EXTRACTION OF *MELOIDOGYNE INCOGNITA* FROM CALADIUM CORMS[†]

R. McSorley,[1] J. J. Frederick,[1] and R. J. McGovern[2]

Department of Entomology and Nematology, P.O. Box 110620, University of Florida, Gainesville, FL 32611-0620,[1] and University of Florida, Gulf Coast REC—Bradenton, 5007 60 St. E., Bradenton, FL 34203-9324, U.S.A.[2]

RESUMEN

McSorley, R., J. J. Frederick y R. J. McGovern. 1999. Extracción de *Meloidogyne incognita* de cormos de caladium. Nematrópica 29:245-248.

La extracción de *Meloidogyne incognita* de cormos de caladium (*Caladium* × *hortulanum*) fue investigada en seis experimentos cortos. Varios métodos de preparación de cormos fueron examinados. La maceración de los cormos con una licuadora fue más efectiva para la recuperación de nematodos agalladores de la raíz, que la extracción con hipoclorito de sodio. El pelado de los cormos seguido con maceración solamente, resultó en una suspensión más limpia para el conteo, sin una perdida significante ($P > 0.10$) de nematodos, en comparación a la maceración del cormos completo. Cuando los cormos sin cuticula se maceraron en la liquadora y se incubaron, la mayoría (84.5%) de los nematodos, se recuperaron después de tres días de incubación, comparado a los totales obtenidos a los 6 días.

Palabras claves: Caladium × *hortulanum*, cultivos-raíz, métodos de extracción, nematodos agalladores de la raíz, ornamentales de follage.

Bulky plant substrates such as bulbs, corms, or enlarged storage roots can present difficulties for nematode extraction. Maceration of these plant parts can result in large amounts of debris or viscous substance within the sample to be counted, and incubation may result in the release of compounds toxic to nematodes (McSorley, 1987). For these reasons, a mist chamber is usually recommended for the extraction of plant-parasitic nematodes from these substrates (Hooper, 1990; McSorley, 1987). However, not all laboratories have mist chambers, and so there remains a need for simpler and less expensive extraction methods.

Caladium (*Caladium* × *hortulanum* Birdsey, fam. Araceae; syn. *C. bicolor* Vent.) is an important foliage ornamental crop in Florida. However, root-knot nematodes, particularly *Meloidogyne incognita* (Kofoid and White) Chitwood, are important pests of caladium (Rhoades, 1964), and one survey of the crop revealed that 52.8% of 4466 soil samples and 27.9% of 4894 corm samples collected from caladium contained *Meloidogyne* spp. (Esser, 1973). Caladiums are propagated vegetatively from corms or pieces of corms and so root-knot nematodes and other endoparasites can be transmitted on planting material. Therefore, it is important to have a convenient method for the assay of root-knot nematodes on caladium corms.

A preliminary trial revealed that root-knot nematodes could be conveniently extracted from caladium corms using the sodium hypochlorite (NaOCl) method of Hussey and Barker (1973). The objective of the experiment reported here was to compare the efficacy of the sodium hypochlorite method with traditional methods such as incubation or maceration for the extraction of *Meloidogyne* spp. from

[†]Florida Agricultural Experiment Station Journal Series No. R-06939.

caladium corms. Subsequent experiments were conducted to optimize the amount and part of the corm to be incubated and the time of incubation.

Caladium corms were obtained from a field infested with *M. incognita* in south Florida, washed, air-dried, and stored at room temperature (ca. 20-22°C) as typical of industry practices. Corms of the caladium cultivar 'White Christmas', having an average weight of 13.0 g, were used for the first experiment. Four different methods were used for the initial step of extraction of *M. incognita* from corms: 1) using a modification of Hussey and Barker's (1973) sodium hypochlorite technique, a corm was shaken vigorously in a solution of 0.525% NaOCl for 30 sec and extracted eggs collected on a 25-µm (U.S. #500) sieve; 2) the same procedure as #1, except an NaOCl concentration of 0.262% was used; 3) a corm was cut up into eight pieces; 4) a corm was cut into eight pieces and macerated in 200 cm^3 water in a blender (Waring Commercial Blender) for 30 sec. Following the initial extraction step, any materials obtained (nematode eggs, all pieces from a cut corm, or macerated corm) were incubated in a uniform manner. Materials were incubated in a modified Baermann device similar to that developed by Rodriguez-Kabana and Pope (1981). A double layer of standard tissue paper (Kimwipes, Kimberly Clark Corp., Roswell, GA) was used in the Baermann trays, which were covered and incubated at 25°C. Second-stage juveniles were removed from the Baermann trays after four and seven days, and counted. Each treatment was replicated 10 times, with each replicate consisting of a single corm. Data were transformed by $\log_{10}(x + 1)$ and analyzed by analysis of variance followed by Duncan's multiple-range test. Untransformed data are presented in all cases.

In a second experiment, the numbers of nematodes extracted from whole corms or skin peeled from corms were compared. Preliminary examination of corms had revealed that most of the root-knot nematode females were located near the surface of the caladium corm. This was anticipated, since root-knot nematodes establish permanent feeding sites in the vascular bundles, which in caladium are located near the periphery of the corm. Corms of the cultivar 'Frieda Hemple', with an average weight of 9.2 g, were used. Each corm was cut into eight pieces, macerated, and incubated as before. For the second treatment, each corm was peeled with a knife, and only the skin was macerated and incubated. Each of the two treatments was replicated eight times, and numbers of extracted nematodes were compared using analysis of variance.

The effect of incubation time on juvenile recovery was examined in a third test. Each caladium corm was peeled with a knife and the peeled skin was incubated as before. Nematodes were removed from the Baermann devices after three and six days and counted. This small test was repeated four times, each with a different caladium cultivar. Five corms of each cultivar were used, with each corm constituting a replicate.

Results of the first experiment (Table 1) indicated that the sodium hypochlorite method was inferior to direct incubation of corms or maceration of corms prior to incubation. Root-knot nematode juveniles were recovered more quickly when corms were macerated in a blender prior to incubation than when corms were simply cut into pieces and incubated. However, while juveniles were recovered easily from incubated corms or from macerated + incubated corms, counting was difficult since the suspension to be counted was thick and syrup-like and contained some debris despite the use of a double layer of tissue paper during incubation.

Table 1. Effect of four extraction methods followed by incubation on recovery of *Meloidogyne incognita* second-stage juveniles from 'White Christmas' caladium corms.

Extraction treatment before incubation	Nematodes recovered per corm following incubation		Number of corms from which nematodes were recovered	
	4 days	7 days	4 days	7 days
0.525% sodium hypochlorite	0 b[y]	0.5 c	0	3
0.262% sodium hypochlorite	0 b	1.6 bc	0	5
Cut up corms	1.3 b	40.5 ab[z]	4	7
Macerated corms	19.7 a	21.0 a	9	9

[y]Data are untransformed means of 10 replications. Means in columns followed by the same letter do not differ ($P \leq 0.05$) according to Duncan's multiple-range test performed on $\log_{10}$-transformed data.
[z]Unusual mean separation resulted from recovery of a very high number (216 nematodes) from one corm receiving this treatment. This resulted in a high numerical average for this treatment, even though the average of the log-transformed data (on which statistical analysis is based) for this treatment (0.78) was lower than that for the macerated corm treatment (1.05).

Examination of infected caladium corms confirmed that nearly all of the root-knot nematode females present were relatively superficial. In the second experiment, when corm skins were macerated and incubated, root-knot nematode juveniles were recovered from 5 of 8 corms tested, with a mean number of 5.5 nematodes/corm. When whole corms were cut up, macerated, and incubated, nematodes were recovered from 4 of 8 corms, with a mean of 9.8 nematodes per corm (most of these from one corm). The difference in numbers recovered was not significant (F = 0.017, based on $\log_{10}$-transformed data). The nematode suspensions recovered when peeled corms were used were much easier to count. Although data were not collected on the times needed to complete various portions of the nematode assay procedure, the time taken to peel corms is much less than the extra time needed to count the dirty suspensions obtained if whole corms are used.

When peeled corm skins are macerated and incubated, most nematodes can be recovered fairly quickly, as indicated by the results of the last group of tests (Table 2). The cumulative numbers of nematodes obtained after six days of incubation are not much greater than the numbers obtained after three days. Across the four tests (each with a different cultivar), an average of 84.5% of the total number of nematodes recovered were obtained after three days of incubation.

Based on these experiments, the maceration of skin peeled from corms followed by Baermann incubation for three days appeared to be the optimal method for assay of *M. incognita* in caladium corms. Good nematode recovery and relatively clear suspensions for counting were provided by this method. When peeling corms, it is easy to distinguish the dark brown skin from the white or yellow internal portion. This method should be useful for surveys of *M. incognita* infection of caladium corms. Provided that a representative proportion of the nematode population is relatively superficial, extraction of peeled material may provide a convenient means for assay of endoparasitic nematodes in other difficult plant substrates as well.

Table 2. Recovery of *Meloidogyne incognita* second-stage juveniles after three and six days of incubation following maceration of peeled skin of caladium corms. Four tests, each with a different caladium cultivar.

Caladium cultivar	Corm wt (g)	Nematodes recovered per corm		% of recovery after 3 days
		3 days	6 days	
Rosebud	18.9[z]	7.8	9.6	81.2
Pink Beauty	10.6	27.0	29.2	92.5
Freida Hemple	8.6	29.2	37.2	78.5
Candidum	7.6	7.2	8.4	85.7

[z]Data are means of five replications.

ACKNOWLEDGMENTS

The authors thank Joseph Mullanack for technical assistance and Nancy Sanders for manuscript preparation.

LITERATURE CITED

ESSER, R. P. 1973. Nematodes associated with caladium in Florida. Plant Disease Reporter 57:558-560.

HOOPER, D. J. 1990. Extraction and processing of plant and soil nematodes. Pp. 45-68 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, U.K.

HUSSEY, R. S., and K. R. BARKER. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025-1028.

McSORLEY, R. 1987. Extraction of nematodes and sampling methods. Pp. 13-47 *in* R. H. Brown and B. R. Kerry, eds. Principles and Practice of Nematode Control in Crops. Academic Press, Sydney, Australia.

RHOADES, H. L. 1964. Effect of hot water treatment of seed corms and soil fumigation for control of root knot on yield of caladiums. Plant Disease Reporter 48:568-571.

RODRIGUEZ-KABANA, R., and M. H. POPE. 1981. A simple incubation method for the extraction of nematodes from soil. Nematropica 11:175-185.

Received: *30.VI.1999*

Recibido:

Accepted for publication: *23.VII.1999*

Aceptado para publicación: