

# Plant-Parasitic Nematodes Detected in Andean Tubers from Argentina and Bolivia

PAOLA LAX<sup>1\*</sup>, MARCELO E. DOUCET<sup>1</sup>, CLAUDIA GALLARDO<sup>2</sup>, SUSANA MURUAGA DE L'ARGENTIER<sup>2</sup> & HUGO VILTE<sup>2</sup>

<sup>1</sup>Laboratorio de Nematología, Centro de Zoología Aplicada, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, C.C. 122, C.P. 5000, Córdoba, Argentina. E-mail: plax@com.uncor.edu

<sup>2</sup>Cátedra de Zoología Agrícola, Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, Alberdi 47, C.P. 4600, San Salvador de Jujuy, Jujuy, Argentina

\* Postdoctoral Fellow, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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**Summary** – Lax, P., M.E. Doucet, C. Gallardo, S. Muruaga de L'Argentier & H. Vilt. Plant-parasitic nematodes detected in Andean tubers from Argentina and Bolivia.

The presence of nematodes was evaluated in the skin and underlying parenchyma of several varieties of Andean tubers (Andean potato, 'Oca' and 'Papalisa') from Argentina and Bolivia. *Nacobbus aberrans* was widely distributed in the Andean region. Other phytophagous nematodes detected were *Meloidogyne incognita*, *M. javanica*, *Globodera* sp. and *Pratylenchus* sp. Two different species of the Family Anguinidae, *Hexatylus* sp. and *Aphelenchus avenae*, were also found. Furthermore, results of two different methods usually used to evaluate tuber sanitation were analyzed: processing of skin and underlying parenchyma and closed bag bioassays, the former appearing as the most efficient.

**Keywords:** *Globodera*, *Meloidogyne*, *Nacobbus aberrans*, nematode survey, *Oxalis tuberosa*, *Pratylenchus*, *Solanum tuberosum* subsp. *andigenum*, *Ullucus tuberosus*.

**Resumo** – Lax, P., M.E. Doucet, C. Gallardo, S. Muruaga de L'Argentier & H. Vilt. 2006. Nematóides fitoparasitas detectados em tubérculos na Argentina e Bolívia.

A presença de nematóides foi avaliada na casca e no parênquima adjacente de muitas variedades de tubérculos andinos (batata andina, 'oca' e 'papalisa'), oriundos da Argentina e Bolívia. *Nacobbus aberrans* mostrou-se largamente distribuído na região Andina. Outros nematóides fitoparasitas foram detectados: *Meloidogyne incognita*, *M. javanica*, *Globodera* sp. e *Pratylenchus* sp. Duas diferentes espécies da Família Anguinidae, *Hexatylus* sp. e *Aphelenchus avenae* foram também encontradas. Além disso, resultados de dois diferentes métodos, usualmente usados para avaliar tubérculos infestados, foram analisados: processo da casca e parênquima adjacente e bioensaio do saco fechado, sendo o primeiro o mais eficiente.

**Palavras-chave:** *Globodera*, *Meloidogyne*, *Nacobbus aberrans*, levantamento de nematóides, *Oxalis tuberosa*, *Pratylenchus*, *Solanum tuberosum* subsp. *andigenum*, *Ullucus tuberosus*.

## Introduction

Andean tuber crops are a valuable source of genetic resources and constitute the food base of the inhabitants in the Andean region. Tubers also have medicinal properties

and their production contributes to the socioeconomic development of local people (Terrazas *et al.*, 1997). Among the main plants grown are Andean potato (*Solanum tuberosum* subsp. *andigenum*), 'Oca' (*Oxalis tuberosa*), 'Papalisa' or 'Olluco' (*Ullucus tuberosus*), and 'isaño'

(*Tropaeolum tuberosum*). These crops are characterized by their great diversity. Potato is widely heterogeneous; its several varieties have particular morphological, agronomical and qualitative characteristics (IBTA/PROINPA, 1994). The same applies to the numerous native varieties of 'Oca' (Cadima *et al.*, 2004).

In the Andean region, species of plant-parasitic nematodes may produce significant yield losses to potato crops and reduce tuber quality. Among these pathogens are representatives of the genera *Globodera*, *Meloidogyne*, and the species *Nacobbus aberrans* (González & Franco, 1997). The latter has also been noted as a limiting factor to 'Papalisa' production (Cadima *et al.*, 2003; Condori *et al.*, 2003) and as a pathogen of 'Oca' (CIP, 1996; Ramos *et al.*, 1998).

Given their intimate relationship with the host, these endoparasitic nematodes can be easily dispersed. *Meloidogyne* spp. and *N. aberrans* have the ability to infect not only the potato roots but also the tubers, and to develop their cycle inside the parenchyma. Furthermore, cysts of *Globodera* spp. may be spread by transport of the soil attached to the tuber (González & Franco, 1997). The objective of this work was to evaluate the presence of plant-parasitic nematodes on different varieties of Andean tubers from different localities of Argentina and Bolivia.

## Materials and Methods

In May, July and September 2005, 65 samples of stored Andean tubers corresponding to the 2004-2005 crop cycle were collected (harvested in February-March 2005). Samples were acquired directly from growers in local markets or their houses. A total of 25 varieties of Andean potato, two of 'Papalisa' and three of 'Oca' were obtained. The variety names were provided by the growers. Table 1 indicates the tubers analyzed and their origin.

Each sample was divided in two parts, which were processed following two methods commonly used to evaluate tuber sanitation. In one, 20 tubers were peeled and the skin and underlying parenchyma were processed using the technique proposed by Costilla (1985). The resulting material was observed under stereoscopic microscope to detect the presence of nematodes. Specimens were identified according to the morphological characteristics defining each taxon detected. In the other part, 10-15 tubers per sample were used for a closed bag bioassay (Ortuño *et al.*, 1996) to detect infections by *Nacobbus*, *Globodera* and

*Meloidogyne*. A plastic bag was filled with 400 g of sterile soil with soil moisture at field capacity and a tuber was planted. The bags were closed, wrapped in newsprint, and stored in the dark at about 25°C. After 40 days the root surface in each repetition was examined for the presence of white females or cysts belonging to the genus *Globodera*. When galls were detected, tissues were dissected under stereoscopic microscope to confirm if galls were produced by nematodes of the genus *Nacobbus* or *Meloidogyne*.

## Results and Discussion

### Nematode detection in skin and underlying parenchyma of tubers

#### Plant-parasitic nematodes (Table 2)

Phytophagous nematodes were detected in 57% of the samples analyzed, *N. aberrans* being the species most frequently found (51%). Third (J3) and fourth stage (J4) juveniles, and immature females of this nematode were observed. In samples obtained a few months after harvest, J3 and J4 were more frequent and immature females were scarce, whereas potatoes that had been stored for longer periods (samplings conducted in July and September) contained a higher number of immature females. Mature females inside the tuber parenchyma were occasionally observed. To confirm this observation, potatoes from some samples analyzed in May were processed in August, and similar results were obtained. This indicates that the nematode does not remain confined to the skin in quiescence, but can continue to develop inside the tuber.

*N. aberrans* was the most widely distributed species in northwestern Argentina (54% of the total localities considered). Ortuño *et al.*, (2005) reported that *N. aberrans* also exhibits the widest geographic distribution among potato infecting nematodes in Bolivia, and that it is found isolated or associated with *Globodera* sp. and/or *Meloidogyne* spp. as we observed in the samples from Argentina.

Globose females with egg masses of *M. incognita* and *M. javanica* were detected to a lesser degree (8% and 9%, respectively). Both nematode species were detected simultaneously in the potato varieties 'Tuni' and 'Negra' from the locality of Alfarcito (Argentina).

Cysts of the genus *Globodera* were extracted from the eyes of 'Collareja' variety from Colanzuli and Santa Victoria (province of Salta) (Figure 1). Recently, the species *G.*

Table 1. Locality of sample and variety of tuber analyzed.

Origin	Locality	Tuber variety	
Argentina			
<u>Prov.: Jujuy</u>			
Dep.: Humahuaca	Aparzo	Azul (1)	
	Chaupi Rodeo	Colorada (1), Churqueña (1), Redonda (1), Runa (1)	
	Huasadurazno	Azul (1)	
	Humahuaca	Collareja (1)	
	Ocumazo	Azul (1)	
	Palca de Aparzo	Azul (2), Colorada (2)	
	Ronque	Collareja (1)	
	Dep.: Santa Catalina	Casira	Collareja (1), Malcacha (1)
		Dep.: Tilcara	Alfarcito
	Colonia San José		Ojo de señorita (1)
San José	Collareja (1)		
Dep.: Tumbaya	Yacoraite	Collareja larga (1)	
	Patacal	Ojo de señorita (1)	
	Purmamarca	Colorada redonda (1), Collareja redonda (1), Chacarera blanca (1)	
Dep. Valle Grande	Santa Ana	Cuarentilla (1), Runa (1), Tuni blanca (1)	
	Valle Colorado	Colorada (1)	
	Valle Grande	Abajeña (1)	
Dep.: Yavi	La Quiaca	Waych'a (1)	
	Yavi	Collareja (2), Criolla (1)	
<u>Prov.: Salta</u>			
Dep.: Iruya	Campo Carreras	Colorada (1), Collareja (1), Ojosa (1), Redonda (1)	
	Colanzuli	Collareja (1), Chacarera (2), Oca amarilla (1), Oca colorada (1), Runa (1), Tuni (1)	
	Iruya	Colorada (1), Collareja (1), Chacarera redonda (1), Oca amarilla (1), Oca rosada (1), Papalisa (1), Papa verde lisa (1)	
	Pueblo Viejo	Ojosa (1), Redonda (1), Rosada (1)	
Dep.: Santa Victoria	Santa Victoria	Collareja (1)	
Bolivia			
<u>Prov.: Cochabamba</u>			
Dep.: Cochabamba	Cochabamba	Oca colorada (1), Papalisa (1), Negra (1), Sani (1), Waych'a (3)	
<u>Prov.: Tarija</u>			
Dep.: Tarija	Villazón	Imilla (1)	

Abbreviations: Prov. = Province; Dep. = Department.

The number of samples analyzed of each variety is indicated in parentheses.

Table 2. Plant-parasitic nematodes detected in different varieties of Andean tubers.

Nematode	Locality	Tuber variety
<i>N. aberrans</i>	Campo Carreras	Colorada, Ojosa, Redonda
	Casira	Collareja, Malcacha
	Cochabamba	Sani, Waych'a
	Colanzuli	Chacarera, Tuni
	Chaupi Rodeo	Colorada, Churqueña, Redonda
	Iruya	Colorada, Collareja, Chacarera redonda
	Palca de Aparzo	Azul, Colorada
	Pueblo Viejo	Ojosa, Redonda
	Purmamarca	Collareja redonda
	San José	Collareja
	Santa Ana	Cuarentilla, Runa, Tuni blanca
	Yavi	Collareja
	<i>M. javanica</i>	Humahuaca
<i>M. javanica</i> + <i>M. incognita</i>	Alfarcito	Negra, Tuni
<i>N. aberrans</i> + <i>Globodera</i> sp.	Colanzuli	Collareja
	Santa Victoria	Collareja
<i>N. aberrans</i> + <i>M. incognita</i>	Colanzuli	Runa
	Chaupi Rodeo	Runa
	Villazón	Imilla
<i>N. aberrans</i> + <i>M. javanica</i>	Campo Carreras	Collareja
	Ronque	Collareja
	Yavi	Collareja
<i>Pratylenchus</i> sp.	Cochabamba	Oca colorada

*pallida* was first detected in continental Argentina, in a locality of the same province (Lax *et al.*, 2005), attacking roots of 'Colorada' and 'Ojosa' Andean potato (Doucet *et al.*, 2005 a, b). Further studies should be conducted to confirm the species identity of the two new *Globodera* populations found.

'Collareja' variety, one of the most widely used in northwestern Argentina, showed high susceptibility to the attack of *N. aberrans* (90% of samples), *M. javanica* (40%), and *Globodera* sp. (20%) with some samples simultaneously infected by *N. aberrans*-*Globodera* sp. or *N. aberrans*-*M. javanica*.

In this work, females of *Pratylenchus* were extracted from the skin of 'Oca colorada' from Cochabamba (Bolivia). It has been noted that species of this genus may infect

potato tubers (CIP, 1996). However, there are no previous records of the presence of plant-parasitic nematodes attacking 'Oca' tubers.

#### Other nematodes (Table 3)

As with 'Oca', there are no reports of plant-parasitic nematodes associated with 'Papalisa' tubers. Representatives of the Family Anguinidae were detected in samples from Cochabamba, whose genus identity has not been determined yet. The Family comprises species of very different feeding habits, including phytophagy and mycophagy (Siddiqi, 2000). With respect to the latter type, female and juvenile *Aphelenchus avenae* were found in 'Colorada' and 'Azul' Andean potato from Campo Carreras and Palca de Aparzo (Argentina), respectively.

Table 3. Other nematodes detected in Andean tubers.

Nematode	Locality	Tuber variety
Family Anguinidae	Cochabamba	Papalisa
<i>Aphelenchus avenae</i>	Campo Carreras Palca de Aparzo	Colorada Azul
<i>Hexatylus</i> sp.	Casira	Collareja

Numerous filiform females and juveniles at different stages, belonging to the genus *Hexatylus*, were found in 'Collareja' variety of the locality of Casira (Argentina). This genus comprises species of complex life cycles, represented by a free-living and micetophagous generation and another entomoparasitic generation. It should be noted that the type species, *H. viviparus*, was found for the first time in a diseased potato tuber (Siddiqi, 2000).

The representatives of Anguinidae, *A. avenae* and *Hexatylus* sp., were observed moving in the extraction solution. These nematodes probably develop while feeding on fungus hyphae present on the tuber surface during storage.

### Methodological aspects

The use of the closed bag bioassay to detect *Globodera* spp. and *N. aberrans* is helpful to evaluate the level of sanitation of tubers and soil in seed potato production (Ortuño *et al.*, 1996). The analysis of tuber skins yielded similar results to those obtained with the bioassay (infections by *Globodera* sp. and *N. aberrans* and non-infected tubers). However, in those samples where *Meloidogyne* spp. were detected in the processed skin and underlying parenchyma, no galls were observed in any repetition using the bioassay, which might indicate that the latter method is not suitable to evaluate the presence of these nematodes in tubers. This difference might be caused by the greater depth at which *Meloidogyne* is located inside the tuber parenchyma, whereas *N. aberrans* and *Globodera* spp. are found at a more superficial level, being able to infect new roots faster. Another advantage of the processed skin and underlying parenchyma method is that the presence of other nematodes, such as: *Pratylenchus* sp., *Hexatylus* sp., *A. avenae*, and specimens of the Family Anguinidae, can be detected.

The Andean potato varieties 'Ojosa' and 'Colorada' from Campo Carreras were obtained from a plot where high

numbers of *G. pallida* were previously detected. The closed bag bioassay performed on tubers from this plot did not reveal cysts or white females of the nematode on roots in any repetition. This suggests that the results obtained from the bioassay are not an indicator of the sanitary conditions of the plot of origin (only of the tubers used), unless the experiment is conducted using raw soil of the same plot.

Costilla (1985) and Ortuño *et al.* (2005) suggested washing the tubers with running water before processing, so that attached soil and organic residues are removed, and the surface is clean. The same process is recommended for a molecular diagnostic method for detecting *Nacobbus* in potato tubers (Atkins *et al.*, 2005). In the tubers analyzed, it was observed that J3, J4 and immature females of *N. aberrans* are not always found under the lenticels, but at times may be directly attached to the potato cuticle (Figure 1). Previous experiments showed that when the water used for washing was filtered through a 40-mm sieve, numerous representatives of the species that were on the skin were retained. It was also observed that submerging the skins in water in Petri dishes for 6 h allowed nematode detachment. Therefore, it is clear a deep cleaning of the tuber is not necessary for diagnostic purposes, especially when the aim is to quantify the level of infestation. A similar situation would occur with cysts of the genus *Globodera* that might be located on the tuber surface.

### Considerations

Tuber production in the Andean region has great economic importance for rural populations (Ludo *et al.*, 1999). Part of the production is marketed between neighbouring communities through buying, selling, and barter (Ludo *et al.*, 1999). The detection of the phytophagous nematodes *N. aberrans*, *Globodera* sp., *Meloidogyne* spp. and *Pratylenchus* sp. alone or combined in the tubers analyzed reveals a highly serious issue, especially when the tubers are used as seeds. Nematodes are being widely

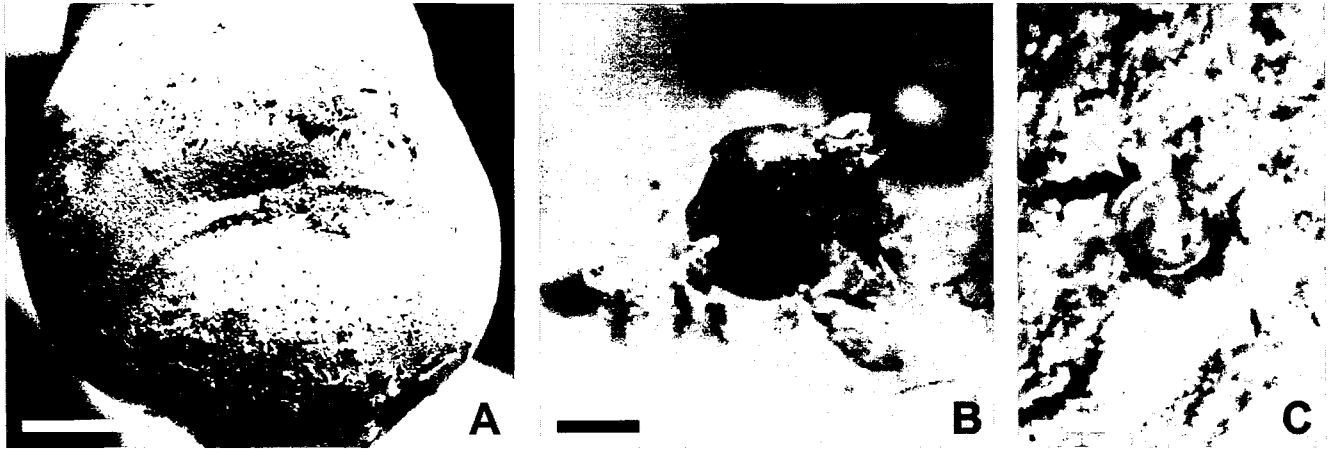


Figure 1. Nematodes detected in Andean potato: A). *Globodera* sp. cyst in the eye of 'Collareja' tuber. B) *Idem* A, greater magnification. C) Quiescent juvenile of *Nacobbus aberrans* on the tuber skin. (Scale bars: A = 1 cm; B = 250  $\mu$ m; C = 200  $\mu$ m).

dispersed to very diverse areas that may or may not be infested. Not only the vector crop but also other hosts will be subsequently damaged, especially in the cases of *Meloidogyne* spp. and *N. aberrans*, which are characterized by their marked polyphagia. This characteristic has been confirmed in *N. aberrans*, since it was found on 17 Andean potato varieties in this work.

The results of this survey indicate the need to: i) control the sanitary conditions of tubers that will be used as seeds by analyzing the skin and underlying parenchyma, since this method ensures fast detection of harmful phytophagous nematodes and other nematode species; ii) accurately identify the species of pathogens found in Andean tubers and determine their biological characteristics; iii) determine areas that are free of or infested by harmful nematodes; iv) evaluate the susceptibility to parasitism of the most commonly grown varieties in a region by the nematode species detected; and v) organize outreach programs aimed at disseminating knowledge among growers and technicians. Accomplishing these objectives will contribute significantly to better management of nematodes that damage tuber crops in the Andean region.

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