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An *Aphelenchoides* sp. nematode Parasitic of *Polianthes tuberosa* in the Mekong Delta

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Abstract: Polianthes tuberosa is a commercially valuable flower crop in the Mekong Delta of Vietnam that is propagated by the harvesting and planting of bulbs. The cultivation of *P. tuberosa* is complicated by an endemic nematode infection that damages a high proportion of the plants. Based on morphological criteria and ribosomal RNA gene sequencing, we have determined that the infection is caused by an *Aphelenchoides* sp. nematode and is most likely *Aphelenchoides besseyi.* By scoring various parts of harvested plants with flowers for the presence of viable nematodes over a period of six months, we determined that the nematode is an ectoparasite that can survive the intercrop periods, especially in the bulbs that are used to plant new crops. A comparison of farming practices in the Mekong Delta failed to identify useful control methods, but rather indicated that fields that have cultivated *P. tuberosa* for the longest periods suffer the worst damage from the nematode infection. Finally, we demonstrated that the nematode is capable of infecting 30 rice cultivars but does not cause the white tip disease usually associated with *A. besseyi* infection.

Key words: Aphelenchoides besseyi, biological control, host-parasitic relationship, Mekong Delta, plant disease loss, *Polianthes tuberosa*, rRNA sequence, survival, Vietnam.

Polianthes tuberosa L., Sp. Pl. (1753) 316 is a bulb crop usually grown for its meter-long flower spikes that produce some 20 fragrant white florets (Morris, 1984; Huxley et al., 1992). The culture of *Polianthes* dates back to the Aztecs in Mexico, who used the essential oil of the plant to flavor chocolate. The plant is said to be among the first plants to be taken back to the Old World by the Spanish; *P. tuberosa* was among the plants included in the garden of a well-known Sevilian physician, Simon Tovar, who died in 1596 (Morris, 1984). The blooms open from the bottom up and can last up to two weeks if the stems are recut periodically. In the Mekong Delta of Vietnam, *P. tuberosa* is today a symbol of purity and spirituality and is grown commercially for cut flowers used in temples and pagodas in the worship of ancestors during weddings or funerals. Many farmers dedicate up to a quarter of their land to its culture, often in intercropping or rotation with rice or vegetables. The crop is highly valuable, fetching as much as 10 times the value of a rice crop over a given area. Unfortunately, the culture of *Poliantes tuberosa* is a risky business in the Mekong Delta; more often than not, the crop is severely damaged or completely destroyed by nematodes of the genus *Aphelenchoides.*

Aphelenchoides besseyi Christie is one of the best known members of its genus because it causes "white tip disease" in rice (Fortuner and Orton Williams, 1975; Nandakumar et al., 1975; OEPP/EPPO, 2004). In rice, *A. besseyi* is an ectoparasite that enters the florets and reproduces sexually with a generation time of about 10 days at 25°C; the nematodes then desiccate and survive anhydrobiotically as adults and fourth-stage juveniles for up to several months or years within seeds. Typically, the nematodes revive and emerge to attack the seedlings when the seeds are soaked in water to start a new crop.

We report the first results of a study funded by the Swedish International Development Cooperation Agency that aims to control nematode damage on *P. tuberosa* in the Mekong Delta. The aim of the study was to assess the frequency with which *P. tuberosa* crops are infested with nematodes in the Mekong Delta, to identify the responsible nematode, and to determine its sites and duration of persistence.

Materials and Methods

Poliantes tuberosa harvesting and greenhouse cultivation: Infected bulbs were collected from severely infested fields. For some experiments, the bulbs were stored in open air under a roof and tested at various time intervals for the presence of viable nematodes or used for planting. For greenhouse cultivation, autoclaved soil was used together with fertilizer, and one bulb was planted per 20-cm-diam. plastic pot. The soil was watered once or twice daily. Each pot was supplied with the following fertilization regimen: 0.5 kg organic fertilizer (ash and ground coconut rinds, pH 5.6–6.8) was used at planting; 10 d after planting, 0.1 g of $(NH_4)_2HPO_4$ was added together with 0.6 g urea; and 0.16 g of NPK (16-16-8) fertilizer and 0.3 g urea were added every 15 d until flowering.

Nematode extraction from plant tissues: A modified Baermann funnel method was used to extract nematodes from plant tissues. Plant tissues were cut into small pieces roughly 2 to 4 mm in diameter and placed as a 1-cm-thick layer onto a porous tissue supported by a wire mesh (1-mm aperture) glued to the bottom of a plastic ring. The sample was then submerged in tap water in a glass petri dish, but held at 1 cm above the bottom of the dish, and nematodes were allowed to crawl out of the tissue and into the water for 1 to 2 hr. The water was then poured into a beaker, replaced with new water, and the nematodes allowed to emerge for

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another 1 to 2 hr, producing another sample. Five samples were produced in this way, with one exception: for the fifth and final sample, nematodes were allowed to emerge during an overnight incubation. The pooled samples were homogenized by stirring, 1-ml samples were examined under the microscope, the nematodes counted and the result extrapolated to the whole extract. For some experiments, growing plants were divided into flowers, plant and leaves, bulb and roots, and the nematodes extracted from each part separately. For other experiments, 10 to 20 bulbs of different post-harvest age (1, 2 or 3 mon) were divided into seven sub-parts. In some experiments to test for ectoparasitism, infected flowers were gently bathed in water or dry-brushed above a water container; the water sample was then observed by microscopy to detect nematodes.

Nematode extraction from soil: Soil samples were taken from three severely infected fields in Cai Lay, Tiền Giang province. For each tested field, 10 0.5-kg samples of soil were collected from the base of infected plants at random locations within the fields. Nematodes were extracted with a modification of a decanting and sieving method adapted from Barker (Barker, 1985). Briefly, after removal of rocks, roots and other large components, 500 g soil was placed in a bucket containing 5 liters water, mixed by stirring, then allowed to settle until the water became nearly still. All except for the heaviest sediments was then poured through a 1-mm-aperture mesh into a second bucket and extracted twice as follows. Sediments were allowed to settle and the supernatant was poured through a 100-µm-aperture mesh into a clean bucket; the material caught in the mesh was backwashed into a 250 ml beaker while the filtrate from the bucket was allowed to settle once again, and then filtered through a 50-µm-aperture mesh. Material caught in the mesh was backwashed into the 250 ml beaker. Active nematodes were extracted from the beaker contents by means of the modified Baermann funnel method.

Testing of water for presence of nematodes: Five 0.5-liter samples were analyzed from each of 10 infected fields in Cai Lay, Tiền Giang province. For each tested *P. tuberosa* field, five 0.5-liter water samples were taken from random sites of irrigation canals and filtered through a 50-µm-aperture mesh; the material on the mesh was backwashed into petri dishes and examined under the microscope.

Photography of nematodes: Nematodes were mounted on 2% agarose pad, paralyzed by one drop of 100 mM levamisole and examined with a Zeiss Axioplan differential interference compound microscope (except for the male tail in Fig. 2G, which was photographed under bright field illumination and using a yellow filter). Digital images were acquired on an attached AxioCam camera (Zeiss, Göttingen, Germany).

PCR amplification of rRNA gene sequences from nematodes: The small ribosomal subunit RNA (18S) gene was amplified using what are considered universal primers for nematodes (Floyd et al., 2002): SSUfor 5′-AAAGATTAAGCCATGCATG-3′ and SSUrev 5′-CATTCTTGGCAAATGCTTTC-3′. The large ribosomal subunit RNA (28S) gene was amplified using primers designed according to a known *Aphelenchoides* sequence: 28Sfor 5′-AGAGAGTGCAAGAGAACGTG-3′ and 28Srev 5′-GATGGGTCGAATAGTCTGGC-3′ (GenBank accession number AY508109). The PCR reaction was performed on single nematodes using a protocol developed for *Caenorhabditis elegans* (Plasterk, 1995). Briefly, individuals were lysed in 2 µl nematode lysis buffer (50 mM KCl, 2.5 mM MgCl2, 10 mM Tris, pH 8.3, 0.45% Nonidet P-40, 0.45% Tween-20, 0.01% gelatin and 100 µg/ml proteinase K) for 1 hr at 60°C. The proteinase K was then denatured with a 15-min incubation at 95°C. For the PCR reaction, 5 µl 10× Pfu Ultra buffer, 1 µl 50 mM $MgSO_4$ 1.25 µl 10 mM dNTP, 2 µl each primer (10 µM stock), 37.8 µl water and 1 µl 5 U/µl Pfu Ultra (Stratagene, La Jolla, CA) were added to the lysed nematodes. Following an initial 4-min denaturation of the template at 94°C, 35 PCR cycles were performed with the following parameters: 94°C (35 sec), 52°C (1 min), 72°C (2 min). Clean, single bands were produced from these reactions, and the DNA was extracted from the gel with a Gel Extraction Kit (Qiagen AB; Solna, Sweden). The DNA was cloned into the pGEM-T Easy vector (Promega, Madison, WI) following the instructions of the supplier and sent to MWG AG Biotech (Ebersberg, Germany) for sequencing. For each of the two primer pairs used, DNA from two individual nematodes recovered from infected *P. tuberosa* plants in the Tiền Giang province was amplified and sequenced, producing in each case identical sequences which have been deposited in the GenBank database (accession numbers EU084036 and EU084037).

Cultivation and inoculation of rice with nematodes from P. tuberosa: 30 rice varieties from the Mekong Delta were challenged with nematodes and later scored for nematode infection and disease symptoms. For each rice cultivar, 10 grains were allowed to germinate in a germination tray for 5 d along a 30-cm line in water-saturated soil containing 10% organic fertilizer. Each seedling was then inoculated with a water suspension of 200 nematodes extracted from *P. tuberosa* tissue. At 25 d after sowing, the seedlings were transferred to a plastic pot (5 plants/pot). Each pot contained 1 kg soil and 100 g of sterilized coconut rinds and was water-saturated. Two days later, a second nematode inoculum was added to the pots, this time using 300 nematodes/plant. Supplements were added as follows: 0.2 g urea was added to each pot on d 5 of cultivation and 0.1 g urea plus 0.3 g K_2HPO_4 was added on d 35. Plant height was measured at 45, 65 or 95 d after planting, and the number of fertile and sterile stems, as well as the number of nematodes per 100 sterile, milk or filled grains

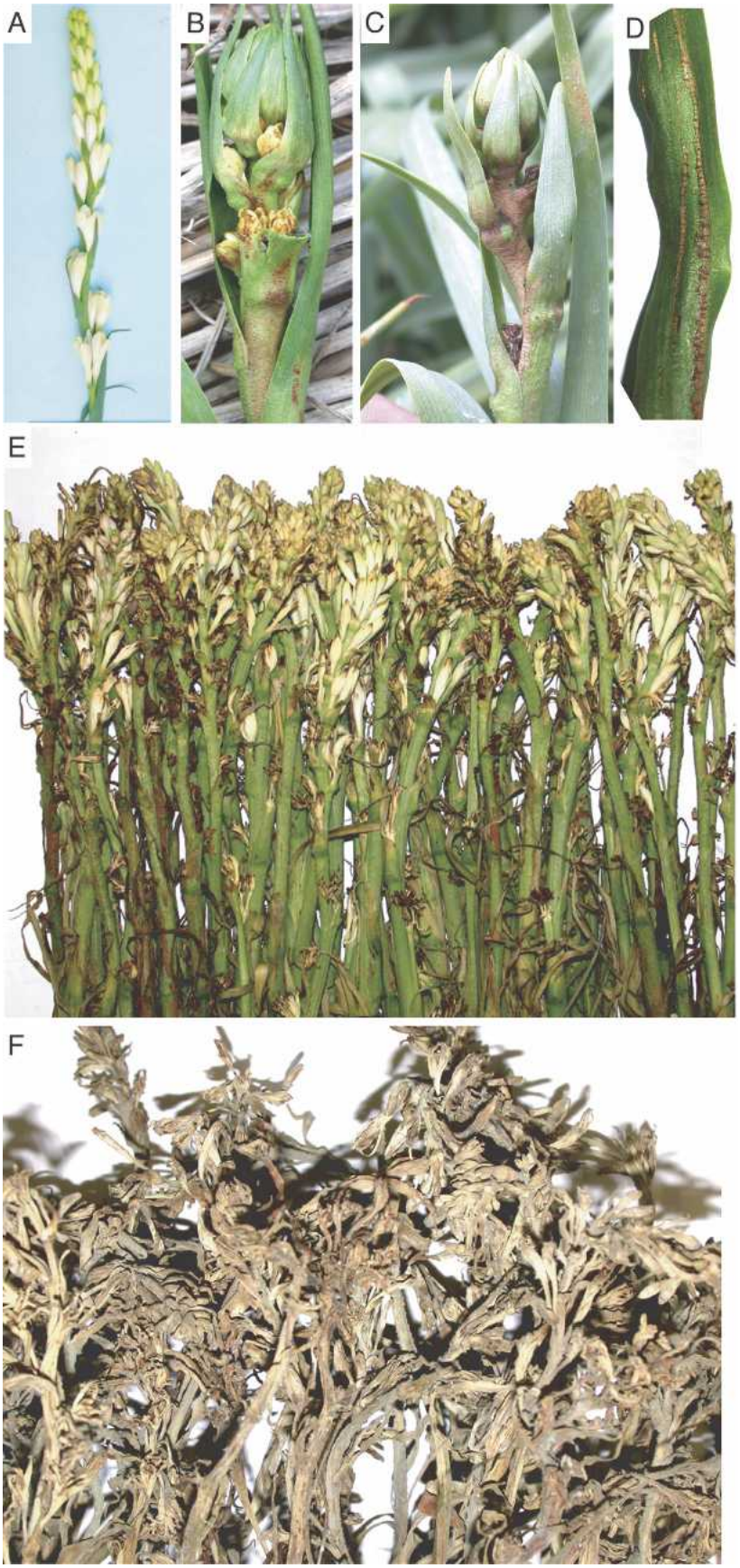

FIG. 1. Effects of *Aphelenchoides* sp. infection on *P. tuberosa*. A) Healthy control stem with abundant white florets. B–D) Infected plants. Note the browned stem, leaves and florets, as well as the distorted skin of the leaves on the stem. E–F) Infected stems 2 d and 1 mon after harvest, respectively.

with hulls attached, was also assessed at 95 d after planting.

Acid fuchsin staining: Plant tissues were stained with acid fuschin to visualize nematodes within plant tissues (Dropkin, 1980). In short, dissected plant tissues were briefly rinsed in water to remove soil, then immediately wrapped in a wet cotton cloth and immersed in boiling lactophenol (20% phenol, 20% lactic acid, 40% glycerol and 20% water) containing 0.05% red acid fuschin. The samples were then washed at room temperature with water and blotted off, and the plant tissue was cleared by incubation in lactophenol for 1 to 2 d.

Survey of cultivators: We surveyed farmers from five different provinces (Tiền Giang, An Giang, Đồng Tháp, Can Tho City, Hâu Giang) and assessed their methods of bulb treatment, crop rotation, use of fertilizer or disinfectant as well as the number of years during which they have cultivated *P. tuberosa* crops. This was done by direct interviews with the farmers.

Results and Discussion

Symptoms and nematode identification: Healthy *P. tuberosa* plants produce a stalk approximately 1 m in length and decorated with up to 20 white, blemish-free florets (Fig. 1A). In contrast, diseased plants produced stunted stalks bearing florets that were yellowed and browned that, in severe cases, failed to open (Fig.

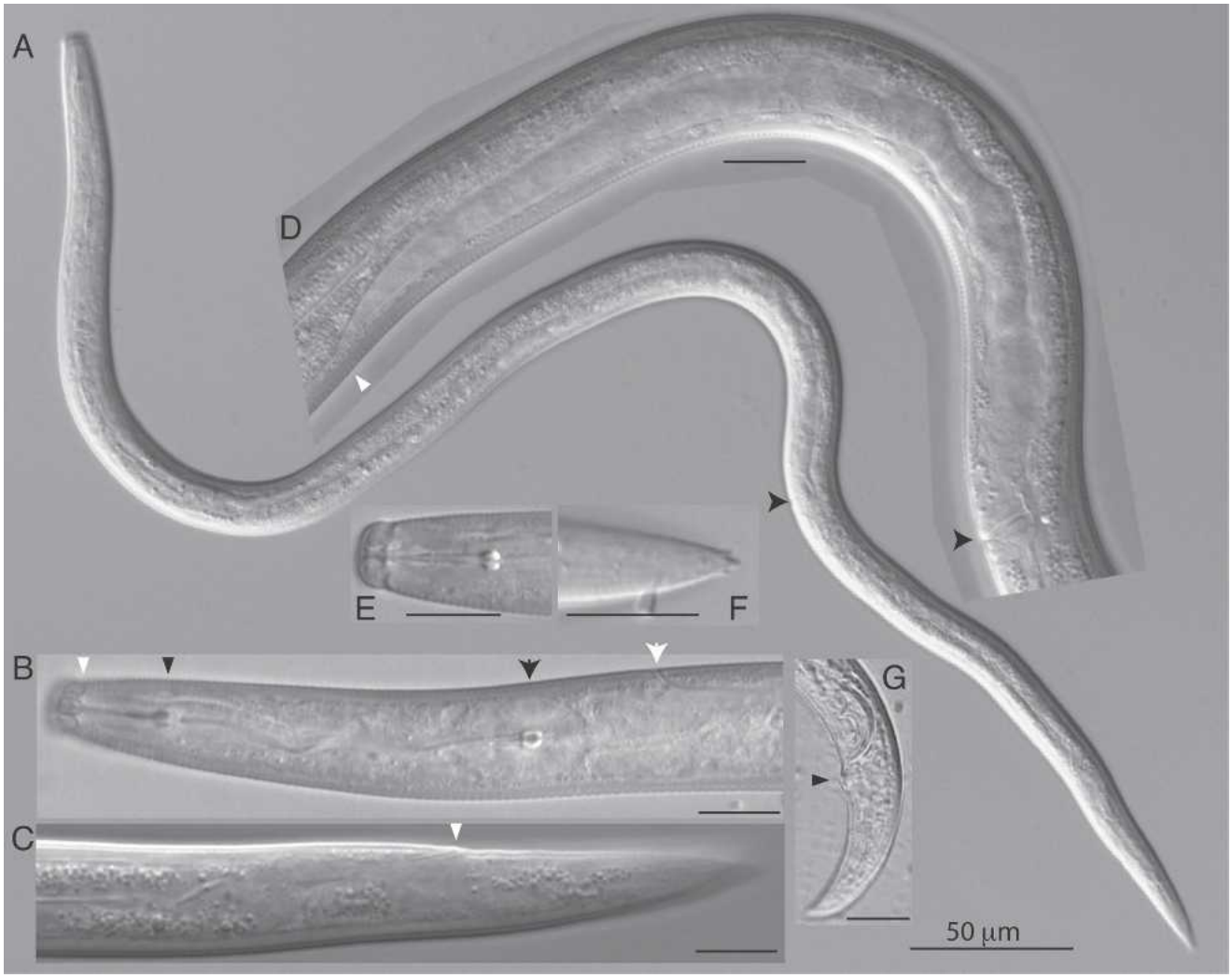

Fig. 2. Morphology of the nematode infecting *P. tuberosa* and partial sequence of its 18S and 28S rRNA. The position of the vulva is indicated in (A) and (D) by a black arrowhead, and the white triangle in (A) indicates the anterior end of the gonad arm. In (B), the white triangle points to the posterior end of the lips, the black triangle to the posterior end of the stylet, the black arrowhead to the posterior bulb of the pharynx, and the white arrowhead to the excretory pore. In (C), the anus is indicated by a white triangle. (E) and (F) are enlargements of the head and tail, respectively. (G) Mail tail, with the anus indicated by a black triangle. The morphological features are consistent with the nematode being *Aphelenchoides besseyi*. In particular, the cuticle was marked by fine striae (each about 1 µm thick), there were six amalgamated lips, the stomatostylet was short (10–13 µm), the ovary was outstretched anteriorly with oocytes arranged in tandem or multiple rows, and the vulva was located about three quarters of the body length away from the anterior end. The excretory pore was located posterior to the oesophageal bulb, approximately level with the anterior part of the nerve ring. The female was approximately 600 µm long, and the tail was conoid with a star-shaped mucro. The scale bars for B–G represent 10 µm in length.

1B,C), as well as having long, dry lesions on the leaves (Fig. 1D). The infected flowers and shoots first show browning symptoms on their most tender emerging parts, which is suggestive of ectoparasitic feeding that compromises the development of the mature structures. These infected plants could be stored under ambient conditions for several months, and nematodes isolated from their various parts (Fig. 1E,F). The morphology of the nematode infecting *P. tuberosa* (Fig. 2A–F) is perfectly consistent with it being *Aphelenchoides besseyi* (Mai et al., 1996; OEPP/EPPO, 2004), which is in agreement with an earlier, limited study of a nematode parasite of *P. tuberosa* (Holtzmann, 1968). Identification was further supported with "molecular barcodes" (Floyd et al., 2002; Powers, 2004) by partially sequencing two rRNA genes. A BLAST search of the GenBank database revealed that the most homologous sequences to the obtained 18S rRNA sequence were those of *Aphelenchoides ritzemabosi* (GenBank DQ901554) and *Aphelenchoides besseyi* "isolate 98" (GenBank AY508035), with 602/628 (95%) and 595/643 (92%) nucleotide identities over their most homologous regions, respectively. Based on the morphology, and in particular the tail shape and position of the excretory pore, the nematode that infects *P. tuberosa* cannot be *A. ritzemabosi* (OEPP/EPPO, 2004).

With respect to the 28S rRNA that we obtained, the two most homologous sequences were those of *Aphelenchoides besseyi* isolate 754a_3fe from Russia (GenBank DQ328684) and *Aphelenchoides besseyi* isolate 98 (GenBank AY508109), with 405/405 (100%) and 356/380 (93%) nucleotide identities over their most homologous regions, respectively. Based on morphology, partial sequence of rRNA genes and its ability to parasitize rice (see below), we conclude that the nematode that infects *P. tuberosa* in the Mekong Delta is most likely *Aphelenchoides besseyi*. However, the reader should note that the rRNA gene sequences of other members of the

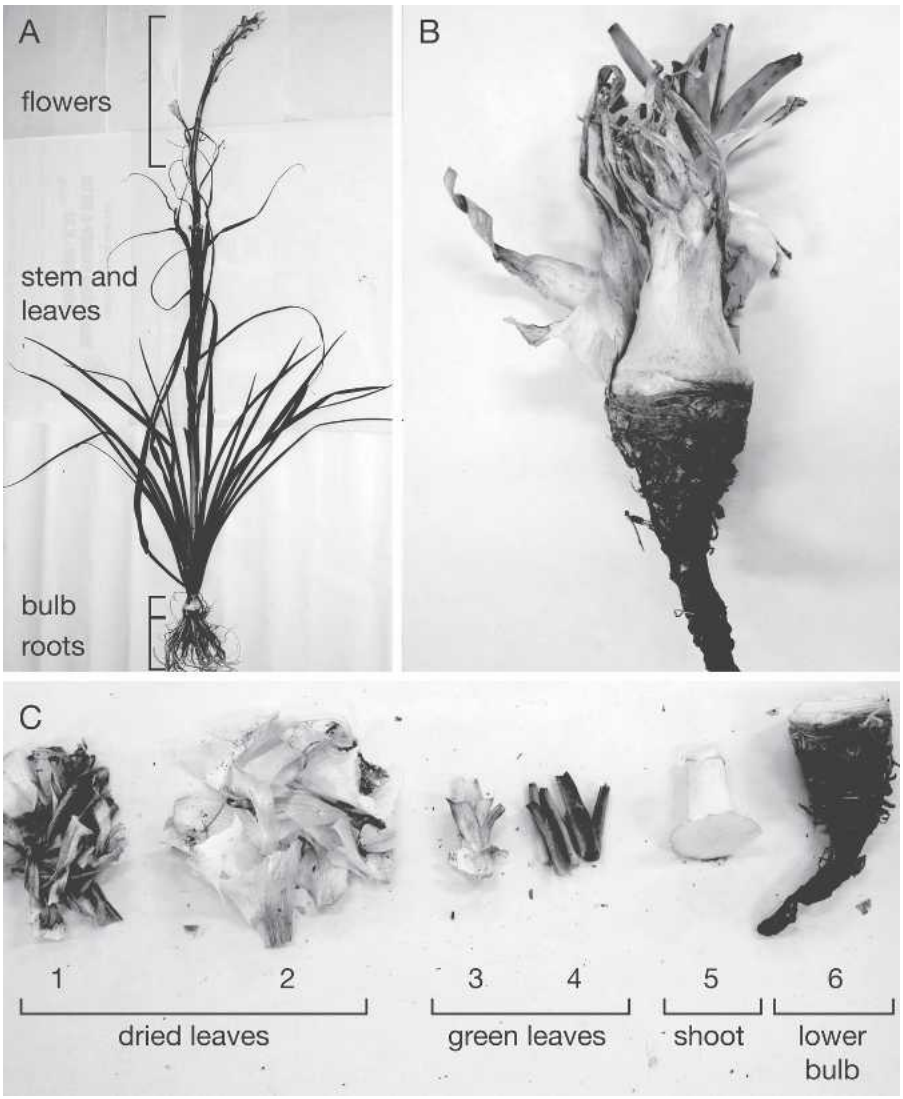

FIG. 3. Identification of the various parts of the *P. tuberosa* plants and bulbs dissected for nematode infestation counts. A) Whole plant with labels for the various parts. B) Typical bulb used for planting. C) Various parts of a dissected bulb as used in this study.

TABLE 1. Number of nematodes found in bulb parts 1 to 6 (see Fig. 3) at 1, 2 and 3 mon after harvest. Numbers are means ± standard deviation.

Plant part no.[a]	1 mon (n = 20)	2 mon (n = 10)	3 mon (n = 20)
1	21 ± 31	9 ± 14	0 ± 1
2	7 ± 15	16 ± 14	17 ± 62
3	1 ± 3	1 ± 4	2 ± 6
4	13 ± 41	2 ± 4	nd[b]
5	13 ± 26	6 ± 12	13 ± 53
6a	22 ± 38	49 ± 66	3 ± 4
6b	0 ± 0	0 ± 0	0 ± 0
Total	77 ± 76	84 ± 78	35 ± 120

[a] Plant part numbers refer to the following bulb components. 1 and 2 are the upper and lower dried leaves attached to the bulb, respectively. 3 and 4 are the upper and lower green leaves attached to the bulb, respectively. 5 is the shoot, 6a is the exterior of the bulb and 6b is the interior of the bulb. See also Fig. 3.
[b] Not determined.

Aphelenchoides genera are currently not available for comparison and that the *Aphelenchoides* genus comprises at least 138 valid species and is currently in "urgent need of a major revision" (Hunt, 1993). For these reasons, we choose here to err on the side of caution and refer to the *P. tuberosa* pest simply as *Aphelenchoides sp.*

TABLE 2. Effect of planting infected *P. tuberosa* bulbs on flower yield.

	Field 1 (n = 20)	Field 2 (n = 20)
Nematodes per bulb[a]	58.8 ± 65.5	77.6 ± 83.0
Number of infected plants with symptoms of infection[b]		
1 MAP	2	4
2 MAP	5	4
3 MAP	6	5
4 MAP	6	5
Frequency of plants with infected spikes 4 MAP[c]	7 of 15 (46.7%)	5 of 12 (41.7%)
Number of nematodes per infected spike 4 MAP[c]	186 ± 120 (n = 7)	269 ± 135 (n = 5)

[a] The average number of nematodes per bulb ± the standard deviation is given.
[b] Twenty bulbs from each field were allowed to grow in pots kept in the greenhouse, and the numbers of plants exhibiting symptoms of nematode infection were counted 1, 2, 3 and 4 mon after planting (MAP).
[c] The presence of nematodes in the spikes of plants that produced spikes (15 from Field 1; 12 from Field 2) was ascertained, and the average number of nematodes per infected spike was also counted.

Persistence of infectious Aphelenchoides sp in plant tissues: Growing but infected plants were divided into flowers, plant and leaves, bulb and roots according to Fig. 3A.

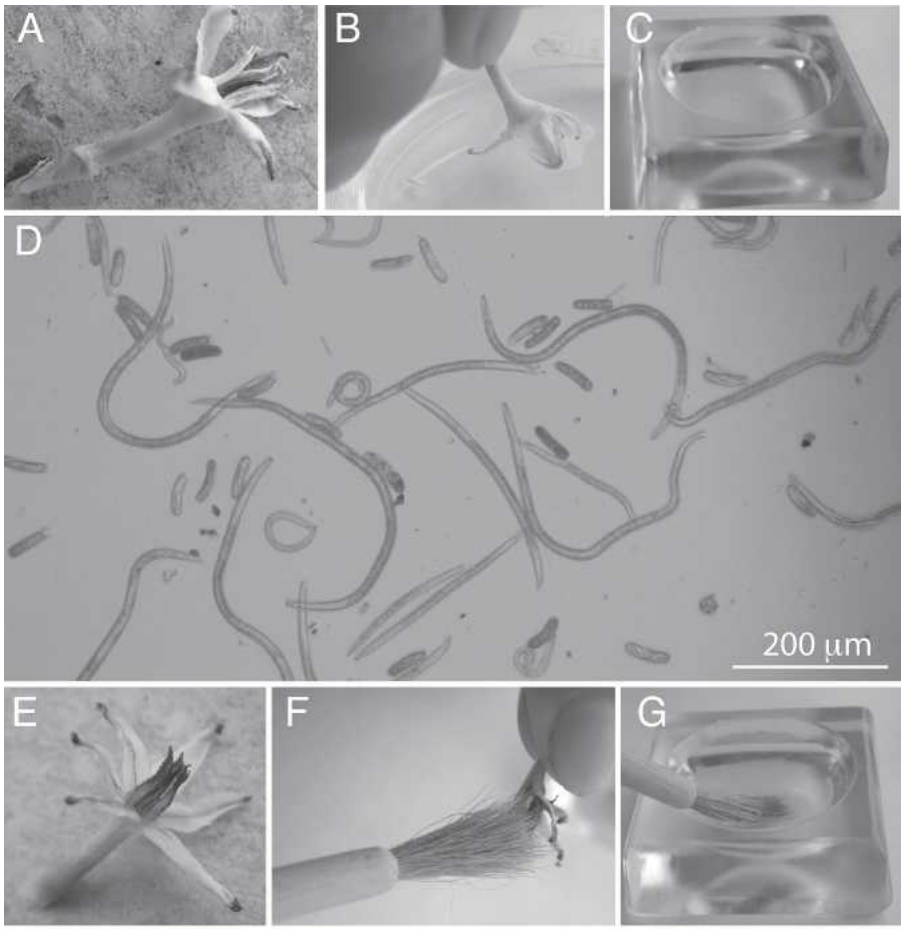

FIG. 4. Evidence of ectoparasitism. A-C) Show how an infected flower is gently bathed in water then inspected in an observation glass to reveal a multitude of *Aphelenchoides* sp. nematodes of various stages, including eggs, juveniles and adults (D). E–G) Alternatively, an infected flower can be gently brushed over water to also produce a mixed nematode population, as in (D).

TABLE 3. Effect of post-harvest flower age on numbers of *Aphelenchoides* sp.

Age of sample	Number of nematodes[a]
2 d	3,974 ± 1,465
1 mon	887 ± 892
2 mon	121 ± 83
3 mon	71 ± 107
4 mon	125 ± 174
5 mon	71 ± 45
6 mon	37 ± 43

[a] The number of nematodes found in 4 flowers/plant at various time points post-harvest. Each data point represents the average from 15 different plants ± the standard deviation.

Living nematodes were recovered from all parts except the roots. Typically, *P. tuberosa* is cultivated by replanting bulbs that have been stored under dry, open-air conditions for a month or more. When dissecting and examining such bulbs of various ages, we found that nematodes can be recovered from all external parts, but never from within the plant tissue itself (Fig. 3B,C and Table 1). Nematodes were most numerous in the top of the dried leaves attached to the bulb and on the skin covering the lower bulb in 1 and 2-mon-old samples. In 3-mon-old bulbs, large numbers of nematodes were found almost exclusively in the lower part of the dried leaves and the more hydrated shoot in the upper part of the bulb (Table 1). Plants grown in sterile soil but from bulbs collected from an infested field often developed severe infection and showed the disease symptoms described earlier (Table 2). The propagation of *P. tuberosa* crops from infected bulbs is therefore a likely source of persistent infestation in the fields.

Aphelenchoides nematodes are usually ectoparasitic (Perry and Moens, 2006), but *A. besseyi* may be endoparasitic in some host species, as suggested by a brief study on *P. tuberosa* cultivated in Hawaii (Holtzmann, 1968) and another on *Ficus elastica* Roxb. ex Hornem. (Marlatt, 1966). The study by Holtzmann focused exclusively on the leaf symptoms, which differed from the leaf symptoms that we observed in that they tended to be much larger, restricted to the center of the leaf blade, water-soaked and caused leaves to droop; none of these features were found in the *P. tuberosa* grown in the Mekong Delta. The discrepancies in symptoms may be due in part to the much more humid climate of the Manoa Valley (average annual rainfall of 4,190 mm) compared to that of the region studied in the Mekong Delta (average annual rainfall of 1,800 mm). Holtzmann (1968) did not directly address the question of whether the nematode infection was endo- or ectoparasitic in nature. In our study of *P. tuberosa*, we found that the greatest numbers of nematodes were extracted immediately after immersion of infected bulb, shoot or flower tissues in water, suggesting that these nematodes were living on plant surfaces. From flower tissues, extracted nematode numbers 0–5, 6–10, 11–30 and 31–60 seconds after immersion were (N = 10): 9,641 ± 2,289, 3,096 ± 727, 833 ± 482 and 508 ± 221. All nematode stages can also be recovered from infected flowers that are gently washed or dry-brushed, indicating that the complete life cycle is supported outside the plant tissue (Fig. 4). Consistently, no nematodes were ever found within stained tissue from infected plants, and showed no sign of being confined by plant tissue compartments. Our results indicate that the nematode is mostly ectoparasitic on *P. tuberosa* and suggest that the leaf symptoms are consequences of the nematodes feeding on the tender emerging meristems and tissues. Similarities exist between the infectious cycle of *Aphelenchoides sp* on *P. tuberosa* and that of *A. besseyi* on rice, where it causes white tip disease (Fortuner and Orton Williams, 1975; Mai et al., 1996). In both cases, the nematode is an ectoparasite that infests the part of the

TABLE 4. Comparison of *Polianthes tuberosa* farming practices and *Aphelenchoides* sp. infection among five different regions of the Mekong Delta.

	Tiền Giang province	An Giang province	Đồng Tháp province	Can Tho city	Hậu Giang province
Fields surveyed	20	10	7	10	5
Infected fields (%)	100	100	100	70	60
Severity of infection[a]	Severe	Moderate	Severe	Light	Light
Bulb treatment	Sun-drying and storage 1–1.5 mon, disinfect with pesticides	None- fresh bulbs used for planting	Sun-drying 1 week, storage 30–45 d	Dry for 7–10 d—No storage	None or insecticide (e.g., methidathion)
Intercrop	Rice	Almost always with vegetables	Rice-vegetables-orchards	Vegetables	Rice-orchards
Land rotation	Change every year	Change	Change, usually after 2 yr	After 1–2 yr	nd[c]
Organic fertilizer	no	yes	yes	Some yes	no
Soil disinfection[b]	no	Dry under the sun	no	Dry under the sun	no
Years of *P. tuberosa* cultivation	>10 yr	1–3 yr	2.5–10 yr	1–2 yr	1–2 yr

[a] All plants within five randomly picked areas of one square meter along a "W" sampling pattern were scored for infections. Severe, substantial, moderate and light correspond to >40%, 20–39%, 10–19% and <10% of plants being infected in a given field, respectively.
[b] "Dry under the sun" means that the soil was turned by plowing and allowed to rest for 1-2 mon before replanting.
[c] Not determined.

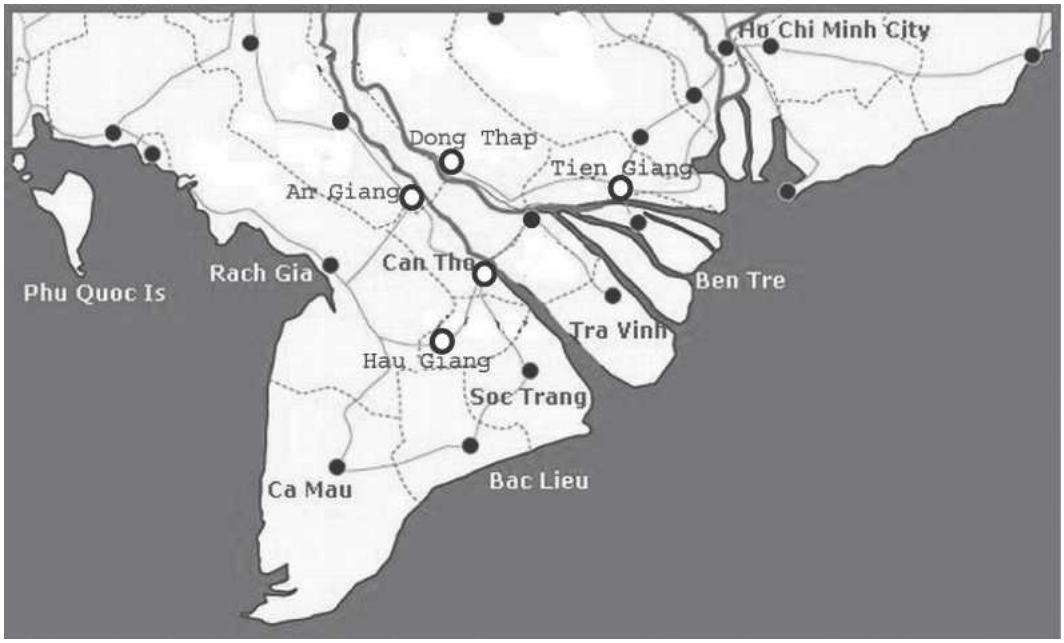

FIG. 5. Map of the Mekong Delta showing the various locales from which *P. tuberosa* samples were obtained (open circles).

plants used for propagation (grain in rice, bulbs in *P. tuberosa*), where it can persist as a long-lived form able to withstand several months of storage before resuming growth when returned to the field (Huang and Huang, 1974; Togashi and Hoshino, 2001, 2003).

Survival of Aphelenchoides sp. on dried flowers and soil: Dried flowers that fall to the ground in an infested field may be a possible source of infection. We tested this hypothesis by counting the nematodes found in flowers 2 d to 6 mon after harvest from the field. Numbers of viable nematodes declined rapidly during the first 2 mon, and then leveled off at about 2% of initial values for up to 6 mon after harvest (Table 3). One interpretation is that once a relatively short-lived population of the nematode has died during the first 2 mon, there is left a long-lived population that exhibits little decline over time.

Another possible source of infection in the field is infested soil. In severely infected fields, the numbers of nematodes often exceeded several hundred per kilogram of soil, and the great majority of these nematodes were morphologically identical to those found in the *P. tuberosa* samples. The mean numbers of nematodes collected from 10 0.5-kg samples of soil in each of three different fields were: 591 ± 263, 759 ± 719 and 221 ± 115. In contrast, no nematodes were collected from the

TABLE 5. Number of *Aphelenchoides* sp. found in seeds of 19 cultivars of rice from four different districts of the Mekong Delta.

Sample no.	Rice variety	Number of nematodes per 300 seeds[a]	Months in storage	(District-Province)
1	Trắng Chim	0	3	Phong Hoà-Lai Vung-Đồng Tháp
2	Móng Chim	0	3	Phong Hoà-Lai Vung-Đồng Tháp
3	504 Thuồng	0	3	Phong Hoà-Lai Vung-Đồng Tháp
4	504 Cần Thỏ	0	1	Phong Hoà-Lai Vung-Đồng Tháp
5	504 Đài Loan	0	1	Phong Hoà-Lai Vung-Đồng Tháp
6	504 Chùm	0	1	Phong Hoà-Lai Vung-Đồng Tháp
7	OM 1940	0	1	Phong Hoà-Lai Vung-Đồng Tháp
8	Bạc Bụng	0	3	Cai Lậy-Tiền Giang
9	Tài Nguyên	30	3	Long Hồ-Vĩnh Long
10	Nông Nghiệp	0	3	Long Hồ-Vĩnh Long
11	OM1490	0	3	Long Hồ-Vĩnh Long
12	OM1960	0	3	Long Hồ-Vĩnh Long
13	OM2517	0	3	Long Hồ-Vĩnh Long
14	VND 95-20	0	3	Nông Truồng Sông Hậu
15	OM 2718	6	3	Nông Truồng Sông Hậu
16	Nù	15	4	Tân Quới-Bình Minh-Vĩnh Long
17	OM Lùn	0	4	Tân Quới-Bình Minh-Vĩnh Long
18	504 Gốc nhật	0	4	Tân Quới-Bình Minh-Vĩnh Long
19	504 Nhị Vàng	0	4	Tân Quới-Bình Minh-Vĩnh Long

[a] The nematodes were counted from grains consisting of the true fruit together with the hull.

water that fills the irrigation canals surrounding the fields.

Effect of farming practices on Aphelenchoides sp.: From surveys of farmers, it emerged that no effective measures are currently in use to control the nematode infection and that the healthiest crops are in fields in which *P. tuberosa* has been cultivated for the least number of years (Table 4).

Pathogenicity of Aphelenchoides sp. on rice grown in the Mekong Delta: Rice or vegetables are the most common intercrops for the farmers who cultivate *P. tuberosa* (Table 4). We found that rice cultivated in the Mekong Delta was rarely infected by nematodes (Table 5), but nevertheless was a host able to sustain reproduction of the nematodes recovered from infected *P. tuberosa* (Table 6), although without causing signs of disease. The numbers of nematodes found in the grains of infected rice were comparable to the numbers previously published for pathogenic infections of rice by *A. besseyi* (Nandakumar et al., 1975), but no significant differences in growth or fertility were found between controls and nematode-infected plants for any of the tested rice varieties (data not shown). The rice cultivars tested therefore appear to be examples of symptom-less but susceptible hosts, as often emerge from screening for resistance (Starr et al., 2002).

TABLE 6. Numbers of nematodes extracted from grains of 30 rice cultivars inoculated with nematodes during sprouting, then allowed to grow to maturity in the greenhouse.

	Nematodes/100 grains[a]		
Rice cultivars	Sterile grain	Milk grain	Filled grain
504 Cần Thỏ	30.8	4.7	76.8
504 Chùm	40.3	9.7	76.7
504 Đài Loan	19.0	1.8	112.2
504 Gốc Nhật	47.8	1.0	67.7
504 Nhị Vàng	38.0	22.5	137.5
504 Thuồng	40.7	6.2	155.2
AS 996	18.7	0.8	35.5
Bạc Bụng	50.0	4.8	348.8
Hàm Châu	31.5	9.0	50.7
Jasmin 85	9.8	1.0	103.8
Móng Chim	44.8	1.0	188.8
MTL 325	36.2	5.7	76.0
Nông Nghiệp	39.3	1.7	73.5
OM 1490	11.7	0.0	31.0
OM 2395	14.5	0.0	37.0
OM 2517	23.0	0.0	47.2
OM 2717	48.2	13.8	129.0
OM 2718	13.5	9.3	28.7
OM 3238	31.7	3.7	62.0
OM 4495	25.3	5.5	33.0
OM 4498	25.0	19.7	92.2
OM 4872	23.7	18.8	51.0
OM Lùn	26.2	8.7	70.8
OM1940	41.2	6.7	85.3
OM1960	85.8	21.8	73.5
OM2492	71.0	16.2	221.5
OMCS 2000	2.7	0.8	8.8
Tài Nguyên	273.0	2.5	853.2
Trắng Chim	37.3	9.3	85.3
VND 95-20	19.7	1.8	112.2

[a] The nematodes were counted from grains consisting of the true fruit together with the hull.

A plan of action: *Aphelenchoides besseyi* can survive in dried rice seeds for over 20 months (Huang and Huang, 1974; Huang and Chiang, 1975). Management of *A. besseyi* often involves soaking of rice seeds in aqueous emulsions of nematicides and subsequent air-drying of the seed for several days (Hoshino and Togashi, 2000). The seeds are later soaked again in water to induce sprouting. An analogous approach should be tested on *P. tuberosa* bulbs, and the cost-benefits evaluated. Hot water treatments have also been used to combat *Aphelenchoides* infection of rice seeds (Fortuner and Orton Williams, 1975), and preliminary results suggest that this method is promising on *P. tuberosa* (Cuc and Pilon, unpublished observations). The careful monitoring of infection levels within bulbs considered for replanting should also strongly be encouraged.

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