# Note

# First Report of a Foliar Nematode *Aphelenchoides fragariae* (Aphelenchidae) on *Stachys riederi* var. *japonica*, a Medicinal Plant, in Korea

Zakaullah Khan<sup>1</sup>, Seon-Hye Son<sup>1</sup>, Hyeon-Dong Shin<sup>2</sup> and Young Ho Kim<sup>1\*</sup>

<sup>1</sup>Department of Agricultural Biotechnology and Center for Plant Molecular Genetics and Breeding Research, Seoul National University, Seoul 151-921, Korea

 $^2$ Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Korea

(Received on January 10, 2008; Accepted on January 28, 2008)

Leaf blight symptoms and abnormal defoliation were found on *Stachys riederi* var. *japonica*, a medicinal plant, grown at Hongcheon, Gangwon province, Korea in 2007. A foliar nematode, *Aphelenchoides fragariae* was isolated from infected leaf tissues. The symptoms observed on host plant were leaf blotching, discoloration and browning of tissues, tan-colored interveinal necrotic lesions and large dead patches of necrotic tissues. This is the first report of *A. fragariae* occurring on *S. riederi* var. *japonica*.

**Keywords**: Aphelenchoides fragariae, description, new record, Stachys riederi var. japonica, symptomatology

Foliar nematodes include several species of the genus *Aphelenchoides* that commonly feed inside tender leaves of higher plants. *A. fragariae* (Ritzema Bos, 1890) Christie, 1932 is an ecto- and endoparasite of the above ground plant parts. The nematodes have a wide host range including several ornamental plants and occur in many countries (Dunn, 1997). The nematodes infect young leaves through stomata (natural openings) or directly through tender leaf tissues.

Once infected inside, the nematode feeds on the mesophyll cells, causing large portions of the leaf to become chlorotic. The chlorotic sections subsequently turn necrotic. These necrotic lesions are usually bounded by large veins, and in severe cases, the entire leaf can dry and fall down (Sanwal, 1959).

In Korea, *A. fragariae* is a common pest of strawberry (Park et al., 2005), on which it causes strawberry crimp disease but has also been reported on several other plants (Choi, 2001; Khan et al., 2007).

Stachys riederi var. japonica Miq., a perennial plant well known for its medicinal value, belongs to the family Labiatae. S. riederi var. japonica is also called as Seok-Jam-Pul that has been used for centuries as traditional

\*Corresponding author.
Phone) +82-2-880-4675, FAX) +82-2-873-2317
E-mail) yhokim@snu.ac.kr

hemorrhage, cough, and skin disease. It has contents of caffeic acid, n-methoxybaicalein, palustrine and palustinoside (Bae, 2000). To our knowledge, this is the first report of a medicinal herb, *S. riederi* var. *japonica* as host of *A. fragariae*.

medicine in Korea. The plant is used for the remedy of

**Nematode occurrence and symptoms.** During routine disease surveys in Korea, leaf blight and severe angular spot symptoms were found on leaves of *S. riederi* var. *japonica* at Seongdong-ri, Bukbang-myeon, Hongcheongun, Gangwon province, Korea in 2007. The detailed symptoms observed on *S. riederi* var. *japonica* leaves were typical of the foliar nematode infection; leaf blotching, discoloration and browning of tissues, tan-colored interveinal lesions, necrotic and large dead patches of tissues (Fig. 1A-D).

Microscopy of infected tissues. Necrotic tissues were excised from infested leaves and fixed in FAA for 24 h, cleaned in 2% sodium hyperchlorite for 10 minutes and stained with 0.07% bromophenol blue in 50% ethanol for 8 h, and rinsed in 50% ethanol. A. fragariae was found in all the diseased leaf tissues (Fig. 1E). It is concluded that A. fragariae can be a damaging pest of S. riederi var. japonica in Korea, which can serve as a source of infestation to other host plants including important horticultural crops such as strawberries. Removing and destroying all infected leaves and plants can help reduce spread of this nematode. To remove and destroy all plants that show any symptoms of infection are efficient control mathod.

**Nematode isolation and identification.** Nematode-infected leaves of *S. riederi* var. *japonica* were cut into 1-cm<sup>2</sup> pieces, and placed into Petri dishes containing water for 48 h for the nematode to emerge. Collected nematodes were killed in water heated to 60°C for one minute and fixed in 4% formalin. For taxonomic studies some specimens were transferred to glycerin by the methods of Seinhorst (1959) and mounted in anhydrous glycerin on glass slides. The

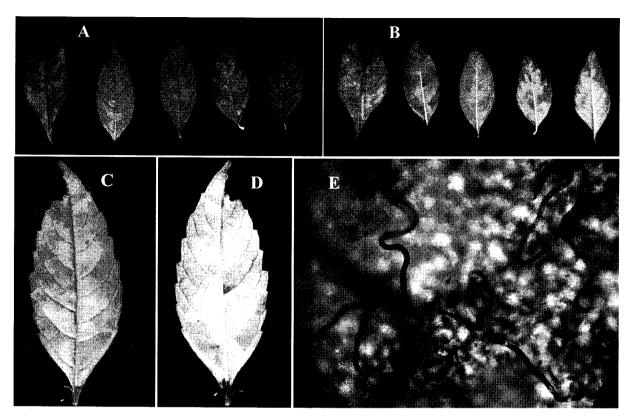


Fig. 1. Symptoms caused by *Aphelenchoides fragariae* on *Stachys riederi* var. *Japonica*. (A-D) Infected leaves showing dark brown angular necrotic lesions, (A & C) showing adaxial surface, (B & D) showing abaxial surface of infected leaves, (E) showing leaf tissues colonized by *A. fragariae*.

nematodes were observed under the light microscope (Axiophot, Zeiss, Germany). Light microscope measurements and photographs were taken with digital photo camera (AxioCam HR, Zeiss, Germany) mounted on the microscope. DeMan's formula was used to determine the pertinent values of measurements. For scanning electron microscopy (SEM) studies, fixed specimens were treated with ultrasonic vibrator to remove any adhering detritus, dehydrated in graded ethanol series, critical point dried, sputter coated with gold, and observed with a scanning electron microscope (JSM-5410LV, Jeol, Tokyo, Japan).

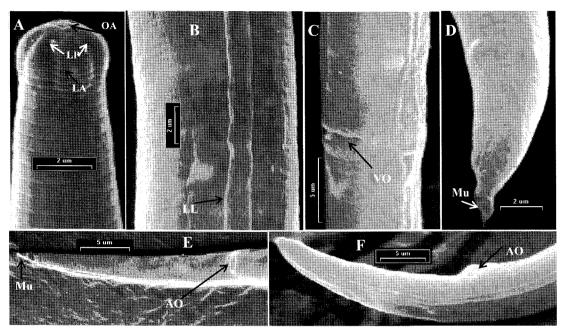
#### Description

### Aphelenchoides fragariae (Figs. 2 & 3)

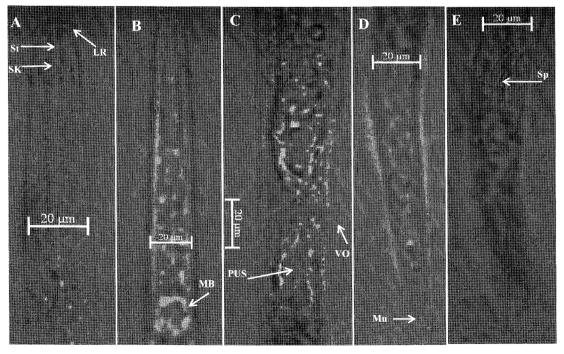
**Measurements:** Female (n = 10): L = 728-845 μm; a = 46.2-56.5; b = 7.6-9.2; c = 16.4-20.2; c' = 4.0-4.5; V = 62.5-70.4%; stylet = 12.0-14.0 μm; tail = 36-50 μm; ABD = 9-11 μm.

Male (n = 7): L = 560-623  $\mu$ m; a = 45.7-53.7; b = 7.3-8.8; c = 15.7-17.5; c' = 3.3-3.8; T = 46-60%; stylet = 10.0-11.2  $\mu$ m; tail = 34-42  $\mu$ m; ABD = 10-11  $\mu$ m; spicules = 16.2-18.5  $\mu$ m.

Female: Body slender; almost straight or curved ventrally when relaxed; tapering posteriorly to pointed terminus with peg-like mucro, 2-3 µm long. Cuticle finely transversely striated, about 0.5 µm wide at mid-body. Lateral fields a narrow band, with two incisures (Fig. 2B). These continue longitudinally for the entire body length, reaching to the tail end (Fig. 2D, F). Lip region almost continuous with body, 3.5-4.0 µm in diameter; 4-5 lip annuli visible with SEM only, and oral aperture circular (Fig. 2A). Stylet slender, 12-14 µm long; anterior portion needle like; basal knobs minute but distinct (Fig. 3A, B). Amphidial apertures small, pore-like, located dorsosubventrally, six outer labial papillae located external to oral disc (Fig. 2A). Esophagus with well developed median bulb (Fig. 3B), rounded to oval in shape, about 9 µm long and 8 µm wide; esophageal glands in a long lobe overlapping intestine dorsolaterally. Vulva in the form of transverse slit (Fig. 2C), lips slightly protruding, located at 62-70% of body length. Vagina anteriorly directed, about half of corresponding body width deep (Fig. 3C). Gonad monoprodelphic, out-stretched with oocytes in a single row. Post-uterine sac extending for more than half of vulva-anus distance (Fig. 3C). Anal aperture crescent-shaped, about 2.5 µm wide (Fig. 2E). Tail elongate conoid ending in minute mucro, 2-3 µm long (Figs. 2D, E



**Fig. 2.** Scanning electron micrographs of *Aphelenchoides fragariae* from *Stachys riederi* var. *japonica*. (A) anterior region showing oral aperture (OA), labial annules (LA) and labial papillae (LP), (B) lateral view of middle body, showing lateral lines (LL), (C) vulval opening (VO), (D-F) female posterior region showing anal opening (AO) and tail terminus with mucro (Mu).



**Fig. 3.** Photo micrographs of *Aphelenchoides fragariae* (1000 x). (A & B) anterior region showing lip region (LR), stylet (St), stylet knobs (SK) and median bulb (MB), (C) vulval region showing vulval opening (VO) and post uterine sac (PUS), (D) female posterior region showing tail and mucro (Mu); E: male posterior region showing spicules (Sp).

& 3D).

Males: Males are similar to female in general morphology but smaller in body length. Testis outstretched, with spermatocytes in a row. Tail arcuate upon death, 3-4 anal body widths long, with a simple blunt terminal mucro. Spicules rose-thorn shaped, dorsal limb about 1.5 anal-body widths long (Fig. 3E). Three pairs of ventro-

submedian caudal papillae present. Bursa and gubernaculum were absent.

**Remarks:** Identification of *A. fragariae* was based on the morphometric data and morphological characteristics of the females and males, which match well with that of *A. fragariae* as given by Choi (1977) from Korea and to those described in Hunt (1993).

## Acknowledgement

The study was supported by the Korean Science and Engineering Foundation (KOSEF) through the Center for Plant Molecular Genetics and Breeding Research.

#### References

- Bae, K. H. 2000. The Medicinal Plants of Korea. Kyo-Hak Publishing Co. Ltd, Seoul, pp 447.
- Choi, Y. E. 1977. Studies on plant parasitic nematodes associated with strawberry. Research Review Kyungpook National University, Korea 23:309-316.
- Choi, Y. E. 2001. Nematoda (Tylenchida, Aphelenchida). Economic Insects of Korea 20. Ins. Korean Supplement 27:392 pp.

- Christie, J. R. 1932. Recent observations on the strawberry dwarf nematode in Massachusetts. *Plant Dis. Rep.* 16:113-114.
- Dunn, R. A. 1997. *Foliar nematodes as pests of ornamental plants*. Institute of Food and Agricultural Science, University of Florida, USA. SP 221, pp. 3.
- Hunt, D. J. 1993. Aphelenchida, Longidoridae and Trichodoridae: Their systematics and bionomics. Wallingford, UK, CABI Publishing, 352 pp.
- Khan, Z., Son, S. H., Moon, H. S., Kim, S. G., Shin, H. D. and Kim, Y. H. 2007. The foliar nematode, *Aphelenchoides fragariae*, on Jerusalem artichoke (*Helianthus tuberosus*) and Weigela (*Weigela subsessilis*). *Nematropica* 37:335-337.
- Kim, Y. H., Riggs, R. D. and Kim, K. S. 1986. A mechanism of density dependent population change in *Heterodera glycines*. *Korean J. Plant Pathol.* 2:199-206.
- Park, S. D., Khan, Z., Yeon, I. K. and Kim, Y. H. 2005. A survey for plant-parasitic nematodes associated with strawberry (*Fragaria ananassa* Duch.) crop in Korea. *Plant Pathol. J.* 21:387-390.
- Sanwal, K. C. 1959. Simple method for rearing pure populations of the foliar nematode, *Aphelenchoides fragariae*, in the laboratory. *Can. J. Zool.* 37:707-711.
- Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 44:67-69.