema bricolense* Ebsary *et al.*, 1989, and *X. californicum* Lambert *et* Bleve-Zacheo, 1979 - have not been reported from Spain.

CONCLUSIONS

Our studies indicate that, although cited, *Ditylenchus destructor* Thorne, 1945, *Nacobbus* Thorne *et* Allen, 1944 and *Xiphinema americanum* Cobb, 1913 are not present in Spain, as a re-examination of the original voucher specimens and the results of new surveys suggest that they were wrongly identified.

Ditylenchus dipsaci is widespread on many different crops and the reports of this species in uncultivated areas of Spain should be re-evaluated.

The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are present in the country, but the distribution of their pathotypes is unclear and has been confirmed only in limited areas of Spain. The potato cyst nematodes are regulated by specific EU directives which require the identification of their pathotypes, their distribution and the implementation of appropriate management and pest exclusion practices to suppress their populations. Proper implementation of these EU directives for PCN in Spain will require extensive surveys for the confirmation and determination of the PCN pathotypes since the majority of the reports for these nematodes date from 1976 or 1987, or they are based on laboratory collections.

The data collected in this study also indicate that the distribution of the regulated dagger nematode *Xiphinema rivesi* Dalmasso, 1969 is confined to the southern half of the Spanish peninsula, Cataluña and the Canary Islands. However, precautions and appropriate sanitation practices should be adopted by nurseries and farmers to avoid the spread of this species to other fruit tree growing areas and vineyards of the country.

The conduction of periodical nematode surveys is an effective strategy that allows early detection and accurate knowledge of the distribution of regulated nematode pests in Spain. However, applied nematologists should perhaps consider preparing a list of all exotic plant parasitic nematodes of potential significance for the agriculture and environment of Spain. Each species on the list should be rated according to the potential risk that it poses. Such a list would serve as a basis for the preparation of pest risk assessment for the nematodes of greatest potential importance by regulatory officials. Pest risk assessments that include identification of the areas most at risk to the introduction and establishment of exotic nematode pests, on the basis of presence of suitable hosts and environmental characteristics, and pathways of introduction through the trade of commodities and plants, can be very useful in preventing or delaying the introduction of unwanted exotic nematode species.

Unfortunately, the intensification of intra and interstate trade of plants and commodities makes Spain and other countries more vulnerable both to the spread of regulated nematodes already present in the country and to the introduction of exotic pests from abroad.

ACKNOWLEDGEMENTS

We would like to thank Drs A. García-Álvarez, M. Escuer, A. Navas and Mr C. Martínez for their collaboration and help.

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