

Nematodes and management of diversity in potato crops in La Rioja (Spain)

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Summary. The aim of the study is to identify the key biotic, environmental and cultural factors in potato production in La Rioja, one of the greatest traditional and highest yielding areas for this crop in Spain. This paper focuses on the study of nematodes and insects that are important limiting factors of the crop. The nematode problem is restricted to *Globodera pallida* and *G. rostochiensis*. No problem was detected with *Nacobbus aberrans* or with *Meloidogyne chitwoodi* and *Ditylenchus destructor*, important pathogens in tropical and temperate environments, respectively. Among the most important pests, *Leptinotarsa decemlineata* is notable. It is concluded that a combination of environmental factors, sanitary measurements and cultural practices, such as crop rotation and organic matter management, foment a great biodiversity, which includes the presence of entomopathogenic nematodes. The management of such diversity is the decisive factor in the design of production systems.

Key words: diversity, La Rioja, nematode problems, potato crops, Spain.

World potato production is estimated at 311 million tonnes annually but, interestingly, 237.9 million tonnes (76.4%) correspond to only six areas of production (Table 1): China (62.0 million tonnes), the European Union (49.0), Russia (32.6), Poland (24.2), India (23.5) and the USA (23.4), according to statistics from the FAO (2000). Even more disturbing are the low outputs in regions such as Asia (16.5 t ha⁻¹), South America (15.1), Africa (11.3) and in countries such as China (16.3), Colombia (16.1), Spain (15.8 on unirrigated land according to MAPA, 1999), Portugal (14.7), Ecuador (12.5), Peru (11.2), Tunisia (10.7), Russia (10.0), Ukraine (8.4), and Bolivia (6.9), which all show values under the world mean, as opposed to the high outputs obtained in other countries such as Belgium and Luxembourg (46.2 t ha⁻¹), Holland (44.8), USA (42.8), Germany (41.8), New Zealand (41.7), United Kingdom (40.3), Denmark (39.5), France (39.4), Israel (38.7) and Ireland (29.4).

It is necessary to delve deeper into the analysis of the production-limiting factors in the cultivation of potato, taking into account the new focuses in

agricultural production, directed at obtaining quality nutritious and environmental products, whilst also taking into consideration the social repercussions of family-orientated subsistence agriculture that does not exclusively reward the development of highly productive agricultural systems but includes their environmental and social functions (Vereijken & van Loon, 1991).

Conventional agricultural production rests on the habitual consumption of agrochemicals. In Spain in 1999, spending on fertilizers and phytosanitary products for horticultural crops reached 1,215 million US \$ (MAPA, 1999), with an outlay of 6,570 US \$ for agrochemicals in the EU (McDougal, 1999). All this in spite of the fact that many of the pesticides used have low efficacy and their indiscriminate use causes serious public health problems (Metcalf, 1993) and environmental contamination (Edwards, 1993). This situation has given rise to public awareness, which is having strong repercussions on the agricultural policies of many countries.

Given this situation, the EU is trying to adapt its agricultural production to the criteria for Sustainable Agriculture, whose object is to produce

quality food with an acceptable cost to society and to the environment (Wijnands & Kroonem-Backbier, 1993). This production system avoids or reduces the use of agrochemicals and is based on crop rotation, the use of crop remains or animal manure, and the management of natural enemies, among other aspects, with the goal of maintaining production, soil structure and fertility, as well as controlling pests and diseases. In Spain there are 2,223 ha of ecological agriculture dedicated to horticultural crops (MAPA, 1999) and it is hoped that this area will continue to increase, given the higher prices that these products bring on the market.

At present, studies to search for alternative procedures in crop protection are of increasing importance. Among them, biofumigation should be mentioned as an alternative that uses organic remains and green manure that, when they decompose in the soil, can regulate populations of pathogenic organisms, preventing them from causing diseases in crops. This alternative has been studied particularly by Bello (1998) and Bello *et al.* (1997, 2000) and Kirkegaard *et al.* (1998). It deals with an ecotechnology that minimizes the environmental impact produced by agricultural activities, since it is compatible with customary production techniques, and at the same time improves the use of natural resources and reduces environmental contamination by preventing or restricting the liberation of agrochemicals to the environment. Moreover, it permits the recuperation of organic remains produced from harvests, which benefits agricultural production itself.

One of the alternatives for controlling pests is the management of natural enemies, which is based on the use of depredators, parasitoids and pathogens. Among these, the work carried out on the use of entomopathogenic nematodes (EPN) from the heterorhabditidae and steinernematidae families, which are obligate parasites, lethal for a wide range of insect species, must be emphasized. The characteristics of these nematodes makes them ideal candidates as biological control agents for pest insects, since they actively search for their host, kill the host rapidly, have high reproductive rates, breed massively *in vitro*, have easy application techniques for the farmer, are compatible with almost all insecticides and finally, imply no risk to the plant or to the environment (Kaya & Gaugler, 1993).

The necessity for a new focus in designing productive systems, in this case for the potato crop, led us in Spain to analyze the possible

production-limiting factors, since, together with Portugal and Poland, Spain has the lowest yields in the EU (Table 1). Therefore, a comparative analysis was made between production and yield for the potato crop in the various autonomous regions of Spain (Table 2). It was found that the community of La Rioja has the greatest output, reaching 25.3 t ha⁻¹ on unirrigated land and 45.2 t ha⁻¹ under irrigation. These values are superior to those of the greater majority of producer countries.

The study was centred on the analysis of biotic and abiotic factors that could be related to the high productivity reached in potato crops in La Rioja, but was also centred on determining the functional value of the crop, in order to develop quality production systems in the region. Thus, the establishment of guidelines is sought for the management of the potato crop, which would serve as a reference for solving low profitability problems in other areas. The context taken in the first place was the study of factors that limit pest and disease problems produced by nematodes and insects, as well as aspects related to soil ecology, with the object of establishing ecological criteria for understanding key elements and processes in the functioning of the crop.

Recognizing the problems that nematodes cause in potato crops due to their pathogenic action as well as to their biogeographical characteristics is basic since tubers used for planting can be a key factor in their dissemination. Emphasis must also be placed on the nematodes that are considered quarantine organisms: *Ditylenchus destructor*, *D. dipsaci*, *Globodera pallida*, *G. rostochiensis*, *Nacobbus aberrans*, *Meloidogyne chitwoodi* and *M. fallax*, not only in order to control their introduction into our crop area, but also because they can be a limiting factor for exportation.

Corresponding to the above exposition, this study seeks to obtain information about environmental factors, the spectrum of phytoparasitic nematodes, insect pests and their natural enemies, especially EPN, associated with the potato crop in La Rioja (Spain). The results obtained from studying the biodiversity associated with this crop will permit the establishment of integrated production management systems (Harrewijn, 1989; Beukema & van der Zaag, 1990; Verma & Shekhawat, 1991).

MATERIAL AND METHODS

For the exploration and identification of soil nematodes, pest-causing insects and biocontrol agents associated with potato crops, 186 samples

were taken in 76 cultivated fields of which 59 supported a potato crop. Simultaneously, a plot was established with an approximate area of 300 m². Three samplings were made: at the time of sprouting, during flowering, and before the harvest. It was sampled regularly at 50 separate points, 1.5 m apart along the length of the plot and every 4 m along its width. This plot is on its way to being converted to the practices of ecological agriculture. The insects found in the aerial part of the plants were collected for later identification. A soil sample of approximately 1 kg was taken at the same points to a depth of 0–20 cm. Samples were transported in plastic bags and were stored at 4°C until analyzed in the laboratory, where the composition of the nematofauna species was determined, particularly the phytoparasitic and entomopathogenic (EPN) nematodes, as well as soil insects that could constitute pests. Soil nematodes were extracted by centrifugation in sugar, decantation and floatation and by using the Fenwick can. Entomopathogenic soil nematodes were isolated from the soil samples by using *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae as bait, according to Bedding & Akhurst (1975). At the same points 50 bait-traps were placed (300 ml plastic containers full of wheat and corn seeds) at 20 cm depth to attract wire worms (*Agriotes sordidus*) and to estimate their population level.

Rearing and maintenance was carried out under laboratory conditions for the species of insects susceptible to being affected by the EPN. These nematodes were maintained and propagated *in vivo* following the Woodring & Kaya method (1988). The study of the effectiveness of the EPN stock, isolated in the field, against pests was carried out by using the Glazer & Lewis bioassays (2000).

RESULTS AND DISCUSSION

If the profitability of the potato crop in Spain (Table 2, Fig. 1) is analyzed, on unirrigated as well as on irrigated land, the highest yields are obtained in the community of La Rioja, and by extension in the regions of northern Spain where environmental conditions are more appropriate for the crop. They present optimum values in reference to annual temperature and rainfall in La Rioja (Fig. 2), as well as to soil characteristics, acidic in type and sandy in texture. This differs from regions located farther south, with higher temperatures that affect tuberization and also where soils with a higher pH predominate, which favours the development of diseases such as potato scab (Adams & Hide,

1981; Marti, 1989).

In relation to limitations from biotic factors, the study centers on the analysis of nematodes and insects because they are considered to be key elements in the production of the potato crop (Lara & Bello, 1983; Martínez-Beringola *et al.*, 1987; Semanayake & Holliday, 1990; Raman & Radcliffe, 1992), not only due to their direct action, but also because of their interaction with other phytopathological problems caused by fungi, bacteria and virus (de Bokx & van der Want, 1987; Burton, 1989).

Phytopathogenic nematodes constitute one of the chief limiting factors to crop production and are one of the most difficult soil-borne organisms to control, since the build-up of their populations cannot easily be recognized until they become a problem. This does not normally happen with aerial pathogens, but soil acts as a buffer. Above all, the enormous variability of the nematofauna population makes it impossible to apply effectively alternatives such as biological control agents or the use of resistant plants.

In Upper La Rioja, where the study was made, phytonematological problems were centered on the species *G. pallida* and *G. rostochiensis*, infecting 44 % of potato fields. Other phytoparasitic nematodes affecting potatoes, from tropical environments and temperate origin, were not found due to the special environmental characteristics of the region.

Among the nematodes from temperate climates, it is possible for *Ditylenchus dipsaci* to be present in other long duration crops, such as in the case of legumes and cereal (Nombela *et al.*, 1985). In some localities in Lower La Rioja, *Meloidogyne arenaria*, *M. hapla* and *M. incognita* were found, the latter in a potato crop. *Meloidogyne chitwoodi* and *M. fallax* were not found, although they constitute a serious problem in southern Holland, the country where the major portion of the seed comes from that is used in La Rioja. Virus vectors for the *Paratrichodorus* and *Trichodorus* genera, as well as other phytoparasitic nematodes were not regarded as causing problems (Lara & Bello, 1983; Arias *et al.*, 2002).

The use of varieties resistant to *G. rostochiensis* in the past several years has led to a selection of virulent *G. pallida* populations. Therefore, it can be considered as the principal problem for potato crops in La Rioja, as well as in the rest of Spain and the entire EU. It is important to realise that the use of potato varieties resistant to *G. rostochiensis* is not effective, especially in Andean countries, due to the great variability of this species, since this region is

Table 1. Worldwide analysis of potato production according to data from the FAO, with special reference to Andean countries and the Mediterranean Basin.

Countries & Geographical Areas	<i>Production</i> (thousands of tons)	<i>Output (t ha⁻¹)</i>	Production per Inhabitant (kg)
Belgium-Luxembourg	3000	46.2	280.7
Holland	8200	44.8	515.9
USA	23404	42.8	85.0
Germany	12633	41.8	153.7
New Zealand	500	41.7	130.6
United Kingdom	6647	40.3	112.7
Denmark	1502	39.5	281.4
France	6652	39.4	113.0
Israel	349	38.7	57.1
Ireland	500	29.4	135.5
Argentina	3500	29.2	95.7
Canada	4569	28.9	148.5
Spain (*)	3138	25.5	77.3
Italy	2078	25.0	36.2
Cyprus	120	24.0	154.2
Egypt	1784	21.5	26.5
Poland	24232	19.4	626.9
Venezuela	352	18.4	14.6
India	23500	18.1	23.2
Morocco	1090	18.0	39.1
Chile	992	17.6	66.1
China	62036	16.3	49.0
Colombia	2705	16.1	64.0
Portugal	1250	14.7	124.9
Ecuador	788	12.5	62.3
Peru	3,187	11.2	124.3
Tunisia	290	10.7	30.7
The Russian Federation	32597	10.0	223.3
Ukraine	13037	8.4	257.0
Bolivia	927	6.9	111.3
North America (USA & Canada)	27973	39.7	91.4
European Union (15 countries)	49014	35.7	130.0
Eastern Europe	33206	17.0	45.6
Asia	113256	16.5	31.2
South America	15144	15.1	44.3
Africa	10110	11.3	12.6
Developed Countries	180471	17.2	138.5
Developing Countries	130817	15.8	28.0
The World	311288	16.6	51.5

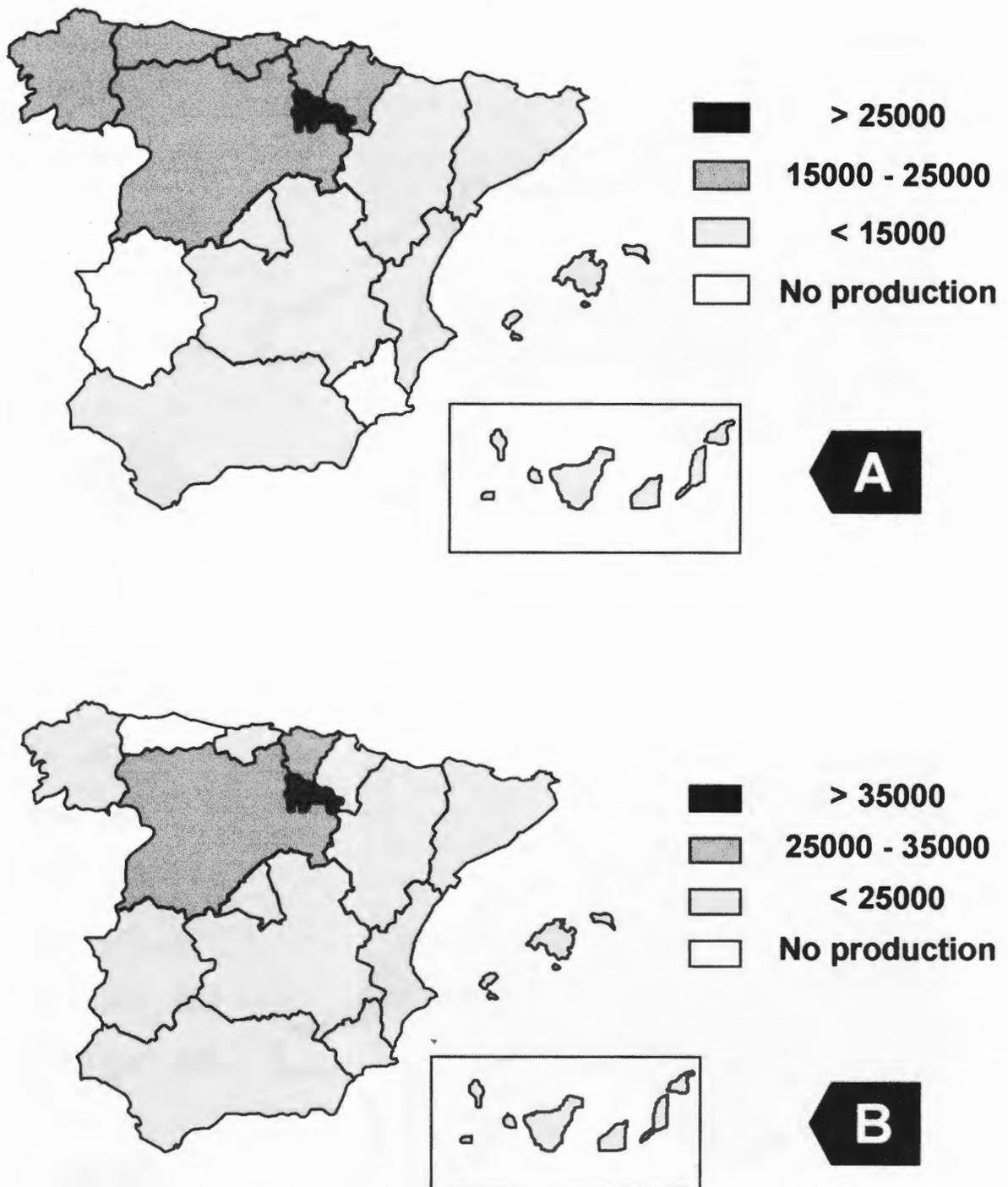


Fig. 1. Average potato crop production in kg ha⁻¹ on unirrigated (A) and irrigated (B) land in Spain.

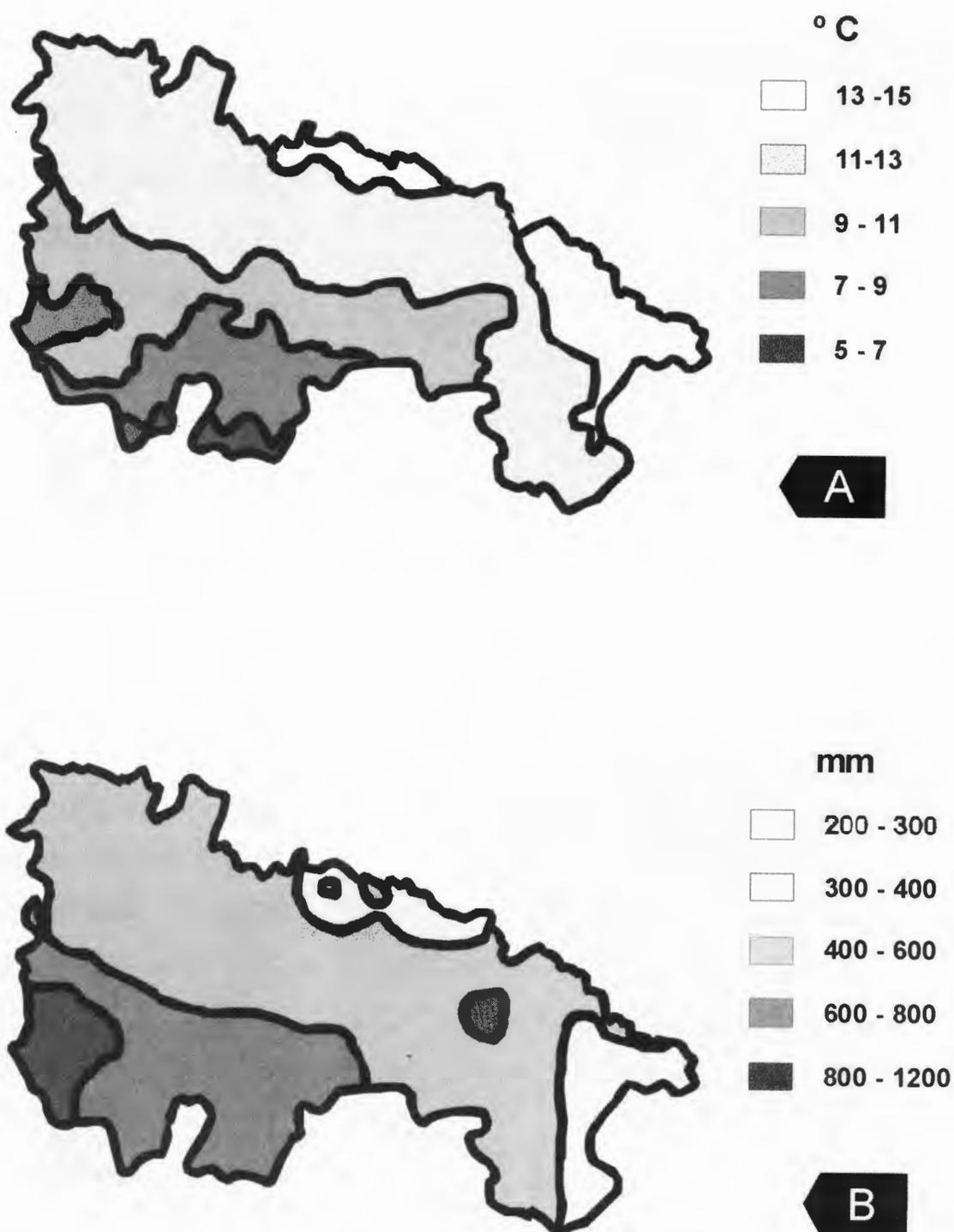
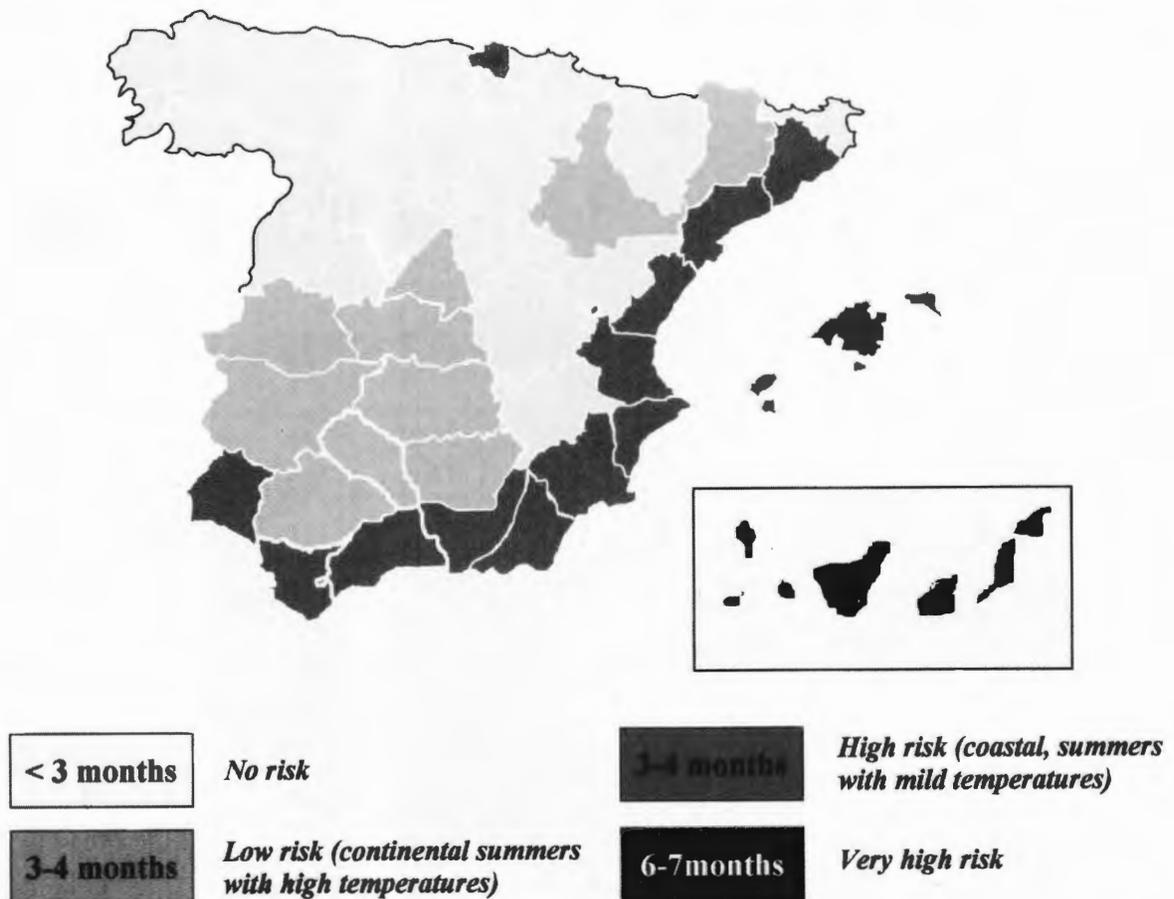


Fig. 2. Isoline maps of the average annual temperature (A) and annual precipitation (B) in La Rioja, Spain.

Table 2. Regional analysis of the potato crop in Spain.

Autonomous Community	Area (ha)			Production	Yield (kg ha ⁻¹)	
	Total	Unirrigated	Irrigated	Community	Total	Unirrigated
1. Galicia	55882	50309	5573	908491	15816	20240
2. Castilla y León	28637	5784	22853	920571	21266	34900
3. Andalucía	26243	2418	23825	498689	11239	19791
4. Castilla - La Mancha	10503	942	9561	226834	6113	23123
5. Extremadura	8550	—	8550	142648	—	16684
6. Canary islands	8075	3104	4971	145538	9753	23188
7. Cataluña	7432	2645	4787	148615	14044	23286
8. Valencia	6267	787	5480	120087	7570	20827
9. Basque region	5009	1773	3236	128357	18650	29447
10. La Rioja	4740	432	4308	205907	25314	45258
11. Balearic islands	3661	425	3236	91881	3521	27931
12. Aragón	3306	198	3108	90803	13596	28350
13. Asturias	3253	3253	—	61875	19021	—
14. Cantabria	2665	2492	173	44855	16476	21947
15. Murcia	2345	—	2345	44274	—	18880
16. Madrid	2037	24	2013	48576	11000	24000
17. Navarra	1461	651	810	27707	16157	21221
Spain	180066	75237	104829	3855708	15776	25459

**Fig. 3.** Areas in Spain at risk from the thermophilic nematode, *Meloidogyne incognita*, based on the months with an average temperature over 20°C.

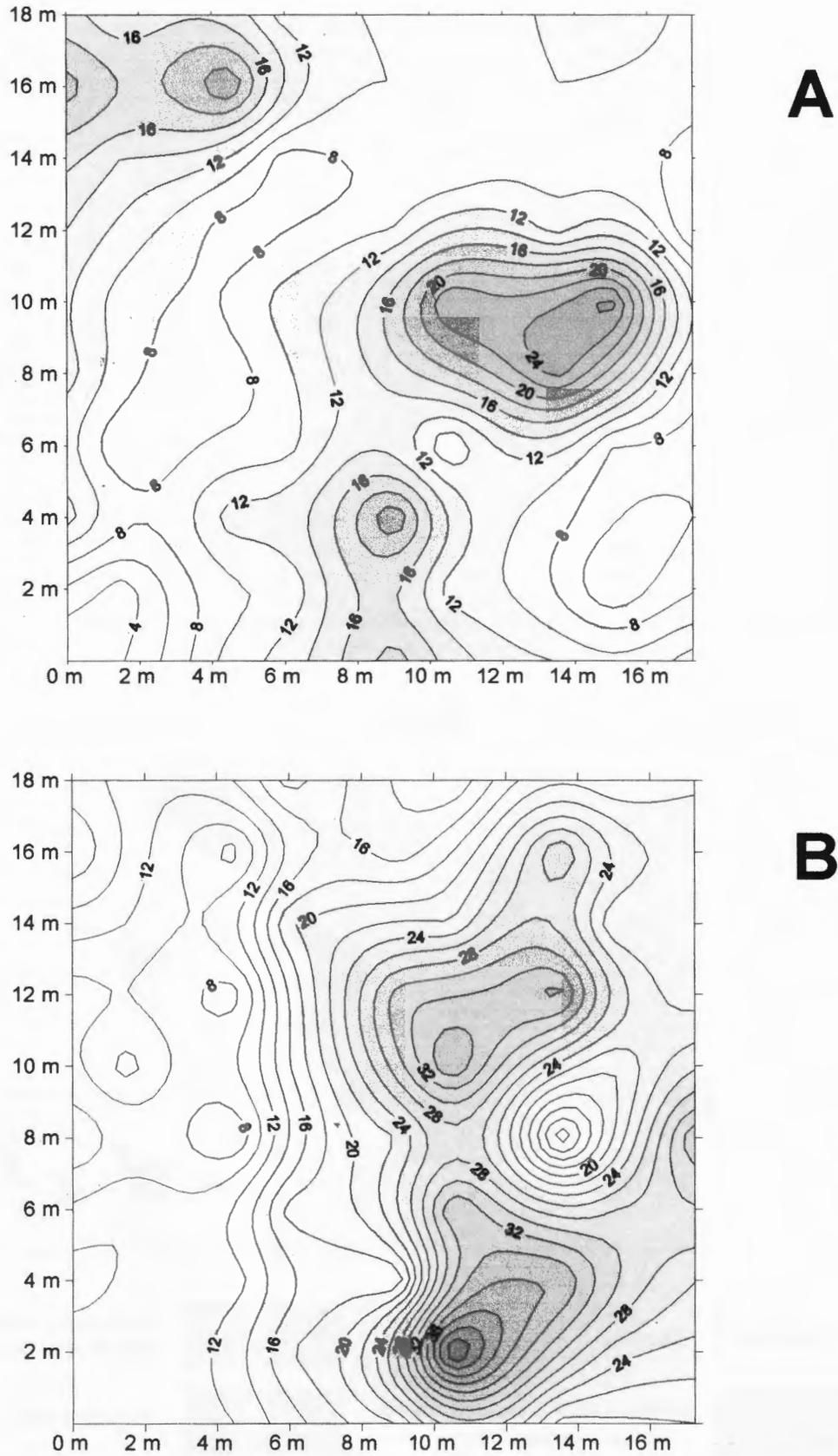
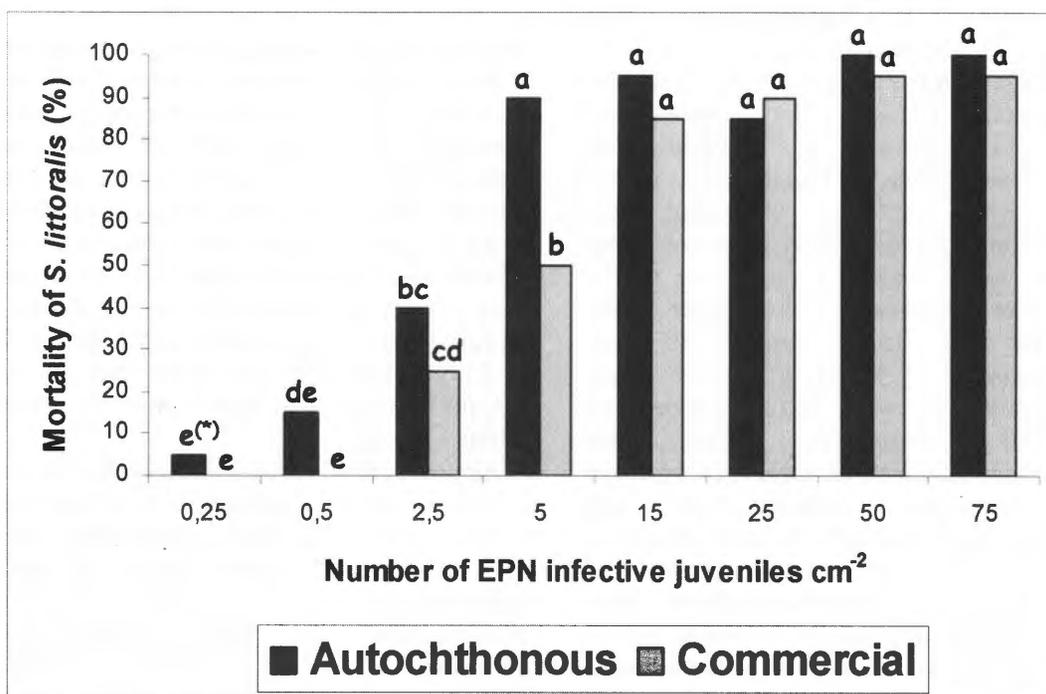


Fig. 4. Spatial distribution of empty *Globodera* cysts in a reconverted ecological plot in La Rioja (Spain), during flowering of the potato plants (A) and before harvest (B); cysts 200 g⁻¹ soil.



(*) Columns with the same letter or letters are not significantly different according to LSD test ($p = 0.05$).

Fig. 5. Dose-response assays on mortality of *Spodoptera littoralis* with autochthonous and commercial strains of *Steinernema feltiae*. Doses of juveniles are regarding to plate surface; values of mortality.

the center of origin for the potato (Ochoa, 1990).

La Rioja, which forms part of the Ebro basin, presents a series of interesting agroecological characteristics. In the first place there are less than three months when average temperatures are higher than 20°C (Fig. 3), which are the thermal requirements necessary for the development of more than three generations of thermophilic nematode species in order to create phytopathological problems. Among traditional agricultural practices the complexity between agriculture and livestock stands out, which permits the establishment of lengthy rotation periods between pastures and fodder crops, while mountains, especially the Pyrenees, are traditional areas for obtaining potatoes from seed, due to a low incidence of nematodes and virus. In the cereal-growing areas of the midlands, the function of fallow land and stubble is important, as well as rotation with legumes and crucifers, which contribute to the reduction of nematode populations. In relation to valleys, the great variety of horticultural crops must be emphasized, due to their importance in crop rotation for the control of potato cyst nematodes. The complement relationship between valley, midlands and

mountains is particularly outstanding, with one of the highest productions of animal manure in Spain, which constitutes a fundamental element in the biological diversification of the soil and in regulating pathogenic soil agents through the use of biofumigation (Bello *et al.*, 2003).

The application of ecological criteria in the management of agricultural systems, especially in potato crops in the highlands of La Rioja, together with the use of resistant varieties have served to regulate populations of *G. rostochiensis* and resulted in the high outputs attained in the past few years. However, the future is uncertain because *G. pallida* is being selected. On a plot that is being reconverted to ecological agriculture and that is subject to a 4-year crop rotation with a use of resistant potato varieties, we found a high number of cysts, but all were empty. This is a consequence of the use of resistant varieties of potato that, are capable of inducing eclosion in *G. rostochiensis* juveniles, which then die because they are incapable of penetrating the root system (Fig. 4). Nevertheless, in the examination of the plot during cultivation, one plant was detected with *Verticillium* wilt, which was also infected by *G. pallida*, indicating the necessity of elaborating

programmes of integrated production that will permit this problem to be controlled in the future (Storey & Evans, 1987; Whitehead, 1986).

The exploration and identification of pests and biocontrol agents in the study plot, enabled the detection of low number of individuals of *Leptinotarsa decemlineata*, although wire worms did not appear in spite of the traps. Entomopathogenic nematodes (EPN) were not found in soil samples taken from the inner part of the field, although their presence was detected along the edges of the crop. In one sample, 149 final-stage *Bibio hortulanus* fly larvae, a secondary pest in horticultural crops, were found infested by EPN, identified as *Steinernema feltiae*. Other natural enemies detected along the edges were the predators *Coccinella septempunctata* and *Hippodamia septemmaculata*. On the other hand, in samplings made from the broccoli crop that replaced potato, very low populations were detected of the aphid *Brevicoryne brassicae*, which presented a high level of parasitism by *Aphidius* sp. The isolated EPN stock was multiplied in the laboratory to carry out bioassays with the object of studying its effectiveness in controlling insects that are pests in potato and in other crops.

The isolated *S. feltiae* stock in La Rioja was infective against Coleoptera such as *Leptinotarsa decemlineata* and *Tenebrio molitor*, or Lepidoptera such as *Spodoptera littoralis*, *Sparganothis pilleriana* and *Trichoplusia ni*. Its virulence was also compared with that of a commercial stock from the same species against *S. littoralis*. In dose-response as well as in penetration trials, the autochthonous stock was more effective and showed greater reproductive potential as well (Fig. 5). It was also observed that clay content over 29% decreases its infectivity.

The potato market is becoming orientated towards an organic production system and differentiated quality products, free from agrochemical residues. Food safety aspects are complemented by other values such as food sovereignty, which attempts to free potato production from the control of a reduced number of countries or multinationals. Also, it must not be forgotten that functional aspects of the crop are key factors in the new strategies for potato production.

In potato cultivation in La Rioja, due to its climatic characteristics and to the diversity of agricultural systems, at present the only nematode that could create problems is *G. pallida*. In the study of the field reconverted to ecological agriculture, among the air-borne insects, only

Leptinotarsa decemlineata was found, although it had no effect on the crop. Wire worms were not found in soil samplings. Among natural enemies, it is important to note the presence of coccinellids predators and of the *Aphidius* sp. parasite, which demonstrated a high level of attack from the *Brevicoryne brassicae* in the broccoli field that followed the potato crop. An autochthonous stock of the *S. feltiae* entomopathogenic nematode that infected pest insects was also isolated. Since it was more effective than the commercial stock tested, it appears to be a very promising biocontrol agent. It is important to use autochthonous EPN species and populations that are adapted to local conditions.

An increase in quality and production in potato is related to the management of environmental factors in the first place, especially climate and soil, through the development of agricultural techniques that combine environmental characteristics with plant requirements, thus permitting a reduction of agrochemicals and achieving rational management of resistant varieties. Control of seed quality must also be taken into account, to prevent the dispersion of highly pathogenic species. The future is in the use of ecological criteria based on the management of biological and environmental diversity, through understanding of how to combine scientific knowledge with the experience of farmers. This would permit crop diversification, which in turn would increase quality and reduce ecological risks, thus achieving a just price that simultaneously takes consumer interests into account.

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García-Álvarez A., Gutiérrez C., Escuer M., Antonio Bello. Нематоды и проблема поддержания биологического разнообразия на картофельных полях Ла Риохи, Испания.

Резюме. Были исследованы основные биотические, экологические и технологические факторы производства картофеля в Ла Риохе, традиционном и дающем основную долю ежегодного урожая районе возделывания этой культуры в Испании. Исследование было сфокусировано на нематодах и насекомых как основных факторах биоты, лимитирующих производство картофеля. Выявленные проблемы с нематодным поражением картофеля ограничивались *Globodera pallida* и *G. rostochiensis*. Не было отмечено поражений нематодами *Nacobbus aberrans*, *Meloidogyne chitwoodi* и *Ditylenchus destructor*, представляющими значительные проблемы в соответственно тропических и умеренных районах возделывания картофеля. Существенный вред приносит среди прочих насекомых-вредителей колорадский жук *Leptinotarsa decemlineata*. Предполагается, что значительное биологическое разнообразие биоты на картофельных полях обусловлено комплексом причин, включающим особенности среды, некоторые приемы культивирования (такие, как севооборот и внесение органических удобрений). Одним из проявлений высокого биоразнообразия на полях Ла Риохи рассматривается присутствие в почвах энтомопатогенных нематод. Поддержание высокого уровня биологического разнообразия рассматривается как важный элемент общего комплекса производства картофеля.
