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Ditylenchus drepanocercus Rediscovered in the Neotropics Causing Angular Leaf Spots on *Miconia calvescens*[1]

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Abstract: During searches for pathogens to be used as classical biocontrol agents for *Miconia calvescens* (velvet tree), a devastating plant invader of Hawaii and French Polynesia, damaging angular leaf spots were found repeatedly. The etiological agent of this disease was identified as the nematode *Ditylenchus drepanocercus*. This nematode has a distinctive falciform appendage at the apex of the tail on both sexes, which allows easy identification. The nematodes were found in the lacunar parenchyma. Infected tissues have abnormally large cells (7 to 13 times the normal size). The lamina at infected areas is chlorotic, slightly thicker, and becomes necrotic with time. The best method of inoculation for this nematode was spraying plants with a suspension containing individuals of various stages on previously wounded leaves. Incubation period was determined to be 20 days.

Key words: aerial parasite, angular leaf-spot, biological control, *Ditylenchus drepanocercus,* invasive weed, *Miconia calvescens,* pathogenicity, taxonomy, velvet tree.

Miconia calvescens DC. (Myrtales: Melastomataceae), commonly known as velvet tree, is a native of the Neotropics that was introduced as an ornamental into several regions of the world. It has become an aggressive invader in Tahiti, Hawaii, and other Pacific islands (Gagné et al., 1992; Meyer, 1996; Meyer and Florence, 1996; Meyer and Malet, 1997). In other regions, such as Australia, there are restrictions in place for its sale and cultivation (Csurhes, 1997).

An exotic plant is normally regarded as an invader when it is widely spread and forms dense stands. Consequently, the management of such weeds usually starts when it is too late for its eradication (Meyer and Malet, 1997). In such cases, biological control is the main method of control (Harley and Forno, 1992). Classical biological weed control projects normally follow a series of steps: (i) determination of the need for weed biocontrol, (ii) search for natural enemies at the center of origin of the weed, (iii) selection of the most effective natural enemies, (iv) determinating the host-range of the natural enemies to verify the safety of their use, (v) introduction and establishment of the agents, and (vi) evaluation of the effect of the natural enemies on the target weed's population (Schroeder, 1983).

In 1995 a cooperative agreement was signed between the Research Corporation of the University of Hawaii (RCUH) and Fundação Arthur Bernardes (FUNARBE), representing the Federal University of Viçosa (Brazil), to undertake a survey of pathogens associated with weedy Melastomataceae native to Brazil, particularly *M. calvescens*, that became invasive in Hawaii. Twelve fungal pathogens, a witches' broom-causing phytoplasm, and a foliar nematode were found (Barreto et al., 2000; Seixas 2002; Seixas et al., 2002). The fungus *Colletotrichum gloeosporioides* f.sp. *miconiae* proved to be host specific and was introduced into Hawaii in 1997 (Barreto et al, 2001, Killgore et al., 1999) and French Polynesia in 2000 (Killgore, pers. comm.). Although the fungus has established and is dispersing naturally, a need is now evident for additional biocontrol agents to complement the effect it is producing.

During a 1997 survey in the state of Amazonas (neighboring areas of Manaus), plants of *M. calvescens* and *M. phanerostila* Pilg. were found showing abundant angular leaf spots. Some leaves were collected and taken to the lab. Dissection of symptomatic tissue revealed the presence of numerous nematodes associated with the lesions. The same nematode was later found on *Miconia* plants in the states of Minas Gerais and Rio de Janeiro. The nematode also was collected during survey visits to Ecuador and Costa Rica.

A distinct form or biotype (or perhaps even a separate species awaiting recognition as a separate species) of *M. calvescens* occurs in Ecuador, Costa Rica, southern Mexico, northern Guatemala, and Belize and has a distinctly different appearance from the *Miconia* occurring in Brazil. These Central American and northern South American populations have a morphology that is similar to that of plants established in French Polynesia and Hawaii. Leaves are up to 1 meter long and are dark green adaxially and purple abaxially (Meyer, 1996). In addition, it has been noted that the leaves of plants belonging to Central American and northern South American populations are more brittle and succulent. Plants in Brazil only occasionally have bicolored leaves, a condition generally restricted to younger shaded leaves. Leaf length reaches a maximum of ca. 39 cm (Baumgratz, 1980; Martins et al., 1996). Another relevant difference is the geographical distribution of the two forms: The bicolored form occurs on the Western slopes of the Andes and the Cordillera Central (Costa Rica) at elevations of between 600 and 1,200 meters;

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the monocolored form occurs generally at altitudes below 700 meters and has a wide distribution on the eastern side of the Andes. Despite those noticeable differences, botanists have so far kept those different forms in the same taxon. *Miconia* Ruiz & Pavon is a clearly defined genus, but its nomenclature is still confused and no recent review of the genus exists (Howard and Kellogg, 1986; Martins et al., 1996).

There are several accounts in the literature of nematodes attacking weeds and there are some publications that deal with the biocontrol potential of some of those species (Orr et al., 1975; Pantone, 1987; Parker, 1986; Robinson et al., 1978; Skinner et al., 1980; Wapshere, 1988; Watson, 1986a). The purpose of this paper is to clarify the identity of the nematode associated to *M. calvescens* and confirm its pathogenicity and provide preliminary steps toward its evaluation as a potential classical biocontrol agent.

Materials and Methods

Examination of symptoms and nematode identification: To observe the symptoms and identify the nematode, diseased leaves of *M. calvescens* were collected in the municipalities of Dionísio and Viçosa (both in the state of Minas Gerais, Brazil) during 1 year. Symptoms were described based on direct observation of lesions by using a dissecting microscope. Effect of the nematode and its distribution on *Miconia* tissues were observed on 25 µm-thick leaf sections made with a freezing microtome using a light microscope. Tissue samples were mounted in lactophenol.

Nematodes were extracted from colonized leaf tissues by placing selected diseased tissue in tap water under continuous aeration by aquarium pumps for 48 hours. The suspension was then sieved through a combination of a 0.84-mm-pore sieve nested over a 0.025-mm-pore sieve. Nematodes were separated from plant tissues, and the individuals that appeared to be alive were removed from the suspension with a finely pointed needle using a stereomicroscope, and then transferred to a drop of sterile distilled water placed on a microscope slide. A cover slip was deposited over each drop and sealed with nail polish before microscopic observations were made. Fifty female and 20 male individuals were examined and measured. Measures were taken with the aid of a drawing tube adapted to a light microscope.

Nematode inoculation: Healthy monocolored *M. calvescens* plants were obtained from cuttings and plantlets collected in the municipalities of Viçosa and Dionísio. Bicolored plants were grown from seeds sent from Hawaii (E. Kilgore, Hawaii Department of Agriculture). Three methods of inoculation were tested: spraying (Orr et al., 1975; Robinson et al., 1978), injection, and deposition (Orr et al., 1975). For spraying and injection, the inoculum consisted of a nematode suspension containing eggs, juveniles of several stages, and adults (639 individuals/ml). Plants were sprayed on both sides of the leaves using a small paint gun. After spraying, the inoculated leaves were pierced repeatedly with a finely pointed needle. Nematodes were injected at several points into both sides of the leaves with a hypodermic syringe. Inoculum deposition was performed as follows: intact lesion tissues cut from diseased leaves and left over healthy humid leaves; macerated infected tissue from diseased plants brushed on both sides of healthy leaves; or 20 to 30 individuals transferred to droplets of water (six drops for each leaf) on the lamina of healthy leaves. The leaf surface under the water droplets was previously injured with a finely pointed needle. Two monocolored and two bicolored *Miconia* plants, each with 3 to 4 pairs of leaves, were used for each of the inoculation methods tested. Plants were left in a mist chamber at 25 °C, for a 12-hour photoperiod, for 48 hours after inoculation, after which inoculated plants were transferred to a temperature-controlled room at 26 °C. Plants were observed weekly for symptoms for 2 months. When plants were inoculated with the deposition of nematodes on droplets of water, the plants were left on the lab bench after the inoculation until the droplets dried, and then transferred to the mist chamber.

Besides attempting to reproduce the disease via artificial inoculation methods, an attempt was also made to promote angular leaf-spot on bicolored plants in the field. This was used as an additional method of testing the infectivity of the Brazilian populations of the nematode (from the municipalities of Dionísio and Viçosa—state of Minas Gerais) to the form of *Miconia* that invades the Pacific islands and then inferring the potential of these nematode populations as a classical biological control agent for introduction into the Pacific islands. Ten potted *Miconia* plants grown from Hawaiian seeds with 3 to 4 pairs of leaves were taken to the Dionísio site and left for 8 months under native *M. calvescens* plants, which were heavily infected naturally by the nematode. These plants were irrigated once a week.

Results

Examination of symptoms and nematode identification: Lesions caused by the nematode (Fig. 1) occured only on leaves. Leaf-spots were angular, vein-delimited, initially pale yellow becoming darker yellow, and finally necrotic and brown. Lesions were 1 to 5 cm^2 and often coalesced over large portions of the lamina. Lesions were easier to observe abaxially on older leaves, and occurrence of lesions on young leaves was rare. The association of nematode-induced lesions with insects caused injuries, and injuries by other means were common (Fig. 2). Microscopically the foliar tissue at lesions appeared slightly swollen compared with healthy tis-

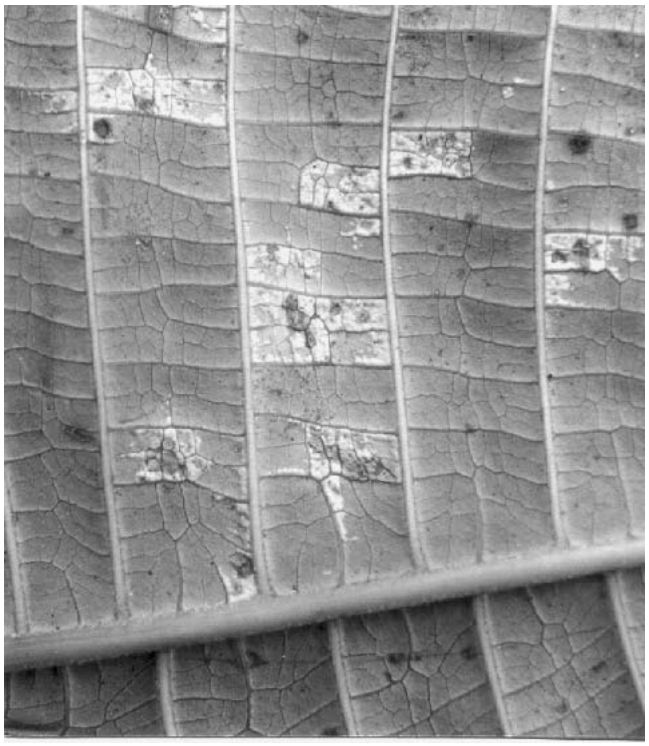

FIG. 1. Angular leaf-spot symptoms of *Miconia calvescens* caused by *Ditylenchus drepanocercus.*

FIG. 2. Angular leaf-spot lesion caused by *Ditylenchus drepanocercus* on *Miconia calvescens* associated with an injury of unknown cause.

sues. Sections from diseased foliar tissue showed that the nematodes were abundant in the lacunar parenchyma, which contained hypertrophied cells. Abnormal cells were 7 to 13 times their normal size. Although numerous in each lesion, the nematodes were restricted to the diseased tissues (Fig. 3).

Nematode samples were preserved by a variety of fixation methods: FA 4:1, FAA, TAF, FG 4:1, FA 4:10 (Southey, 1978). The fixative that yielded the best result was TAF. Samples were maintained in this fixative, but the nematodes were fragile and standard fixation procedures resulted in extensive damage to the specimens. Because of these difficulties, observations of nematode morphology and measurements of structures were made only on freshly killed individuals.

The nematode attacking *Miconia* was identified as a member of *Ditylenchus* Filipjev. It had a thin and striated cuticle, a continuous head that was narrower than the adjacent body, and was low and flattened, a delicate stylet with small knobs, a dorsal esophageal gland opening 1 to 3 μm posterior to the knobs, an esophagus tylenchoid with an inconspicuous metacorpus that was difficult to detect; and an esophageal lumen that was slightly more refractive in procorpus than in isthmus and was a glandular part of esophagus. The female reproductive system was monodelphic and prodelphic with a postvulval uterine sac, with a ratio of the distance from head to vulva and body length (V) equaling 71% to 84%. Males were similar to females but shorter and less numerous, with spicules slightly ventrally bent, gubernaculum simple, and caudal alae almost reaching the tail end. The most distinctive character of the nematode found on *M. calvescens* was a falciform appendage at the apex of the tail of adult individuals of both sexes (Fig. 4). Morphometrics of the nematode from *M. calvescens* and of *Ditylenchus drepanocercus* Goodey, 1953 according to the original description are presented in Table 1. *Ditylenchus drepanocercus* is the only species of *Ditylenchus* described and accepted in the literature (Brzeski, 1991; Fortuner, 1982) as having a tail appendage similar to that observed on the *Miconia* nematode.

Nematode inoculation: The only method adequate for the inoculation of *D. drepanocercus* was spraying a suspension containing individuals at several developmental stages. Symptoms, in the form of a single lesion,

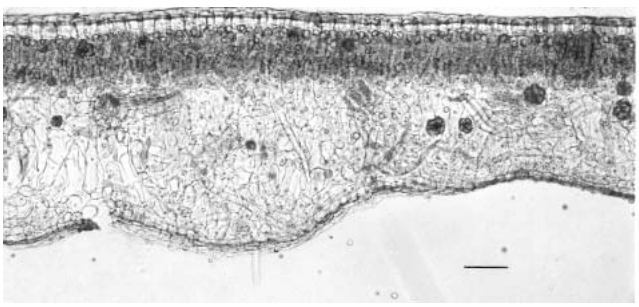

FIG. 3. Section through an angular leaf spot on *Miconia calvescens.* Note numerous *Ditylenchus drepanocercus* in a single lesion. (Scale bar = 50 μm).

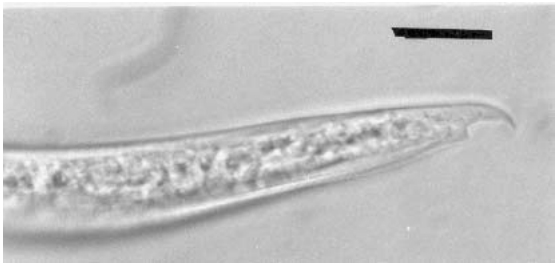

FIG. 4. Tail of *Ditylenchus drepanocercus* associated with *Miconia calvescens*. Note apical falciform appendage. (Scale bar = 10 µm).

were observed 20 days after inoculation of monocolored plants. This lesion was excised from the host leaf and left in water under aeration for 24 hours. When the resulting suspension was examined microscopically, some living individuals of *D. drepanocercus* were found. Bicolored plants grown from Hawaiian seeds left in the field under infected *Miconia* were not infected by the nematodes after 1 year of observation.

DISCUSSION

Foliar disease-causing nematodes are widespread. Some species that can cause leaf or branch galls have been evaluated as biocontrol agents for weeds—e.g., *Nothanguina phyllobia* Thorne (= *Orrina phyllobia* (Thorne) Brzeski) as a bicontrol against *Solanum elaegnifolium* Cav. (Orr et al., 1975; Parker, 1986), *Subanguina picridis* (Kirjanova) Brzeski against *Acroptilon repens* (L.) DC. (Watson, 1986a), and *Anguina amsinckiae* (Steiner & Scott) Thorne against *Amsinckia* spp. (Pantone, 1987). Nevertheless, lesions such as those caused by *D. drepanocercus* on miconia are not common. The only comparable form of leaf gall recorded in the literature is that described by Goodey (1953) for the same nematode species attacking a different host plant. One well-known example of a leaf pathogenic nematode is *Aphelenchoides besseyi* Christie on rice, but symptoms are different, with apical chlorosis commonly accompanied by leaf roll and probably due to the different growth pattern of grasses (Bedendo, 1997; Ou, 1987; Webster and Gunnel, 1992).

The nematode attacking *Miconia* had the general appearance, tail terminis, and morphometrics equivalent to those described by Goodey (1953). Goodey (1953) views the small body size and distinctive falciform tail appendage as basic characters delimiting his species. The nematode attacking *Miconia* has these features and thus is regarded herein as *D. drepanocercus*. In his description of *D. drepanocercus,* Goodey (1953) did not list the number of individuals that were examined, but the number likely was small because the author stated that he had difficulties finding living individuals. This may be one of the reasons for the wider range of some biometric data obtained in the present description as compared with that of Goodey (1953). Further nematode size may be influenced by environmental factors as well as the host from which they are obtained (Goodey, 1952; Smart and Darling, 1963; Sturhan and Brzeski, 1991; Watson, 1986b). Smart and Darling (1963) observed that the length of *Ditylenchus destructor* individuals growing under conditions with a lack or total absence of potassium and no phosphorous was shorter than that of individuals growing where such elements were present. Populations of *S. picridis,* obtained from different plants in the tribe Cynareae, varied in size and structural details and the differences found were not significant enough to separate the taxon into a different species as accepted previously (Watson, 1986b).

More than 500 species of angiosperms are known to be hosts of *Ditylenchus* spp. nematodes. Some species, such as *D. dipsaci,* have a wide host-range. *Ditylenchus drepanocercus* is regarded as a highly host-specific species (Sturhan and Brzeski, 1991). There is only one record (India) of this species—on leaves of *Evodia roxburghiana* Benth. (Sapindales: Rutaceae) (Goodey, 1953). It is surprising to find the same nematode species attacking plants of a different order and family on a different continent. This surprisingly disjunct distribution of *D. drepanocercus* may result from few studies about plant-parasitic nematodes attacking non-crop plants. Future surveys may show a pantropical distribution for this species.

Ditylenchus drepanocercus was shown to have an endoparasitic mode of parasitism. Sections of lesions revealed individuals located on the lacunar parenchyma. The nematode appears to be susceptible to desiccation, as attempts to obtain individuals from infected dried leaf fragments, using the normal water aeration treatment, did not yield live individuals. When lesions were kept in the refrigerator at 10 °C, live individuals were recovered from leaves after 1 week. This coincides with observations of *D. destructor,* which does not survive if the relative humidity is lower than 40% (Sturhan and Brzeski, 1991).

The sole successful instance of inoculation was that of spraying a suspension containing several different stages of the nematode on previously injured leaves; thus, the infective stage of the nematode remains undetermined. For attempted inoculation involving the deposition of nematodes in water droplets on leaves, only seemingly mature individuals were used. As this method did not produce any infection, it can be hypothesized that adults are not capable of producing new infection sites; however, wounds appear to be necessary for infection to take place. One attempted inoculation also involving the use of a suspension of nematodes at various stages but without wounds failed to cause the disease. The lack of wounds may have caused the failure of the method involving the deposition of macerated diseased plant tissue over healthy leaves. Often, when angular spots were observed under the dissecting microscope, they were found to be associated with tissue injuries. Some of these were clearly arthro-

TABLE 1. Biometric data for female and male nematodes obtained from *M. calvescens* and of *Ditylenchus drepanocercus* from *Evodia roxburghiana*[a]

Biometric feature (μm)		Nematode from *Miconia*		*Ditylenchus drepanocercus*	
		Female (50)	Male (20)	Female	Male
Length	Body	416–697	364–585	455–545	420–492
	Esophagus	100–177	67–155	–	–
	Sylet	6–11	6.67–8.98	8–9	8–9
	Tail	21–45	22–40	–	–
	Head to vulva	270–458	–	–	–
	Spicule	–	7–10	–	±10
Diameter	at midbody	8–15	9.5–11.5	–	–
	at anus	5–9.5	8–10.5	–	–
	at vulva	7–13.5	–	–	–
	a	32–66.5	36–54	33–62	38.8–58
	b	3–4.5	2.5–6	4–5.3	3.74–4.6
	c	8–25	10.5–22	16.2–18	14.5–17.3
	V (%)	71–84	–	75–80	–
	T(%)	–	1.5–3.5	–	–
	s	1–2	1–1.5	–	–

[a] Data for nematodes on *Evodia roxburghiana* as described by Goodey (1953).

pod-caused injuries; others were from uncertain causes. Perhaps *D. drepanocercus* is not capable of invading *Miconia* tissue, and perhaps, one or more arthropod vectors may be involved. That *D. drepanocercus* proved to be capable of infecting the monocolored Brazilian form of *Miconia* but not infective for the bicolored Hawaiian form, suggesting that there may be physiological specialization of *D. drepanocercus* that may merit taxonomic recognition at an infra-specific level. It is important to note that this nematode was found in Ecuador and in Costa Rica attacking a bicolored form of miconia—very similar to plants occurring in Hawaii. A high host-specificity among populations of *D. drepanocercus* and genetic difference between the two forms of *Miconia* may explain the failed attempts of inoculating the bicolored form with *D. drepanocercus*.

Many important gaps remain in the information gathered about *D. drepanocercus* and its interaction with *M. calvescens*. Nevertheless, it appears at this stage that *D. drepanocercus* may have potential as a biocontrol agent for *Miconia*.

LITERATURE CITED

Barreto, R. W., E., Killgore, L. S., Sugiyama, and C. D. Santos. 2000. Biological control of *Miconia calvescens,* the 'cancer vert' with fungal pathogens: Present and prospects. Pp 170–171 *in* Third International Weed Science Congress—Abstracts. Corvallis: International Weed Science Society.

Barreto, R. W., C. D. S. Seixas, and E. Killgore. 2001. *Colletotrichum gloeosporioides* f.sp. *miconiae:* O primeiro fungo fitopatogênico brasileiro a ser introduzido no exterior para o controle biológico clássico de uma planta invasoras (*Miconia calvescens*). In: 7 Simpósio de Controle Biológico—Livro de Resumos, 109.

Baumgratz, J. F. A. 1980. Miconias do município do Rio de Janeiro—Seção *Miconia* DC. (Melastomataceae). Rodriguésia 32:73–95.

Bedendo, I. P. 1997. Doenças do arroz (*Oryza sativa* L.). Pp. 85–99. São Paulo: Editora Agronômica Ceres.

Brzeski, M. W. 1991. Review of the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Anguinidae). Revue de Nématologie 14:9–59.

Csurhes, S. M. 1997. *Miconia calvescens* DC. (Melastomataceae) A potential invasive plant in Australia's tropical and sub-tropical rainforests. Proceedings of the First Regional Conference on *Miconia* Control, Papeete, Tahiti, August.

Fortuner, R. 1982. On the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Tylenchida). Revue de Nématologie 5:17–38.

Gagné, B. H., L. L. Loope, A. C. Medeiros, and S. J. Anderson. 1992. *Miconia calvescens:* A threat to native forests of the Hawaiian Islands. Pacific Science 46:390–391.

Goodey, J. B. 1952. The influence of the host on the dimensions of plant-parasitic nematode, *Ditylenchus destructor.* Annals of Applied Biology 39:221–229.

Goodey, T. 1953. On two new species of nematodes associated with leaf-blotch in *Evodia roxburghiana,* an Indian evergreen tree. Thapar Comments 95:95–104.

Harley, K. L. S., and I. W. Forno. 1992. Biological control of weeds, a handbook for practioners and students. Melbourne: Inkata Press.

Howard, R. A., and E. A. Kellogg. 1986. Nomenclatural notes on *Miconia* (Melastomataceae). Journal of the Arnold Arboretum 67: 233–255.

Killgore, E. M., L. S. Sugyiama, R. W., Barreto, and D. E. Gardner. 1999. Evaluation of *Colletotrichum gloeosporioides* for biological control of *Miconia calvescens* in Hawaii. Plant Disease 83:964.

Martins, A. B., J. Semir, R. Goldenberg, and E. Martins. 1996. O gênero *Miconia* Ruiz & Pav. (Melastomataceae) no Estado de São Paulo. Acta botânica brasilica 10:267–316.

Meyer, J. Y. 1996. Status of *Miconia calvescens* (Melastomataceae), a dominant invasive tree in the Society Islands (French Polynesia). Pacific Science 50:66–76.

Meyer, J. Y., and J. Florence. 1996. Tahiti's native flora endangered by the invasion of *Miconia calvescens* DC. (Melastomataceae). Journal of Biogeography 23:775–781.

Meyer, J. Y., and J. P. Malet. 1997. Study and management of the alien invasive tree *Miconia calvescens* DC. (Melastomataceae) in the islands of Raiatea and Tahaa (Society Islands, French Polynesia): 1993–1996. Manoa, Hawaii: Cooperative National Park Resources Studies Unit, University of Hawai'i at Manoa. (Technical Report 111).

Orr, C. C., J. R. Abernathy, and E. B. Hudspeth. 1975. *Nothanguina phyllobia,* a nematode parasite of silverleaf nightshade. Plant Disease Reporter 59:416–418.

Ou, S. H. 1987. Rice diseases. 2nd ed. Slough: CAB.

Pantone, D. J. 1987. Host range of *Anguina amsinckiae* within the genus *Amsinckia.* Revue de Nématologie 10:117–119.

Parker, P. E. 1986. Nematode control of silverleaf nightshade (*Solanum elaeagnifolium*): A biological control pilot project. Weed Science 34:33–34.

Robinson, A. F., C. C. Orr, and J. R. Abernathy. 1978. Distribution

of *Nothanguina phyllobia* and its potential control agent for silver-leaf nightshade. Journal of Nematology 10:362–366.

Seixas, C. D. S. 2002. Controle biológico de *Miconia calvescens* DC. (Melastomataceae) com fitopatógenos. D.S. thesis, Universidade Federal de Viçosa, Viçosa, Brazil.

Seixas, C. D. S., R. W. Barreto, and K. Matsuoka. 2002. First report of a phytoplasm-associated witches' broom disease of *Miconia calvescens*. Plant Pathology 5:801.

Schroeder, D. 1983. Biological control of weeds. Pp. 42–78 *in* W. W. Fletcher, ed. Recent advances in weed research. Slough: CAB International.

Skinner, J. A., C. C. Orr, and A. F. Robinson. 1980. Histopathogenesis of the galls induced by *Nothanguina phyllobia* in *Solanum elaegnifolium*. Journal of Nematology 12:141–150.

Smart, G. C., Jr., and H. M. Darling. 1963. Pathogenic variation and nutritional requirements of *Ditylenchus destructor*. Phytopathology 53: 374–381.

Southey, J. F. 1978. Plant nematology. London: Her Majesty's Stationery Office.

Sturhan D., and M. W Brzeski. 1991. Stem and bulbs nematodes, *Ditylenchus* spp. Pp. 423–464 *in* W. R. Nickle, ed. Manual of agricultural nematology. New York: Marcel Dekker, Inc.

Wapshere, A. J. 1988. Prospects for the biological control of Silverleaf Nightshade, *Solanum elaegnifolium,* in Australia. Australian Journal of Agriculture Research 39:187–197.

Watson, A. K. 1986a. Biology of *Subanguina picridis,* a potential biological control agent of Russian knapweed. Journal of Nematology 18:149–154.

Watson, A. K. 1986b. Morphological and biological parameters of the knapweed nematode, *Subanguina picridis*. Journal of Nematology 18:154–158.

Webster, R. K., and P. S. Gunnel, eds. 1992. Compendium of rice diseases. St. Paul: APS Press.