COMUNICACION—COMMUNICATION

QUARANTINE DETECTION OF NEMATODES AND PROCEDURES FOR THEIR ERADICATION FROM VEGETATIVELY PROPAGATED MATERIALS IMPORTED BY BRAZIL

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RESUMEN

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El Centro Nacional para las Investigaciónes de los Recursos Genéticos y Biotecnologia (CENARGEN) es responsable de la introducción, cuarentena de pos-entrada e intercambio de germoplasmas vegetales para los programas de investigaciónes brasileños que incluyen instituciones de investigaciones y universidades. El Laboratorio de Nematologia del CENARGEN tiene facilidades para la cuarentena de pos-entrada y la inspección de todos los germoplasmas propagativos vegetales. Resultados de la inspección entre 1981-94 son presentados teniendo en cuenta la incidencia, identificación y la irradicación de los nematodos asociados con materiales propagativos vegetales. Algunos de los mas importantes generos detectados durante este periodo fuerón *Globodera, Ditylenchus, Pratylenchus y Xiphinema.* Aproximadamente el 64% de los germoplasmas vegetales infectados fueron satisfactoriamente saneados utilizando métodos de cultivo de tejidos. Algunos nematodos no fueron erradicados de aproximadamente el 24% de las asepciones inrecladas las que fueron posteriormente incineradás. El resto de los germoplasmas infectados (aproximadamente el 12%) fue saneado mediante el tratamiento con agua caliente o con el tratamiento con el nematicida carbofuran.

Palabras clave: Brasil, cuarentena, nematodos erradicados.

The principal objective of the Centro Nacional de Recursos Genéticos e Biotecnologia(CENARGEN) is to provide the research system in Brazil with germplasm of sufficient variability for research programs. To accomplish its mandate, CENARGEN participates in botanic exploration to collect, classify and conserve germplasm, and is responsible for importation and introduction of exotic material. CENARGEN has imported germplasm into Brazil since its inception in 1974 and has experienced a significant increase in its accessions in the past several years.

While the importation of plant germplasm is a fundamental aspect of agricultural development in Brazil, the introduction of plant materials increases the risks of introducing exotic pathogens and pests at a rate commensurate with the increasing accession levels. In response to those risks, CENARGEN established the Section of Plant Germplasm Exchange, with the purpose of safely managing plant importation through a process of inspection and quarantine.

Fewer accessions of vegetative propagative material are received by CENARGEN than seed; however, vegetative plant material has the capacity to carry more pathogens and pests than seed. Therefore, detection and eradication of pathogens and pests carried on vegetative propagative material imported into Brazil is considered to be a high priority objective.

The purpose of this communication is to review results of the plant inspection program during the period 1981 to 1994 with regard to the detection of plant para-

sitic nematodes in vegetative plant material. In addition, we describe the efficacy of three methods to eradicate nematodes from vegetative plant material prior to its release to plant breeders.

Several methods were used to detect nematodes in plant germplasm. Microscopic examination of plant tissue was used to detect symptoms (galls, lesions) or signs (females, egg masses) of sedentary plant-parasitic nematodes. Plant material also was processed (20°C±2°C) using a modified tray technique to recover motile nematodes (Whitehead and Hemming, 1965). Our modification included the use of a smaller tray size (53 × 21 × 5 cm), and the use of one layer of plastic net (2-mm aperture) to support Kimwipe tissue. Distilled water was added in sufficient quantity to wet the tissue. After 4-6 hrs of extraction, the water was passed through a 25 µm sieve. The contents of the sieve were backwashed into a beaker prior to observation for the presence of nematodes.

To detect nematode cysts, pieces of propagating plant tissue were placed in a flat-bottomed glass flask with 500 ml of distilled water and automatically agitated for 10 min. A sucrose solution (400 g/L water + 12.5 ppm of Separan) was added, and the flask was agitated for an additional 10 min. The suspension was poured into a flask and left for 10 min before passing 1/3 of the suspension through a Whatman filter paper. The material that was retained on the filter paper was examined with a dissecting microscope (Carvalho *et al.*, 1953).

Generally, we attempted to eradicate nematodes using heat or carbofuran. If those methods were unsuccessful, meristem cultures were tried. Vegetative propagation tissue was treated with hot water (52°C) for 20 min. Bulbs were soaked for 15 min in a solution (2.4 g a.i./L) of carbofuran (Tenente *et al.*, 1986 and 1994) and then planted in sterilized soil and maintained in the greenhouse until new bulbs were produced and inspected. However, meristem tissue culturing was the most successful technique for nematode eradication. Meristems were grown (20-22°C) in specific media in an incubator with 12 hr of light Plantlets were adapted to soil under greenhouse conditions at 18-25°C. Plants successfully generated with this technique were checked for nematodes, using the previously described methods, prior to release.

Most nematodes were recovered as juveniles, thereby preventing identification to species (Table 1). For crops such as potato, garlic, peanut and grape, the most economically important genera detected were *Globodera, Xiphinema, Meloidogyne* and *Ditylenchus.* These and previous results (Tenente, 1985; Tenente *et al.*, 1993) clearly indicate the need for an effective inspection and quarantine service to mitigate the risks of introducing and disseminating exotic pests in the imported plant germplasm (Tenente and Manso, 1987).

The hot water treatment eradicated *Aphelenchoides, Cryptaphelenchus, Helicotylenchus, Pratylenchus* and *Tylenchus* from pineapple and *Aphelenchoides* from mango (Table 1). *Aphelenchoides* and *Ditylenchus dipsaci* were eradicated from infested garlic bulbs by immersion in carbofuran solution. Nematodes were eradicated and plants regenerated from approximately 64% of the infested accessions using meristem tissue culture. Although nematodes were eradicated from tissues of *Arachis hypogaea, Amorphophalus* sp., *Humulus* spp. and *Tulipa generiana,* the meristem-derived plantlets did not survive transplantation to the greenhouse. Our nematode eradication methods were ineffective for approximately 24% of the infested germplasm accessions which included *Acassia, Agave, Brownea, Eriobotrya, Humulus, Lignum, Teconua,*

Table 1. Nematodes detected in vegetative propagating material imported into Brazil (1981-1994).

Plant germplasm	Origin	Detected nematodes	Eradication techniques
Acassia cyanophilla	Mexico	*Aphelenchus* sp.	Not successful[z]
		Ogma sp.	" "
Agave sisalana	Tanzania	*Aphelenchoides* sp.	Not successful
		Tylenchus sp.	" "
		Xiphinema sp.	" "
Allium sativum	Italy	*Aphelenchoides* sp.	Tissue culture
		Tylenchus sp.	" "
	Peru	*Aphelenchoides* sp.	Tissue culture
		Pratylenchus sp.	" "
	Chile	*Ditylenchus dipsaci*	Tissue culture
	USA	*Aphelenchoides* sp.	Carbofuran 35%
		Ditylenchus dipsaci	" "
Amorphophalus sp.	Indonesia	*Brachydorus* sp.	Tissue culture
Ananas comosus	France	*Aphelenchoides* sp.	52°C/20 min
		Helicotylenchus sp.	" "
		Pratylenchus sp.	" "
	Martinique	*Aphelenchoides* sp.	52°C/20 min
		Cryptaphelenchus sp.	" "
		Pratylenchus sp.	" "
		Tylenchus sp.	" "
Arachis hypogaea	Colombia	*Aphelenchoides* sp.	Tissue culture
		Ditylenchus sp.	" "
		Tylenchus sp.	" "
Arracacia xanthorriza	Ecuador	*Aphelenchoides* sp.	Tissue culture
Brachiaria brizantha	USA	*Ditylenchus* sp.	Tissue culture
Brownea latifolia	Trinidad and Tobago	*Tylenchus* sp.	Not successful
Eriobotrya japonica	Israel	*Aphelenchoides* sp.	Not successful
		Nothotylenchus sp.	" "
Humulus spp.	USA	*Dorylaimus* sp.	Not successful
		Tylenchus sp.	" "
Hyacinthus orientalis	USA	*Aphelenchoides* sp.	Tissue culture
Hypahrenia spp.	Colombia	*Aphelenchoides* sp.	Tissue culture
Lignum vitae	Trinidad and Tobago	*Aphelenchus* sp.	Not successful
		Dorylaimus sp.	" "

[z]Not successful = Nematodes not eradicated and materials were incinerated.

Table 1. (Continued) Nematodes detected in vegetative propagating material imported into Brazil (1981-1994).

Plant germplasm	Origin	Detected nematodes	Eradication techniques
		Helicotylenchus sp.	" "
		Tylenchus sp.	" "
Mangifera indica	USA	*Aphelenchoides* sp.	52°C/20 min
Mentha sp.	Peru	*Aphelenchoides* sp.	Tissue culture
Mentha villosa	Peru	*Pratylenchus* sp.	Tissue culture
Musa sp.	Ecuador	*Aphelenchoides* sp.	Tissue culture
		Helicotylenchus sp.	" "
	Martinique	*Ditylenchus* sp.	Tissue culture
	Phillippines	*Meloidogyne* sp.	Tissue culture
		Xiphinema sp.	" "
Narcissus sp.	USA	*Aphelenchoides* sp.	Tissue culture
Opuntia ficus-indica	Mexico	*Dorylaimus* sp.	Tissue culture
		Tylenchus sp.	" "
Oreganum vulgare	Peru	*Aphelenchoides* sp.	Tissue culture
Pinus spp.	Mexico	*Criconemella* sp.	Tissue culture
		Tylenchus sp.	" "
Piper wichmanii	USA	*Ditylenchus* sp.	Tissue culture
Prosopis sp.	USA	*Aphelenchoides* sp.	Tissue culture
Saccharum officinallis	USA	*Paurodontus gracilis*	Tissue culture
Solanum spp.	Holland	*Aphelenchus* sp.	Tissue culture
		Aphelenchoides sp.	" "
		Globodera sp.	" "
	USA	*Aphelenchoides* sp.	Tissue culture
		Aphelenchus sp.	" "
		Ditylenchus sp.	" "
		Meloidogyne sp.	" "
	Canada	*Ditylenchus dipsaci*	Tissue culture
	Peru	*Ditylenchus* sp.	Tissue culture
		Tylenchus sp.	" "
Teconua sp.	Trinidad and Tobago	*Tylenchus* sp.	Not successful
Thaumatococcus danielli	Ghana	*Aphelenchoides* sp.	Not successful
		Ditylenchus sp.	" "
		Tylenchus sp.	" "
Tulipa generiana	USA	*Aphelenchoides* sp.	Tissue culture

[z]Not successful = Nematodes not eradicated and materials were incinerated.

Table 1. (Continued) Nematodes detected in vegetative propagating material imported into Brazil (1981-1994).

Plant germplasm	Origin	Detected nematodes	Eradication techniques
Vitis spp.	France	*Aphelenchoides bicaudatus*	Tissue culture
		Ditylenchus sp.	" "
		Trichodorus sp.	" "
	Africa	*Dorylaimida* (Order)	Tissue culture
	USA	*Aphelenchoides* sp.	Tissue culture
		Ditylenchus sp.	" "
		Xiphinema sp.	" "
Zenziber sp.	Italy	*Aphelenchoides* sp.	Not successful
Xanthosoma sagihifolium	Venezuela	*Aphelenchoides* sp.	Tissue culture
		Ditylenchus sp.	" "

[z]Not successful = Nematodes not eradicated and materials were incinerated.

Thaumatococcus, and *Zenziber.* All remaining tissues of the latter genera were incinerated.

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