COLONIZATION OF FOLIAR TISSUES OF AN AQUATIC PLANT, *ANUBIAS BARTERI* SCHOTT, BY *RADOPHOLUS SIMILIS*

P. S. Lehman,[1] N. Vovlas,[2] R. N. Inserra,[1] L. W. Duncan,[3] and D. T. Kaplan[4]

Florida Department of Agriculture and Consumer Services, DPI, Nematology Section, P.O. Box 147100, Gainesville, FL 32614-71000, U.S.A.,[1] Istituto di Nematologia Agraria, CNR, via Amendola 165/A, 70126 Bari, Italy,[2] University of Florida, CREC, 700 Experiment Station Road, Lake Alfred, FL 33850-2299, U.S.A.,[3] and USDA, ARS, Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 33728, U.S.A.[4]

ABSTRACT

Lehman, P. S., N. Vovlas, R. N. Inserra, L. W. Duncan, and D. T. Kaplan. 2000. Colonization of foliar tissues of an aquatic plant, *Anubias barteri* Schott, by *Radopholus similis*. Nematropica 30:63-75.

In this paper evidence is presented for the reproduction of *Radopholus similis* in foliar tissues of *Anubias barteri* Schott. Burrowing nematodes colonized petioles and leaves of *A. barteri* in addition to the rhizomes. The nematode invaded the epidermis and the mesophyll of leaves causing cavities and cell disruption in the epidermis, palisade parenchyma and spongy parenchyma. Cavities extended from the spongy parenchyma into the periphery of the vascular bundles disrupting the regular flow of nutrient solution in the leaf tissues. Nematode feeding and migration also damaged the palisade parenchyma and cell chloroplasts inducing chlorosis and small brown lesions on the blades of the infected leaves. The burrowing nematodes recovered from *A. barteri* reproduced on sour orange and Duncan grapefruit in the greenhouse and on sour orange in the laboratory. In greenhouse tests, the citrus race of *R. similis* from citrus and the population from *A. barteri* reproduced on four *Anubias* species. Morphological and morphometric characteristics of *R. similis* from *Anubias* spp. did not differ from the *R. similis* citrus race from citrus.

Key words: anatomical alteration, *Anubias*, aquatic ornamentals, burrowing nematode, citrus, citrus race, histopathology, mesophyll, *Radopholus similis*, regulatory nematodes.

RESUMEN

Lehman, P. S., N. Vovlas, R. N. Inserra, L.W. Duncan y D. T. Kaplan. 2000. Colonización de los tejidos foliares de la planta acuática, *Anubias barteri* Schott, por *Radopholus similis*. Nematrópica 30:63-75.

En este reporte, se presenta evidencia de la reproducción de *Radopholus similis* en los tejidos foliares de *Anubias barteri* Schott. Los nematodos barrenadores, colonizaron los peciolos y las hojas de *A. barteri*, además de los rizomas. El nematodo invadió la epidermis y el mesofilo de las hojas de *A. barteri*, causando cavidades y rotura de las células epidérmicas, parenquimáticas y tejido esponjoso. Las cavidades en el parénquima esponjoso, se extendieron a la periferia de los receptáculos vasculares, interrumpiendo el flujo regular de la solución de nutrientes en los tejidos de la hoja. La alimentación y migración de los nematodos dañaron también al parénquima de palizada y los cloroplastos celulares, induciendo clorosis y pequeñas lesiones necróticas en los bordes de las hojas infectadas. Los nematodos barrenadores recuperados de *A. barteri*, se reprodujeron en naranjo agrio y toronja Duncan en el invernadero y en naranjo agrio en el laboratorio. En pruebas de invernadero, los aislamientos de *R. similis* de anubias y de cítricos, se reprodujeron en cuatro especies de *Anubias*. Las características morfológicas y morfométricas de *R. similis* de *Anubias* spp., no se diferenciaron de aquellas provenientes de raíces de cítricos.

Palabras claves: alteración anatómica, *Anubias*, cítricos, histopatología, mesófilo, nematodo barrenador, nematodos reguladores, ornamentales acuáticas, *Radopholus similis*.

INTRODUCTION

In many banana growing areas of the world, the burrowing nematode (BN), *Radopholus similis* (Cobb) Thorne, is recognized as a serious economic pest. In the 1950s, the citrus industry in Florida was threatened by a disease known as spreading decline which was caused by the citrus race of BN. When it was determined that this race of BN only occurred on citrus in Florida, other citrus producing states (Arizona, California, Texas, and Louisiana) and countries (Argentina, Brazil, Chile, the European Union, Japan, and South Africa) placed regulatory restrictions on the importation of floricultural crops from Florida. There was a special concern because host studies indicated that many ornamental plants are hosts of BN, including populations of the citrus race. Floricultural crops grown in Florida for export to many other states and countries where citrus is grown, must be sampled and certified to be free of BN. Since the mid 1950s, the Nematology Section of the Florida Department of Agriculture and Consumer Services has processed almost one half million samples, primarily for BN certification. Florida nurseries marketing plants in California must sign a compliance agreement which mandates strict sanitation requirements, including growing plants on raised benches and using growing media and plant propagative materials free of all regulated phytoparasitic nematodes.

In 1998, during sampling for routine nematode certification of a Florida aquatic nursery, a BN population was detected in roots of *Anubias barteri* Schott var. *nana* (Inserra, 1998). *Anubias* spp. are aquatic plants native to West Africa and are very popular as aquarium plants. Phytoparasitic nematodes, when in soil, are basically aquatic animals, living and moving in the water film surrounding soil particles, and this suggests that the aquatic environment in which aquarium plants are grown could increase the potential for the natural infection of epigeal tissues. A study was conducted to determine: i) the plant parts of *A. barteri* that were naturally infected by BN, ii) the ability of the *Anubias* BN population to parasitize and reproduce on citrus and other *Anubias* species, iii) the possible differences in the morphology and morphometrics of the *Anubias* BN population compared with those of the BN citrus race infecting citrus. The results of this study are reported in this paper.

MATERIALS AND METHODS

Detection in foliar tissue and histological observations: Burrowing nematodes were initially extracted from the roots of *A. barteri* plants that were sampled for routine California certification. Plants in which BN was detected in the roots were then sampled to determine if nematodes were present in the rhizomes and leaves. Prior to sampling, the leaves and rhizomes were throughly rinsed with tap water. Samples were placed in plastic petri dishes, 5 cm in diameter, containing 5 ml of water. Ten leaves of *A. barteri* were sampled and the blade and petiole of each leaf was subsampled separately. The blade was cut into 5 mm strips prior to placing it in a petri dish. The petiole of each leaf sampled was cut into 1 cm sections, starting at the base of the petiole where it was attached to the rhizome or caulis, and each section was incubated separately to determine the distribution of nematodes in the petiole. After 24 hrs the nematodes that emerged were counted using a stereo-microscope. Chlorotic and necrotic leaf tissue was also dissected and examined under the stereomicroscope for eggs, juveniles, and adults of BN. Because eggs might originate from nematodes other than BN, when embryo-

nated eggs were observed in the leaf tissue they were dissected and the enclosed juveniles identified. Leaf tissue was selected for histological studies from dishes that had high numbers of burrowing nematodes emerging after 24 hrs and from symptomatic areas of the leaves. Leaves were cut in slices 5 mm long, fixed in FAA (formalin, acetic acid, ethanol), dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Embedded leaf slices were sectioned 10 µm thick, stained with safranin and fast-green, mounted in Dammar xylene and examined with the aid of a compound microscope (Johansen, 1940).

Host tests: In preliminary studies, the host status of citrus to the BN population from *A. barteri* was tested in the greenhouse using Duncan grapefruit, *Citrus paradisi* Macfad., and sour orange, *Citrus aurantium* L., seedlings, which are both known to be susceptible to BN citrus race which parasitizes citrus in Florida. Sour orange seedlings were inoculated with 100 BN at the time they were transplanted into 15 cm pots containing 2 000 cm^3 artificial soil mix, Metro-mix 350 (Grace Sierra Horticultural Products Co., Milpitas, CA). At 75 days after inoculation, the roots of the citrus plants were throughly rinsed with water to remove the soil particles and nematodes attached to the root surfaces. Roots were incubated in jars, and the number of BN emerging after 72 hours was counted. The susceptibility of Duncan grapefruit to the *Anubias* BN population was tested in the same manner except that plants were inoculated with 150 BN and the final number of nematodes in the roots was determined at 180 days after inoculation.

A laboratory test was also conducted to compare reproduction on citrus of the BN population from *Anubias* with a population of BN citrus race from citrus in Florida. Surface sterilized sour orange seeds were germinated on water agar and planted in steam sterilized astatula sand (97% sand, 2% silt, 1% clay) in 100 cm^3 glass test tubes (Kaplan, 1994). Seven seedlings at the three-leaf-stage were each inoculated with 100 individuals (juveniles and adults) of either population obtained from carrot disk cultures (O'Bannon and Taylor, 1968). Plants were maintained in a window shelf in the laboratory (22-26°C) where they were watered as needed, but not fertilized. Seventy-three days after inoculation, root systems were rinsed from the tubes, weighed, cut into 2-cm segments, macerated in a blender, and the suspension poured through nested sieves (425 µm and 25 µm aperture) to recover the nematodes. Mean numbers of nematodes per plant and per g fresh root were log transformed and compared using Student's t-test.

In another greenhouse test the reproduction of the two BN populations was compared on four different *Anubias* species. The *Anubias* plants were grown in 5 cm pots containing 100 cm^3 of a 1:1 soil mix consisting of sterile sand and Metromix 350. Each plant was inoculated with 1 000 juveniles and adults of either population obtained from carrot disk cultures. Each treatment was replicated four times. Plants were harvested 7 weeks after inoculation and the number of BN emerging from the roots after 72 hours was determined using the jar incubation method (Young, 1954).

A test was also conducted to verify the ability of BN to infect anubias in an aquatic environment. Bare-rooted anubias were placed in quart jars with enough water to submerse the roots and lower leaves and then inoculated. Two replications of two different varieties of *A. barteri*, var. *glabra* and var. *caladiifolia* were inoculated with 400 juveniles and adults of the BN population from *A. barteri*. Lids were loosely placed on the jars and plants grew well in this aquarium-like environment near a win-

dow in the laboratory. Seven weeks after inoculation, the plants were harvested and the number of nematodes emerging from the roots and leaves after 72 hrs was determined as described previously.

Morphological observations: Specimens of BN population from *A. barteri* were extracted by the jar incubation method. Specimens of the citrus race BN population were extracted with the same procedure from roots obtained from a citrus orchard affected by spreading decline in central Florida. Live specimens of each population were narcotized with low heat and mounted on water agar (Esser, 1986) for measurements. Morphological features and morphometrics of diagnostic value for the genus *Radopholus* (Esser *et al.,* 1984; Ryss and Wouts, 1997; Sher, 1968) were determined for the two BN populations and compared using Student's t-test.

RESULTS

Detection in foliar tissue and histological observations: Burrowing nematodes (BN) emerged from two out of ten leaf blades and three out of ten petioles that were incubated in water, in petri dishes for 24 hrs. A total of 88 nematodes emerged from the leaf blade with the highest density, representing 9 nematodes/cm^2 and a density of 338 nematodes per gram of leaf tissue. The number of nematodes emerging from the petioles ranged from 2 to 10. In all cases, the nematodes that were recovered from petioles were from the 1 cm section taken from the base of the petiole where it was attached to the rhizome; none of the three infected petioles were from leaf blades from which BN emerged.

Symptomatic leaf tissue that was dissected had, in addition to juveniles and females, embryonated eggs that contained second-stage BN juveniles. Anubias leaves infected by *R. similis* were usually discolored and showed small lesions 1-2 mm long and irregularly shaped (Fig. 1). Histological examination of cross sections of leaves infected by nematodes indicated that BN invaded the epidermis and also the mesophyll. In the epidermis BN moved intracellularly causing large cavities (Fig. 2A and B). Nematode feeding and migration disrupted the mesophyll which also showed pronounced cavities and necrosis in the spongy parenchyma, extending into the palisade parenchyma (Fig. 2C-E). All nematode developmental life stages including eggs were observed in the infected leaf tissues (Fig. 2D). Nematode colonization induced cavities which affected the sheath bundle cells (Fig. 2F) and also extended in the vascular bundle peripheral tissues (Fig. 2G) compromising the normal flow of water and nutrients in those tissues. Disruption and damage of the palisade and spongy parenchyma was also evident in leaf sections parallel to the surface of the leaf (Fig. 3A-E). Cavities in the spongy parenchyma obliterated the periphery of vascular bundles and incorporated leaf crystals (Fig. 3B,F). Nematode males were also commonly observed (Fig. 3G).

Omnivorous nematodes of the genus *Butlerius* (Figs. 1 and 4A,B) were found in

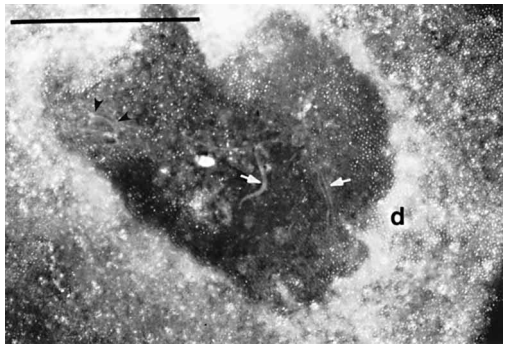

Fig. 1. External symptoms caused by *Radopholus similis* infection on anubias leaf. Note nematodes (arrows) in the small brown lesion which is surrounded by discolored tissues (d). Scale bar = 1mm.

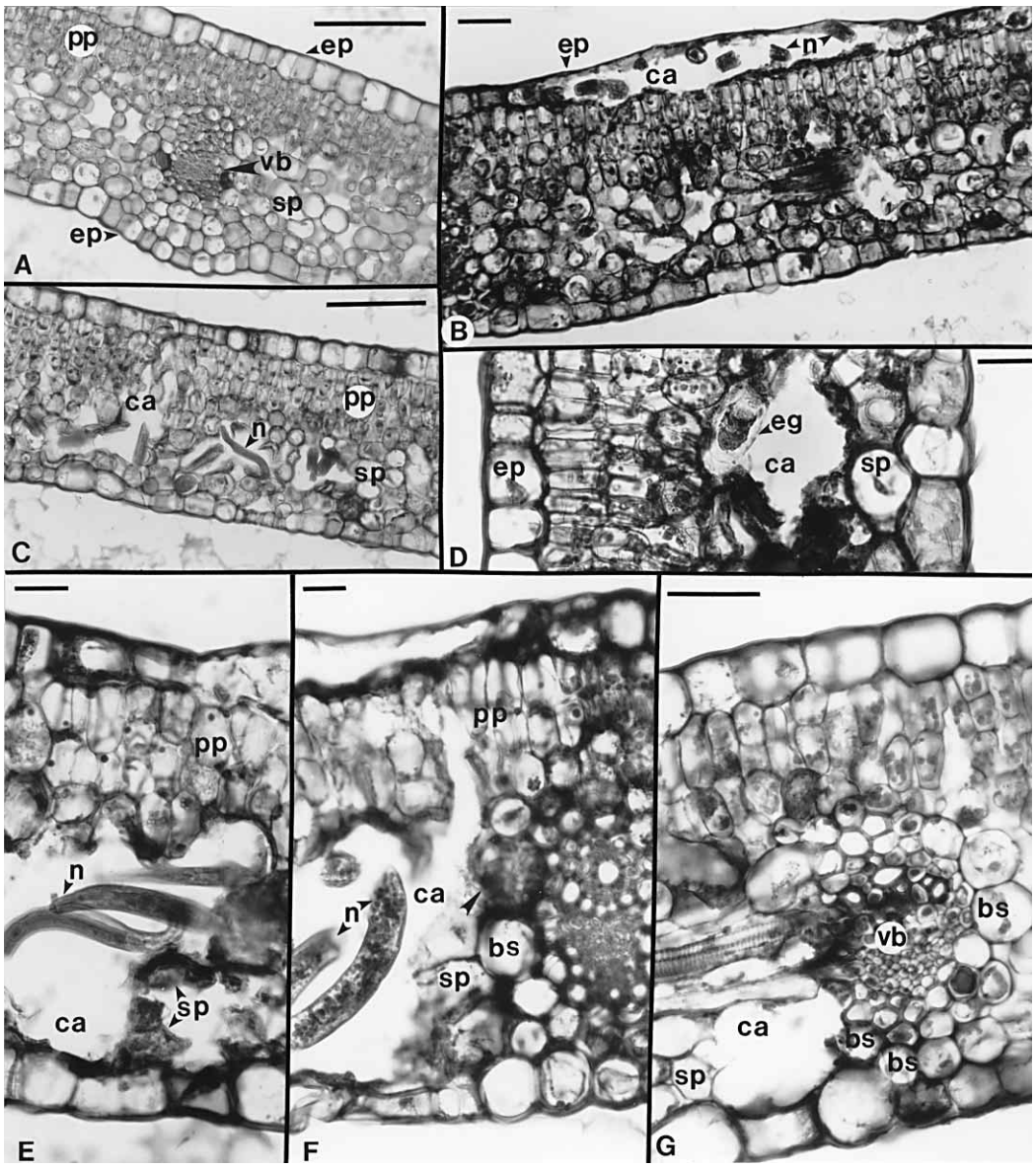

Fig. 2. Cross sections of anubias leaves infected by *Radopholus similis*. A) Non-infected leaf tissues showing the epidermis (ep), palisade parenchyma (pp) rich in chloroplasts, a vascular bundle (vb), and spongy parenchyma (sp). B) A large cavity (ca) in the epidermis (ep) caused by nematode (n) colonization. C) Cavity (ca) with nematodes in the spongy parenchyma and extending into the palisade parenchyma. D) Cavity in the spongy parenchyma containing a nematode egg (eg). E) Nematodes in a large cavity obliterating almost the entire spongy parenchyma of the root section. F) Large cavity obliterating the spongy parenchyma and extending into the palisade parenchyma. Note a bundle sheath cell (arrow) damaged by nematode feeding and migration. G) Cavity extending from the spongy parenchyma into a vascular bundle after obliterating bundle sheath cell and the peripheral cells of the bundle. Scale bars = 119.5 μm in A, 4.5 μm in B, 104 μm in C, 20 μm in D, 13 μm in E, 16.5 μm in F, and 40.7 μm in G.

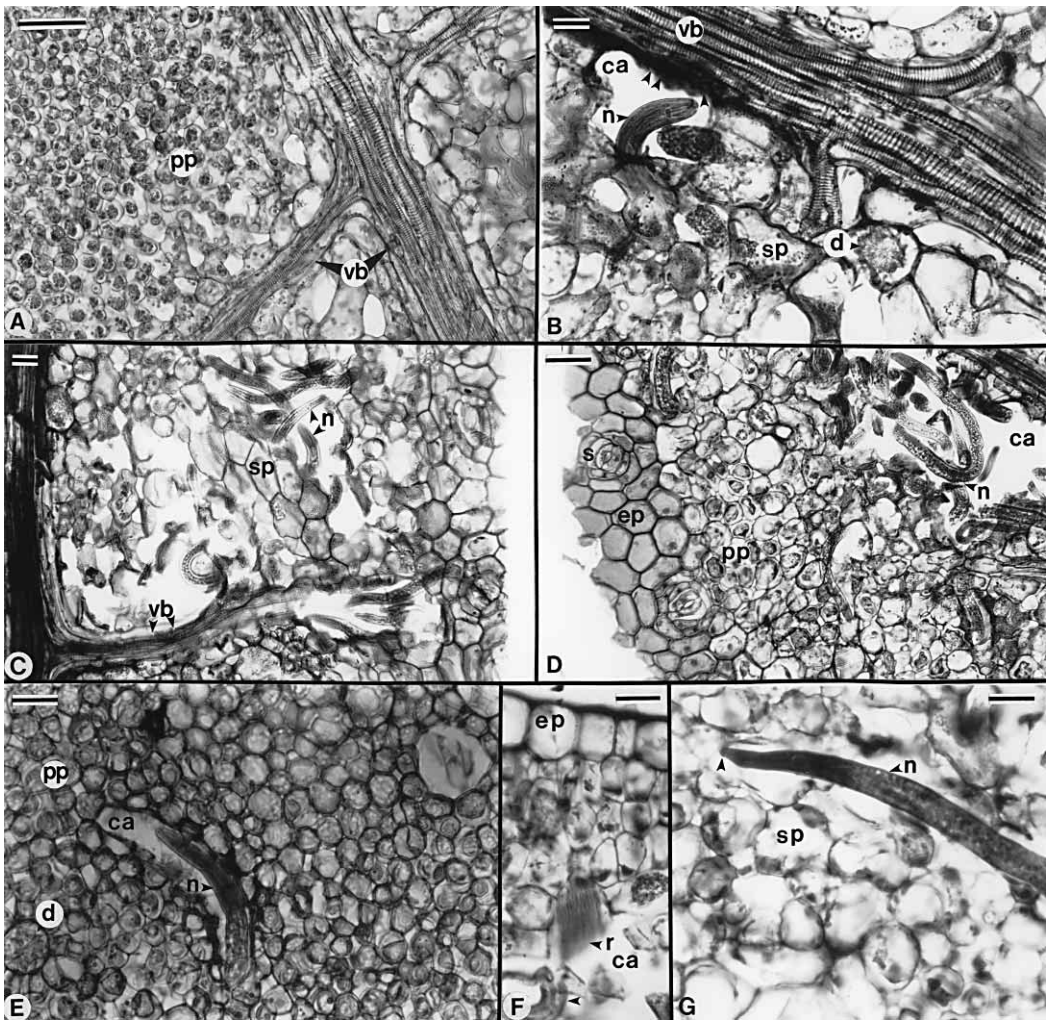

Fig. 3. Sections parallel to the surface of anubias leaves infected by *Radopholus similis*. A) Non-infected leaf tissues showing the palisade parenchyma (pp) and the vascular bundles (vb). B) Cavity (ca) in the spongy parenchyma (sp) adjacent to a vascular bundle. Note nematode (n) and dark necrotic tissues (arrows) of the vascular bundle near the nematode head. d = druse. C) Spongy parenchyma colonized by numerous nematodes. D) Nematodes in a cavity of the palisade parenchyma (pp) below the epidermis (ep). s = stomata. E) Cavity in the palisade parenchyma with a nematode. F) Leaf cross section showing a cavity containing a nematode and a raphid (r). G) A male nematode in the spongy parenchyma. Note the characteristic off-set lip region (arrow) of the *R. similis* male. Scale bars = 60 µm in A, 18 µm in B, 28 µm in C, E, and F, 72 µm in D, 25 µm in G.

leaves infected by BN, especially in decaying leaf tissues infected with bacteria.

Host tests: An average of 63 BN were recovered from the roots of sour orange seedlings 75 days after inoculation with 100 BN per plant from the anubias population. After 180 days, roots of Duncan grapefruit seedlings that were inoculated with 150 BN from the same population had a mean of 607 BN per plant, or 57 BN per g fresh roots. In addition, on average 31 BN were recovered per 100 cm^3 of artifi-

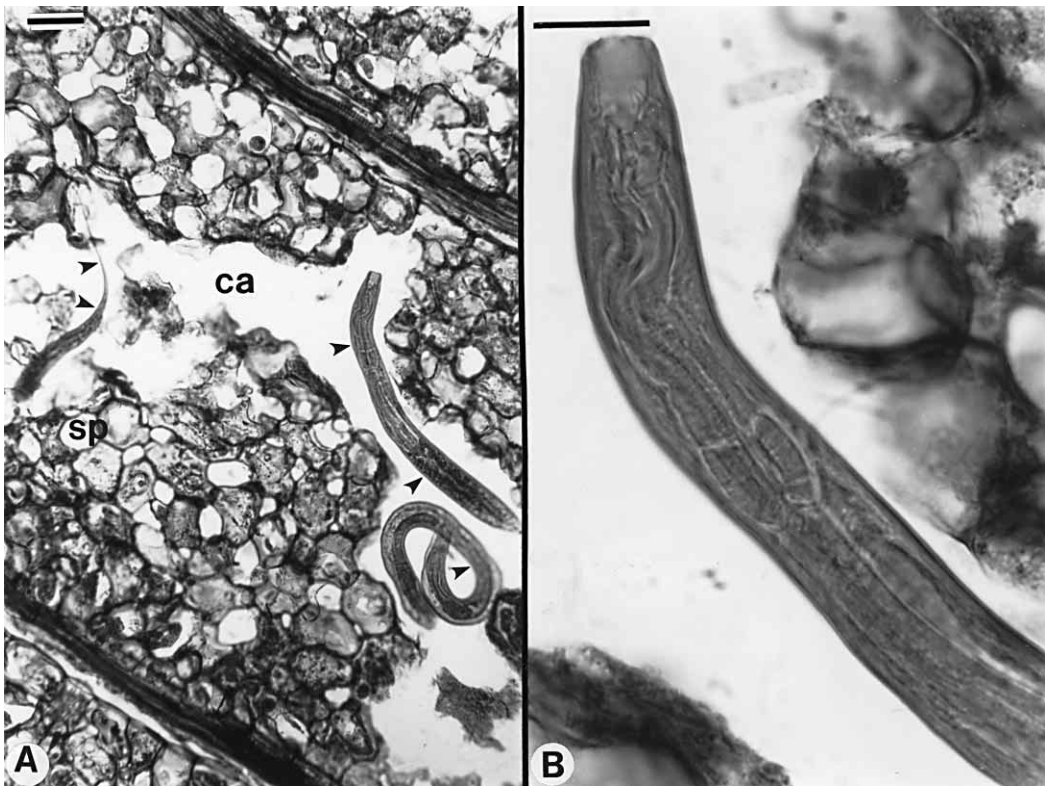

Fig. 4. Sections parallel to the surface of anubias leaves colonized by *Butlerius* sp. A) Cavity in the spongy parenchyma (sp) containing numerous specimens of *Butlerius* sp. B) Anterior portion of the body of a *Butlerius* sp. specimen. Scale bars = 36 μm in A, and 18 μm in B.

cial soil mix sampled from each pot, indicating that the anubias BN population increased at least five fold on Duncan grapefruit seedlings in the greenhouse.

In the laboratory test in which carrot disk cultures of the anubias and citrus race BN populations were used to inoculate sour orange seedlings in tubes, nematodes of both populations reproduced on sour orange seedlings, but the final means per tube and per g fresh root were different ($P = 0.05$) between the two populations. For the anubias population an average of 283 BN per plant and 1019 BN per g fresh roots were recovered, compared to 3 925 per plant and 12704 per g root for the citrus BN population.

The mean number of BN recovered from the roots of *Anubias* spp. inoculated with the BN citrus race and the population from anubias is shown in Table 3. Nematodes of both populations were recovered from the roots of all species and varieties that were inoculated 7 weeks earlier, but numbers recovered from roots of plants inoculated with the anubias BN population were consistently greater than those for the BN citrus population.

The laboratory test in which bare rooted plants were grown in water in jars and inoculated with the anubias population, demonstrated that BN is able to parasitize both the roots and leaves of plants in an aquatic environment. At 7 weeks after

inoculation, a mean of 16 and 45 BN were recovered from *A. barteri* var *caladiifolia* roots and leaves respectively, and 2 and 6 BN from roots and leaves of *A. barteri* var. *glabra*, respectively.

Morphological observations: Morphometrics of diagnostic significance for the two BN populations from anubias and citrus are listed in Tables 1 and 2. Variability and some significant differences among selected morphometric characters of the two populations were observed. BN specimens from the anubias population were larger than the specimens from the BN citrus race population (Tables 1 and 2). However, the length and width ranges of the two populations overlapped among each other and also with those reported in the literature for populations of *R. similis* (Esser *et al.,* 1984; Ryss and Wouts, 1997; Sher, 1968). Morphological features of the head and tail of females and males and mean values of morphometrics of diagnostic values such as stylet length, tail length, and h values of the two populations were similar to those reported for *R. similis*. These findings indicate that the two populations used in our study belong to *R. similis*. These two BN populations differed from *R. bridgei* Siddiqi and Hahn, 1995, a species very close to *R. similis*. The length of the hyaline portion of the tail of the females ranged from 5.5-16.0 µm and 7.5-12.0 µm for the population from anubias and citrus race population, respectively, vs. < 4 µm in *R. bridgei*. Also, the average stylet length of the females from the anubias and citrus populations are greater than in *R. bridgei* (18.0 µm vs. 15.5 µm). The BN citrus race and anubias populations differ also from *R. citri* Machon and Bridge, 1996, which infects citrus in Indonesia (Machon and Bridge, 1995), in the longer tail which ranges 76.5-96.0 µm and 61.9-81.0 µm in the anubias and citrus populations, respectively, compared to 43.0-57.0 µm in *R. citri*. Furthermore, the male stylet of the anubias BN population is not strong as those reported for *R. citri*, but is similar to the weak stylet observed in males of the citrus population, and typical of *R. similis*.

DISCUSSION

To our knowledge, the only commercial aquatic plants reported as hosts of BN are the *Anubias* spp. reported in this paper. After BN was initially recovered from *A. barteri* var. *nana* in the aquatic nursery, additional anubias species were sampled and BN was extracted from the roots of the following plants: *A. barteri* var. *coffeefolia*, *A. barteri* var. *glabra,* and *A. gigantea* Chevalier ex Hutchinson. In addition, *A. afzelii* Schott, *A. barteri* var. *caladiifolia,* and *A. gracilis* Chevalier ex Hutchinson were shown to be hosts in greenhouse studies. *Anubias* species belong to the family Araceae and many other species in this family are good hosts of BN. In Florida nurseries, for example, BN is frequently recovered from the roots of *Epipremnum* and *Philodendron* spp. *Radopholus similis* is capable of reproducing on a wide variety of plant species in 88 families, with at least 365 species reported as hosts for the banana race and 139 species as hosts for the citrus race (Holdeman, 1986a-b). Because many ornamental plants are known to be hosts of both the citrus and banana race of BN, most of the major citrus producing states and countries have regulations to exclude BN occurring on ornamental plants originating in Florida. However, there is little, if any, evidence that the BN intercepted in commercial shipments from Florida's ornamental nurseries belong to the citrus race of BN. In a review of the biology of the citrus and banana races of BN, Holdeman (1986b) stated: "No one has demonstrated that the citrus BN either is, or has been moving in the nursery trade in plants other than citrus". This paper pro-

Table 1. Morphometrics (mean, standard deviation, range; n = 20) of *Radopholus similis* females from anubias and citrus in Florida.

Character	Anubia	Citrus	t[z]
Length (µm)			
L	748.1 ± 47.0 **647.5-837.0**	696.4 ± 60.2 **588.5-794.5**	3.02*
Body width (BW)	25.3 ± 1.7 **22.5-28.0**	22.6 ± 2.1 **18.5-27.4**	4.55**
BW at anus	17.4 ± 0.9 **15.5-19.0**	16.5 1.6 **14.0-19.5**	NS
Esophagus	100.0 ± 6.3 **87.0-111.5**	95.8 ± 6.5 **83.0-106.5**	NS
Base of esophageal lobe to head end	163.7 ± 11.2 **137.5-180.5**	166.1 ± 12.0 **142.0-182.5**	NS
Pharyngeal overlap	63.7 ± 7.6 **50.5-78.0**	71.3 ± 9.5 **53.5-85.0**	2.77*
Excretory pore to head end	102.4 ± 7.6 **87.0-112.5**	99.2 ± 8.5 **85.0-116.5**	NS
DGO	4.3 ± 0.3 **4.0-5.0**	4.8 ± 0.4 **4.5-5.5**	4.46**
Stylet	18.3 ± 0.5 **17.5-19.5**	18.3 ± 0.4 **17.5-19.0**	NS
Stylet knob width	4.8 ± 0.3 **4.0-5.0**	4.2 ± 0.3 **4.0-5.0**	7**
Stylet knob height	2.7 ± 0.3 **2.5-3.0**	2.5 ± 0.0 **2.5-2.5**	NS
Head-vulva	417.5 ± 27.7 **369.0-472.0**	400.2 ± 35.0 **341.0-465.5**	NS
Tail	84.7 ± 5.2 **76.5-96.0**	71.1 ± 5.3 **61.5-81.0**	8.22**
H	8.0 ± 2.3 **5.5-16.0**	9.8 ± 1.3 **7.5-12.0**	3.05*
Ratios			
a	29.6 ± 1.8 **25.7-33.0**	31.0 ± 2.7 **23.4-36.8**	NS
b	7.4 ± 0.5 **6.3-8.4**	7.2 ± 0.4 **6.6-7.9**	NS
b'	4.6 ± 0.4 **3.9-5.4**	4.2 ± 0.3 **3.5-4.8**	3.93**
c	8.8 ± 0.3 **8.2-9.3**	9.8 ± 0.4 **9.2-10.7**	8.78**
c'	4.9 ± 0.3 **4.2-5.4**	4.3 ± 0.4 **3.5-4.8**	5.76**
Percentage			
V (%)	55.7 ± 1.7 **52.0-57.0**	57.5 ± 0.1 **55.0-59.0**	4.20**

[z]Student's t-test; * and ** significantly different at $P = 0.05$ and 0.01, respectively.

Table 2. Morphometrics (mean, standard deviation, range; n = 20) of *Radopholus similis* males from anubias and citrus in Florida.

Character	Anubia	Citrus	t[z]
Length (µm)			
L	643.8 ± 37.7 **590.5-705.5**	608.9 ± 32.7 **562.5-666.0**	NS
Body width (BW)	19.0 ± 0.9 **17.5-20.5**	17.2 ± 0.9 **16.0-18.5**	4.45**
BW at anus	13.5 ± 0.5 **13.0-14.5**	13.4 ± 0.6 **13.0-14.5**	NS
Esophagus	86.6 ± 2.3 **83.0-90.0**	85.9 ± 4.0 **80.0-91.0**	NS
Base of esophageal lobe to head end	127.5 ± 5.2 **120.5-137.5**	130.8 ± 7.1 **121-144**	NS
Pharyngeal overlap	41.0 ± 5.0 **34.0-50.5**	45.5 ± 4.9 **39.0-55.0**	NS
Excretory pore to head end	100.5 ± 5.2 **88.0-105.0**	94.5 ± 3.0 **90.0-98.0**	NS
Stylet	12.7 ± 0.7 **11.5-13.5**	13.3 ± 0.3 **13.0-13.5**	NS
Knob width	1.5 ± 0.3 **1.0-2.0**	0.8 ± 0.4 **0.5-1.5**	4.32**
Testis	180.8 ± 35.5 **107.0-215.0**	202.1 ± 18.0 **165.5-219.5**	NS
Spicule	18.9 ± 0.8 **17.0-19.5**	18.7 ± 0.5 **18.0-19.5**	NS
Gubernaculum	9.9 ± 0.7 **8.0-10.5**	9.4 ± 0.4 **9.0-10.5**	NS
Tail	81.1 ± 6.7 **73.5-94.0**	68.5 ± 3.6 **62.5-73.5**	5.2**
H	6.2 ± 2.4 **3.0-10.5**	5.0 ± 1.2 **3.5-7.5**	NS
Ratios			
a	33.9 ± 2.4 **29.5-38.1**	35.5 ± 2.1 **32.0-38.0**	NS
b	7.4 ± 0.4 **6.7-8.0**	7.1 ± 0.4 **6.5-7.8**	NS
b'	5.0 ± 0.3 **4.5-5.5**	4.6 ± 0.3 **4.1-5.0**	3.1*
c	7.9 ± 0.4 **7.1-8.6**	8.9 ± 0.4 **8.3-9.4**	5.6**
c'	6.0 ± 0.6 **5.0-6.9**	5.1 ± 0.3 **4.6-5.5**	4.54**
Percentage			
T %	28.4 ± 5.5 **17.0-37.0**	33.2 ± 3.1 **29.0-37.0**	NS

[z]Student's t-test; * and ** significantly different at $P = 0.05$ and 0.01, respectively.

Table 3. Mean number of Radopholus similis recovered from roots of anubias species inoculated with *R. similis* populations from anubias and citrus.

	Anubias population		Citrus race population	
	No. nematodes		No. nematodes	
Anubias species	/Plant	/g root	/plant	/g root
Anubias afzelii	8	26	3	9
Anubias barteri				
var. *caladiifolia*	155	103	6	5
Anubias barteri				
var. *glabra (lanceolata)*	38	43	11	12
Anubias gracilis	50	65	5	7
Anubias heterophylla				
var. *congensis*	40	74	2	7

vides evidence for the first time that the citrus race of BN occurred in commercially grown ornamental plants which were sampled for certification purposes and destined for another citrus producing state.

In spite of the report of BN in the caulis of anthuriums (Aragaki *et al.*, 1984), there is no information of *R. similis* infection of the foliar tissues of plant hosts. Microscopic observations and histological studies reported in this paper demonstrate that BN is capable of parasitizing and reproducing in leaf tissues. The intestinal contents of some nematodes recovered from the leaves contained green chloroplast particles. Our observations and experiments indicate that in an aquatic environment or when leaves are in contact with the soil, BN is capable of reproducing in epigeal plant parts of some plants. This nematode behavior complicates sanitation problems in nurseries if propagative cuttings are used from plants grown in BN infested soil. Regulatory officers should be aware of this nematode behavior when BN hosts are sampled for routine inspections. Other nematodes which primarily parasitize roots are reported occasionally as reproducing in epigeal tissue. *Pratylenchus coffeae* is reported to invade the rhizomes of *Aglaonema,* another plant in the family Araceae (Noegel, 1972). *Meloidogne incognita* (Kofoid and White, 1919) Chitwood, 1949 caused severe galling of leaves of *Siderasis fuscata* in a Florida nursery (Miller and DiEdwardo, 1962). Although *Meloidogyne* spp. cause galls primarily on roots, they also are known to naturally infect and cause galls on stems in 18 genera, and on leaves in 3 genera (Lehman, 1985). *Meloidogne javanica* (Treub, 1885) Chitwood 1949 was reported to even reproduce and cause galls on the inflorescence of *Palisota barteri* Hook. f. (Lehman and MacGowan, 1986). These findings suggest that many plant parasitic nematodes that are primarily root feeders are capable of feeding and reproducing on epigeal tissues, which normally escape the attack of nematodes because of moisture fluctuation levels on their surface or because of poor nematode vertical motility in water films.

The extensive damage caused by *R. similis* to leaf tissues of anubias evinces the great ability of this nematode to parasitize

any suitable tissue of the host plant. On the majority of its hosts, BN behaves as a cortical root feeder, which occasionally can disrupt the periphery of the root stele (DuCharme, 1959). Like the root parasitism by BN which involves mainly the cortical parenchyma rich in starch, leaf parasitism by BN also involves tissues rich in carbohydrates such as the palisade and the spongy parenchyma. Disruption of palisade parenchyma is the major cause of chlorosis of nematode infected leaves. As in the case of citrus roots, where the peripheral tissues of the vascular cylinder including endodermis, pericycle, phloematic tissue, and vascular parenchyma are damaged by the nematode (DuCharme, 1959), the periphery of the vascular bundle of anubias leaves is disrupted or obliterated by nematode feeding and migration without any evidence of xylem element damage.

The subsequent colonization of BN parasitized leaf tissues by bacteria and omnivorous nematodes (*Butlerius* spp.) is also similar to the subsequent invasion of secondary organisms reported on citrus root tissues infected by the citrus BN (DuCharme and Price, 1966).

Morphometric variability between the BN citrus race and anubias populations is well documented for other populations of *R. similis* (Esser *et al.*, 1984). Body size difference observed between the two BN populations may be caused by difference in the nutrient content of anubias leaves and citrus roots. Seasonal variation of *Pratylenchus coffeae* body size has been observed on nematode infected citrus trees and it is correlated with seasonal variation of the starch content in the roots (Duncan *et al.*, 1998).

LITERATURE CITED

ARAGAKI, M., W. J. APT, R. K. KUNIMOTO, W. H. KO, and J. Y. UCHIDA. 1984. Nature and control of anthurium decline. Plant Disease 68:509-511.

BALA, G., and F. HOSEIN. 1996. Plant parasitic nematodes associated with anthuriums and other tropical ornamentals. Nematropica 26:9-14.

DuCHARME, E. P. 1959. Morphogenesis and histopathology of lesions induced on citrus roots by *Radopholus similis*. Phytopathology 49:388-395.

DuCHARME, E. P., and W. C. PRICE. 1966. Dynamics of multiplication of *Radopholus similis*. Nematologica 12:113-121.

DUNCAN, L. W., R. N. INSERRA, and D. DUNN. 1998. Seasonal changes in citrus fibrous root starch concentration and body length of female *Pratylenchus coffeae*. Nematropica 28:263-266.

ESSER, R. P. 1986. A water agar *en face* technique. Proceedings of the Helminthological Society of Washington 53:254-255.

ESSER, R. P., A. L. TAYLOR, and Q. L. HOLDEMAN. 1984. Characterization of burrowing nematode *Radopholus similis* (Cobb, 1893) Thorne, 1949 for regulatory purposes. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Nematology Circular 113, Gainesville, FL, U.S.A.

HOLDEMAN, Q. L. 1986a. The burrowing nematode *Radopholus similis sensu lato*. Division of Plant Industry, California Department of Food and Agriculture, Sacramento, CA, U.S.A.

HOLDEMAN, Q. L. 1986b. The burrowing nematode, the citrus pathotype. Division of Plant Industry, California Department of Food and Agriculture, Sacramento, CA, U.S.A.

INSERRA, R. N. 1998. Tri-ology Technical Report. 37 (1): 7. Nematology Section. Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, U.S.A.

JOHANSEN, D. A. 1940. Plant Microtechniques. McGraw-Hill, New York, NY, U.S.A.

KAPLAN, D.T., 1994. An assay to estimate citrus rootstock resistance to burrowing nematodes. Proceedings of the Florida State Horticultural Society 107:85-89.

LEHMAN, P. S., and J. B. MacGOWAN. 1986. Inflorescence and leaf galls on Palisota barteri caused by *Meloidogyne javanica*. Journal of Nematology 18:583-586.

LEHMAN, P. S. 1985. Galls on aboveground plant parts caused by root-knot nematodes. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Nematology Circular 125, Gainesville, FL, U.S.A.

MACHON, J. E., and J. BRIDGE. 1996. *Radopholus citri* n.sp. (Tylenchida: Pratylenchidae) and its pathogenicity on citrus. Fundamental and Applied Nematology 19:127-133.

MILLER, H. N., and A. A. DI EDWARDO. 1962. Leaf galls on Siderasis fuscata caused by root-knot nematode *Meloidogyne incognita incognita*. Phytopathology 52:201-202.

NOEGEL, K. A. 1972. The pathogenicity and interrelationship of *Pratylenchus coffeae* and *Pythium splendens* on Chinese evergreen. PhD thesis, Department of Entomology and Nematology, University of Florida, Gainesville, FL, U.S.A.

O'BANNON, J. H., and A. L. TAYLOR. 1968. Migratory endoparasitic nematodes reared on carrot discs. Phytopathology 58:385.

RYSS, A. Y., and W. M. WOUTS. 1997. The genus *Radopholus* (Nematoda: Pratylenchidae) from native vegetation in New Zealand, with description of two new species. International Journal of Nematology 7:1-17.

SHER, S. A. 1968. Revision of the genus *Radopholus* (Nematoda: Tylenchoidea). Proceedings of the Helminthological Society of Washington 35:219-237.

SIDDIQI, M. R., and M. L. HAHN. 1995. *Radopholus bridgei* n. sp. (Tylenchida: Pratylenchidae) from Indonesia and its differentiation by morphological and molecular characters. Afro-Asian Journal of Nematology 5:38-43.

YOUNG, T. W. 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Disease Reporter 38:794-795.

Received: *2.XII.1999*

Recibido:

Accepted for publication: *10.I.2000*

Aceptado para publicación:

BLANK PAGE USED IN PAGE COUNT