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Pathogenicity of *Aphelenchoides pseudogoodeyi* on the ornamental plants *Asplenium nidus* and *Lilium speciosum* in Brazil

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Summary

Aphelenchoides pseudogoodeyi has recently been reported in association with seeds of forage grasses and rice in Brazil and senescent strawberry plants, in the United States. This nematode is likely a mycophagous species; however, so far, its pathogenicity potential to plants is unclear. This study aimed to verify the pathogenicity of *A. pseudogoodeyi* to two species of ornamental plants. The experiments were conducted by inoculating *A. pseudogoodeyi* onto Bird's-Nest Fern (*Asplenium nidus*) and Oriental Lily (*Lilium speciosum*) leaves, using two inoculation methods (with and without injury). After 40 days of inoculation (DAI) in Bird's-Nest Fern and 5, 10, 20 and 40 DAI in Oriental Lily, the pathogenicity and the host efficiency were evaluated by symptoms observation and by severity, final nematode population and reproductive factor (RF), respectively. Additionally, a histopathological study was performed by inoculating *A. pseudogoodeyi* onto Bird's-Nest Fern for observing anatomical alterations. *A. pseudogoodeyi* was able to cause local necrotic lesions on both Bird's-Nest Fern and Oriental Lily leaves. However, the presence of injury was essential to enable *A. pseudogoodeyi* to penetrate and cause those symptoms in both plant species. Also, the total population of *A. pseudogoodeyi* decreased drastically over time and RF was <1, which characterized these species as poor-host or resistant plants. *A. pseudogoodeyi* penetrated into the foliar tissue and induced a total destruction of the mesophyll and collapse of the cells, with the formation of large intercellular spaces. It is concluded that *A. pseudogoodeyi* is an opportunistic pathogen as injury was required to induce symptoms in Bird's-Nest Fern and Oriental Lily.

Keywords: Aphelenchoididae; Bird's-Nest Fern; Foliar nematode; *Lilium speciosum*

Introduction

The *Aphelenchoides* genus comprises around 200 species, mainly mycophagous or predatory, and some species are also facultative plant-parasites (Hunt, 2008; Sánchez-Monge *et al*., 2015). However, just a few species of *Aphelenchoides* spp. are economically important, including *A. fragariae* Christie, 1932, *A. ritzemabosi* Steiner and Buhrer, 1932 and *A. besseyi* Christie, 1942. *Aphelenchoides ritzemabosi,* the chrysanthemum foliar nematode, parasitizes more than 200 species, most of which belong to the family Compositae, while *A. fragariae,* the strawberry nematode, is reported from more than 250 species, including ferns and members of Liliaceae, Primulaceae and Ranunculaceae (Vovlas *et al*., 2005; Jagdale & Grewal, 2006; Kohl, 2011; Sánchez-Monge *et al*., 2015). *Aphelenchoides besseyi,* the rice white tip nematode, infects many grasses, agronomic crops, strawberry (*Fragaria* spp.),

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common bean (*Phaseolus vulgaris*), Bird's-Nest Fern (*Asplenium nidus*) and several ornamental plants (Chaves *et al*., 2013; Sánchez-Monge *et al*., 2015). More recently, *A. besseyi* emerged as a serious threat to soybean and cotton crops in Brazil (Meyer *et al*., 2017; Favoreto *et al*., 2018), causing losses of up to 100 % in some fields (Meyer *et al*., 2017). Moreover, *A. besseyi* is listed as a quarantine pest for many countries and its presence may mean export barriers.

The association of plant-parasitic and non-pathogenic species of *Aphelenchoides* spp. to plant grass seeds in Brazil has been frequently reported (Sharma *et al*., 2001; Favoreto *et al*., 2011). Among those, de Jesus *et al*. (2016) reported the frequent association of a species identified as *A. fujianensis* with *A. besseyi* in forage and rice seeds. On the other hand, Oliveira *et al*. (2019) described a new species named *A. pseudogoodeyi*, which is associated with *A. besseyi* in senescent strawberry plants in Florida, USA. In this paper, the authors presented evidence that the Brazilian populations, previously identified as *A. fujianensis* by de Jesus *et al*. (2016), are in fact conspecific to *A. pseudogoodeyi*.

The pathogenicity of Florida population of *A. pseudogoodeyi* was assessed on soybean, alfafa, strawberry and Gerbera daisy, which induced local necrotic lesions only on soybean plants, under controlled conditions (Oliveira *et al*., 2019). Thus, the authors concluded that *A. pseudogoodeyi* is mainly mycophagus and may become phytophagous under stress conditions. However, it is still necessary to evaluate the pathogenicity of *A. pseudogoodeyi* in other cultivated plants, due to evidence that this nematode has potential for phytoparasitism. Thus, this study aimed to investigate the pathogenicity of a Brazilian population of *A. pseudogoodeyi* to two ornamental plant species.

Material and Methods

Plant materials of potential hosts

To evaluate the pathogenic potential of the Brazilian population of *A. pseudogoodeyi*, two economically important ornamental plants were used in this study: Bird's-Nest Fern (*Asplenium nidus* L.) and Oriental Lily cv. Entertainment (*Lilium speciosum* Thunb). These plant species were chosen as their susceptibility to plant-parasitic *Aphelenchoides* species (such *A. besseyi* and *A. fragariae*) is widely known (Yu & Tsay, 2003; Sánchez-Monge *et al*., 2015). Healthy seedlings of Bird's-Nest Fern, with circa 30 cm of height, were obtained from a local florist shop. Oriental Lily seedlings were obtained 54 days after the bulbs were sown in 2 L pots, which contained a mixture of substrate (Topstrato HT Hortaliça®) + soil at the ratio of 1: 1 (v / v). Plants were kept in a growth chamber at 25 °C, 12 h photoperiod and water and nutrients were supplied as required.

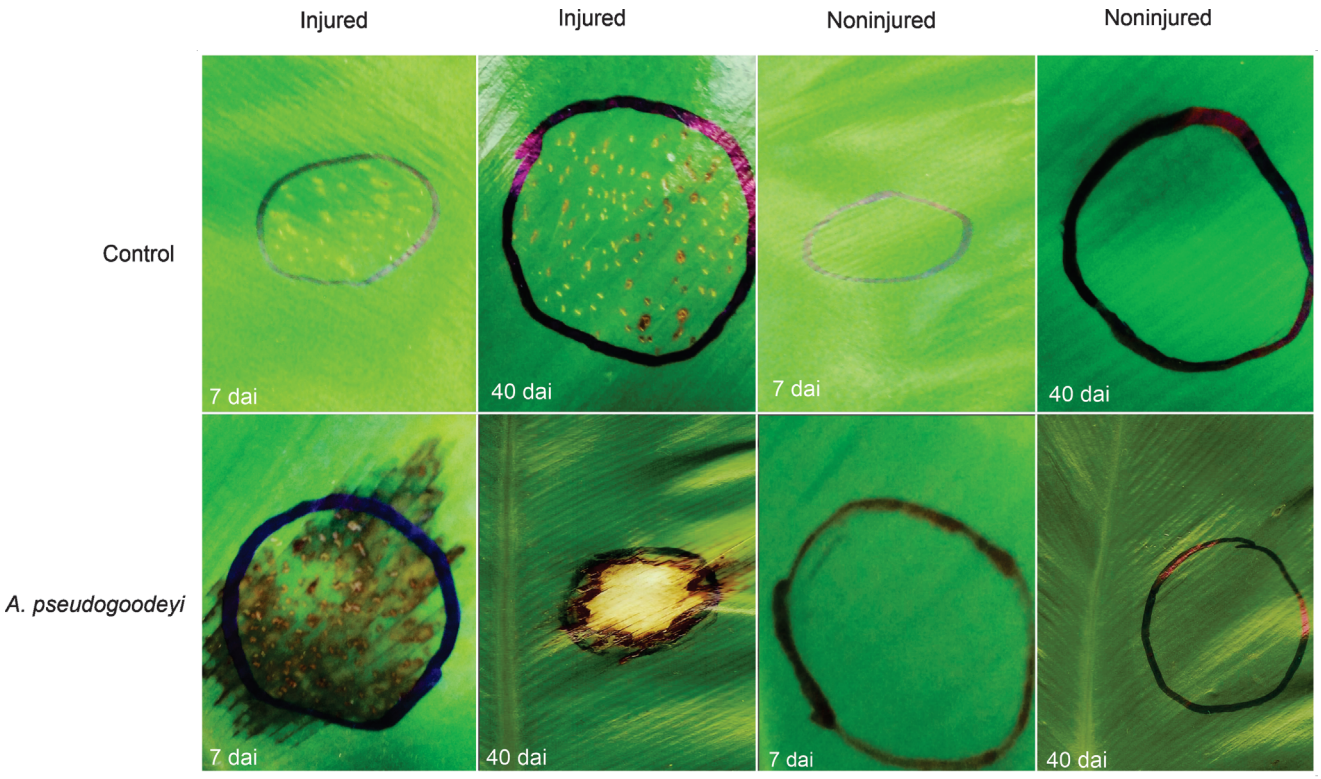

Fig. 1. Symptoms observed at 7 and 40 days after inoculation on Bird's Nest Fern leaves caused by *Aphelenchoides pseudogoodeyi*. The inoculation was performed in the adaxial surface of injured and uninjured leaves.

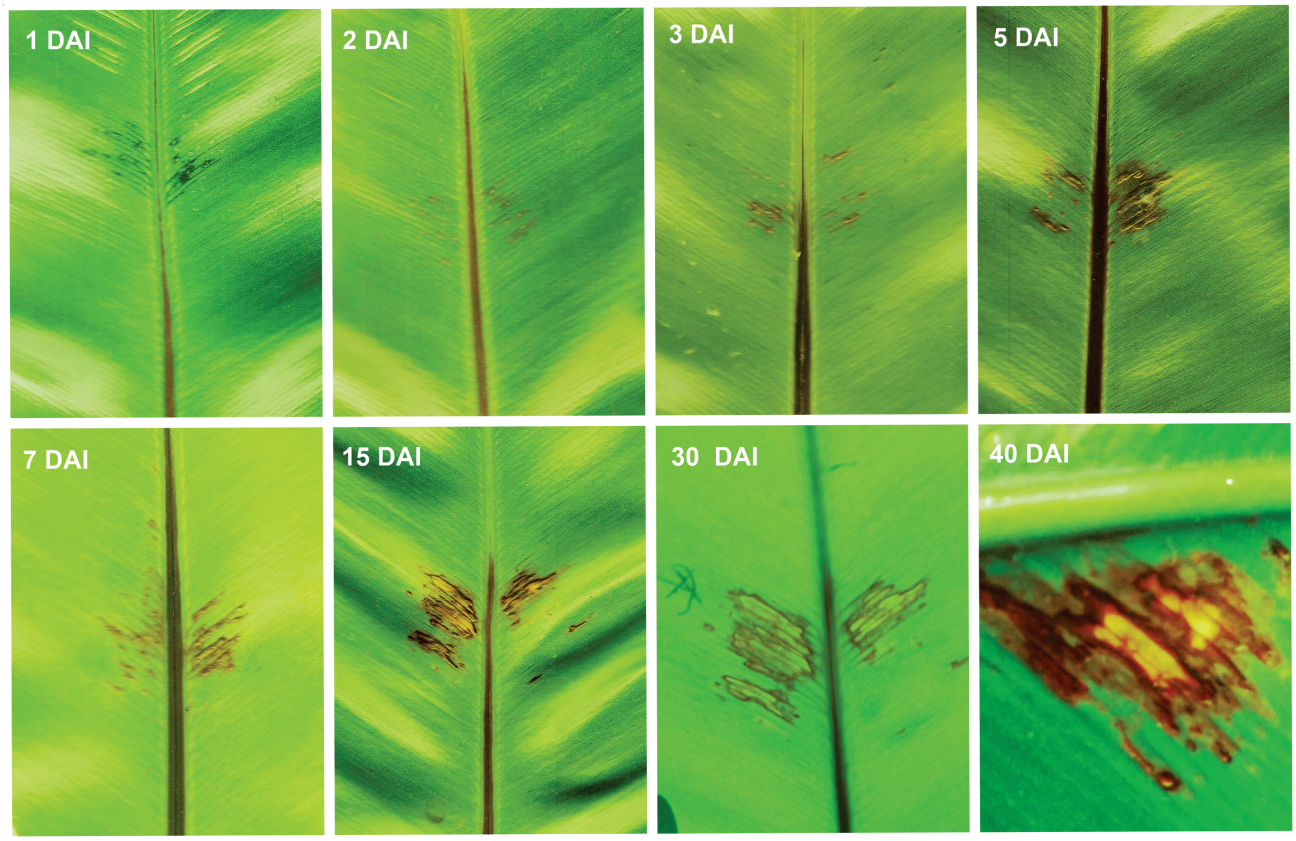

Fig. 2. Progress of symptoms on injured leaves of Bird's Nest Fern caused by *Aphelenchoides pseudogoodeyi* at 1, 2, 3, 5, 7, 15, 30, 40 days after inoculation. The inoculation was performed in the abaxial surface of leaves of Bird's Nest Fern.

Nematode culture and inoculum preparation

The *A. pseudogoodeyi* population used in this study was originally extracted from seeds of *Urochloa brizantha* cv. Marandu, according to Coolen and D`Herde (1972). This population was previously characterized as population DJ17 by de Jesus *et al*. (2016). In order to provide an axenic culture, *A. pseudogoodeyi* population was multiplied in Petri-dishes (9-cm-diam) with *Fusarium solani* colonies. Thus, nematodes extracted from seeds were hand-picked, surface-sterilized in antibiotic solution (200 ppm of ampicillin + 300 ppm of chloramphenicol) for 20 min and transferred to *F. solani* colonies, which were grown on potato dextrose agar medium for 20 days, at 25 °C ± 5 °C (Favoreto *et al*., 2011). Plates were incubated at 25 ºC, in the dark, for about 4 weeks. Next, nematodes were collected by rinsing the lid of Petri-dishes with distilled water into a beaker. Morphological identification was carried out, following the separation of viable nematodes, after 24 h of incubation to Baermann funnels. The resulting suspension was calibrated and used as inoculum in the pathogenicity tests and histopathological study.

Leaf inoculation methods

Two inoculation methods were used: (*i*) without and (*ii*) with leaf injury (Zhen & Agudelo, 2012). The latter consisted of leaves injured with a needle, with 20 punctures in the adaxial or abaxial surface, in the middle leaf region. Next, a piece of cotton was used to cover each inoculation site, where the nematode suspension with 2,000 nematodes (adults + juveniles) was carefully dispensed in each experimental unit. Then, the inoculated leaf site was immediately wrapped in a plastic tape. The plants were covered with transparent plastic bags after inoculation in order to maintain a moist environment. The bags and plastic tape wrapping were removed after 48 h in Oriental Lily and 7 days after inoculation (DAI) in Bird's-Nest Fern.

Pathogenicity tests

Three independent experiments were conducted to study the pathogenicity of *A. pseudogoodeyi* to Bird's-Nest Fern and Oriental Lily, including the histopathology of *A. pseudogoodeyi* on Bird's-Nest Fern leaves. In the first experiment with Bird's-Nest Fern, the treatments consisted of the nematodes inoculated on the adaxial surface: (*i*) of uninjured or (*ii*) mechanically injured leaves. Nematode inoculation was performed on two arbitrarily selected intact leaves of Bird's-Nest Fern. One of the two inoculated leaves from each plant was uninjured and the other was mechanically in-

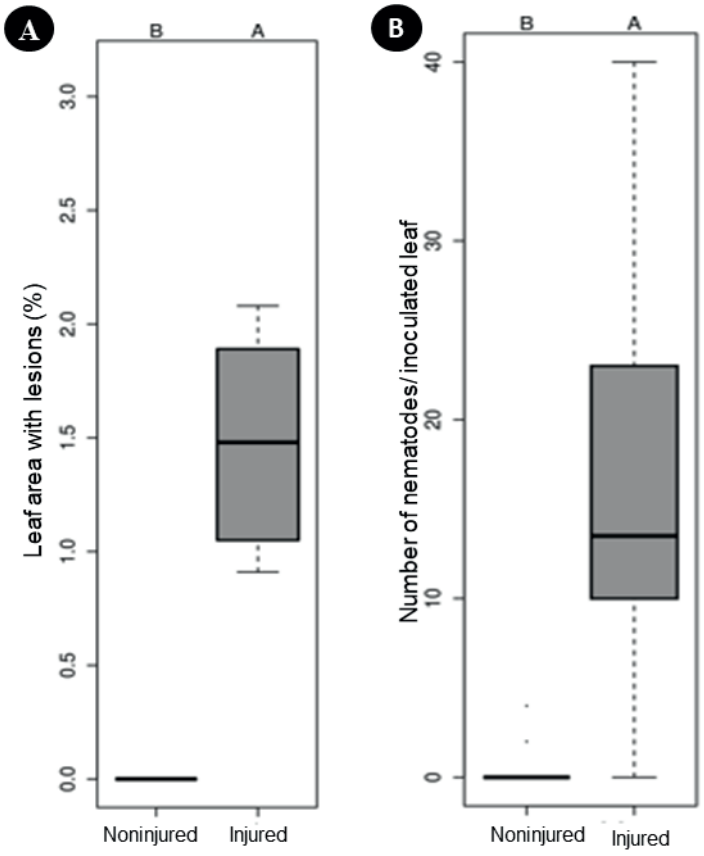

Fig. 3. Effect of injury on (A) symptom severity (expressed as percentage of leaf area with lesions), and (B) final population of *Aphelenchoides pseudogoodeyi* recovered from Bird's Nest Fern 40 days after inoculation. Different upper-case letters indicate that the medians differed significantly according to the Kruskal-Wallis test, at 5 % significance. The horizontal line inside the box represents the median, while the lower and upper horizontal lines of the box indicate the first quartile and third quartile, respectively.

jured on the upper side, as described above. Each treatment was replicated on ten Bird's-Nest Fern plants, and the experimental unit consisted of an inoculated leaf, which received 2,000 nematodes (adults + juveniles). Evaluation was performed 40 DAI, as described below.

Aiming to evaluate the pathogenicity of *A. pseudogoodeyi* to Oriental Lily cv. Entertainment, 54-day-old plants were inoculated, as previously described. However, in this case, the inoculation was performed on the abaxial surface of the leaves. Four leaves per plant were selected and inoculated through the injured or uninjured method. The treatments were arranged in a completely randomized design, following a 2 x 4 factorial scheme [two inoculation methods (with or without injuries) and four evaluation intervals (5, 10, 20 and 40 DAI)], with five replicates.

In all experiments, distilled water was included as the control and was mock inoculated in the same manner as other treatments (with and without injury). The experimental unit consisted of an inoculated leaf. The plants were kept in a growth chamber, at 25 °C, with photoperiod of 12 h.

Data collection

The progress of the symptoms was observed and recorded weekly. The inoculated leaves were collected and the severity of the symptoms was evaluated by calculating the percentage of affected leaf area, using the Quant program (Vale, 2001). Then, the inoculated leaves were sectionated into small pieces and ground with distilled water in a blender for 10 s. The resulting suspension was incubated for 24 h under continuous aeration, supplied by an aquarium pump (Oliveira & Wilcken, 2016). After incubation, it was poured through nested 200-mesh (75 µm) and 500-mesh (25 µm) sieves and the content of the 500-mesh sieve was transferred to a beaker. The nematodes extracted were counted in a Peters chamber under the light microscope (Southey, 1970). The final population of nematodes (*Pf*) in each treatment was used to calculate the reproduction factor (RF) (Oostenbrink, 1966).

Histopathology of A. nidus leaves inoculated with A. pseudogoodeyi

For the histopathology study, a second experiment was set up with Bird's-Nest Fern, in the same manner as described above.

Table 1. Reproduction factor (RF) - Total number of nematodes days after inoculation divided by the initial number of nematode inoculated (2,000). RF ≤1 resistance reaction and RF>1 susceptibility reaction, according to Oostenbrink (1966).

Host plant (Scientific name)	Treatment	Days after inoculation (DAI)	RF (mean ± SD)	Classification
Asplenium nidus	Injured leaf	40	0.008 ± 0.005	Resistant
Lilium speciosum	Injured leaf	5	0.016 ± 0.006	Resistant
L. speciosum	Injured leaf	10	0.018 ± 0.007	Resistant
L. speciosum	Injured leaf	20	0.008 ± 0.011	Resistant
L. speciosum	Injured leaf	40	0.000 ± 0.000	Resistant

However, the inoculation was performed on the abaxial surface of the leaves and the bags and plastic tape wrapping were removed after 48 h. The treatments were arranged in a completely randomized design, following a 2 x 3 factorial scheme [two inoculation methods and four evaluation intervals (1, 3, 5 and 7 DAI)], with five replicates.

In each evaluation interval, inoculated leaves were collected, fragments from the inoculated leaf regions were fixed in 50 % FAA (formaldehyde-acetic acid-ethanol) for 48 h (Johansen, 1940) and dehydrated in an ethanol series (70 %, 85 % and 95 %) (Kraus & Arduin, 1997). Then, those fragments were embedded in glycol methacrylate and submitted to microtomy to obtain cross sections in an autotuning rotary microtome (model RM2155, Leica Microsystems Inc., Deerfield, USA). 6 µm thick sections were placed on glass slides, stained with 0.05 % toluidine blue in 0.1 M phosphate buffer pH 6.8 (O'Brien *et al*., 1964) for anatomical characterization. Additionally, histological sections from the areas inoculated with each treatment were stained with Lugol's reagent for detection of starch in the chloroplasts (Johansen, 1940). All the images were captured by a digital camera (AxionCamHRcZeiss) coupled to a photomicroscope (model AX-70 TRF, Olympus Optical, Tokyo, Japan).

Statistical analyses

The significance of differences in the percentage of the injured leaf area and the final population of nematodes among the treatments was tested using the nonparametric Kruskal–Wallis test ($P < 0.05$) for multiple samples performed using R software (R Core Team,, 2017).

Ethical Approval and/or Informed Consent

The conducted research is neither related to human nor animals use.

Results

Pathogenicity of A. pseudogoodeyi to Bird's-Nest Fern

Symptoms were observed only on injured leaves inoculated with *A. pseudogoodeyi* (Fig. 1). There was no difference between the control treatments and the uninjured leaves inoculated with *A. pseudogoodeyi* (Fig. 1). At the first day after inoculation (DAI), small necrotic lesions were observed in the injured leaves inoculated with *A. pseudogoodeyi* (Fig. 2). Water soaked lesions were observed as early as 7 DAI and progressed from yellow to brown in the course of time. The lesions gradually increased over time, but were limited to the inoculation region (Figs. 1 and 2) corresponding to 1.5 ± 0.42 % (mean ± SD) of the total leaf area, evaluated 40 DAI. At 40 DAI, it was observed that the leaf mesophyll was desiccated, forming a bleached central region with necrotic borders (Figs. 1 and 2).

There were differences in the final population of nematodes (Pf) between the inoculation methods, with and without injury (Fig. 3B). At 40 DAI it was observed a drastic reduction in the total numbers of nematodes in treatments inoculated on both injured (16.3 ± 11.6 nematodes) and uninjured leaves (0.6 ± 0.44 nematodes). At 40 DAI, the mean RF of *A. pseudogoodeyi* for the injured and uninjured leaves were 0.008 ± 0.005 (Table 1) and 0.000 ± 0.000, respectively. In the control treatments, no *A. pseudogoodeyi* was found and the injured leaf grew as well as the uninjured leaf.

Pathogenicity of A. pseudogoodeyi to Oriental Lily

Similar to that observed in Bird's-Nest Fern, *A. pseudogoodeyi* induced symptoms only when it was inoculated in injured leaves of Oriental Lily (Fig. 4). The symptoms were soaked chlorotic patches, which became brown and remained in the region of the injury. The central lesion area became progressively desiccated with necrotic borders. The largest lesioned leaf area was observed at 5 DAI, and decreased gradually at 10, 20 and 40 DAI in injured leaves (Figs. 4 and 5A). The symptoms at 5 DAI were characterized by the presence of a large brownish necrotic region with a drenching aspect, corresponding to the injured region (Fig. 4). At 10 and 20 DAI, necrotic regions with dry appearance were observed (Fig. 4). At 40 DAI, the lesions appeared as dry necrotic regions, circumscribed by brownish necrotic borders (Fig. 4). No symptoms were observed in the control treatments and on the uninjured leaves inoculated with *A. pseudogoodeyi* (Fig. 4).

There was no significant difference in the final population of nematodes (Pf) between Oriental Lily inoculated leaves, with and without injury, at the intervals of evaluation times (Figs. 5B-C).

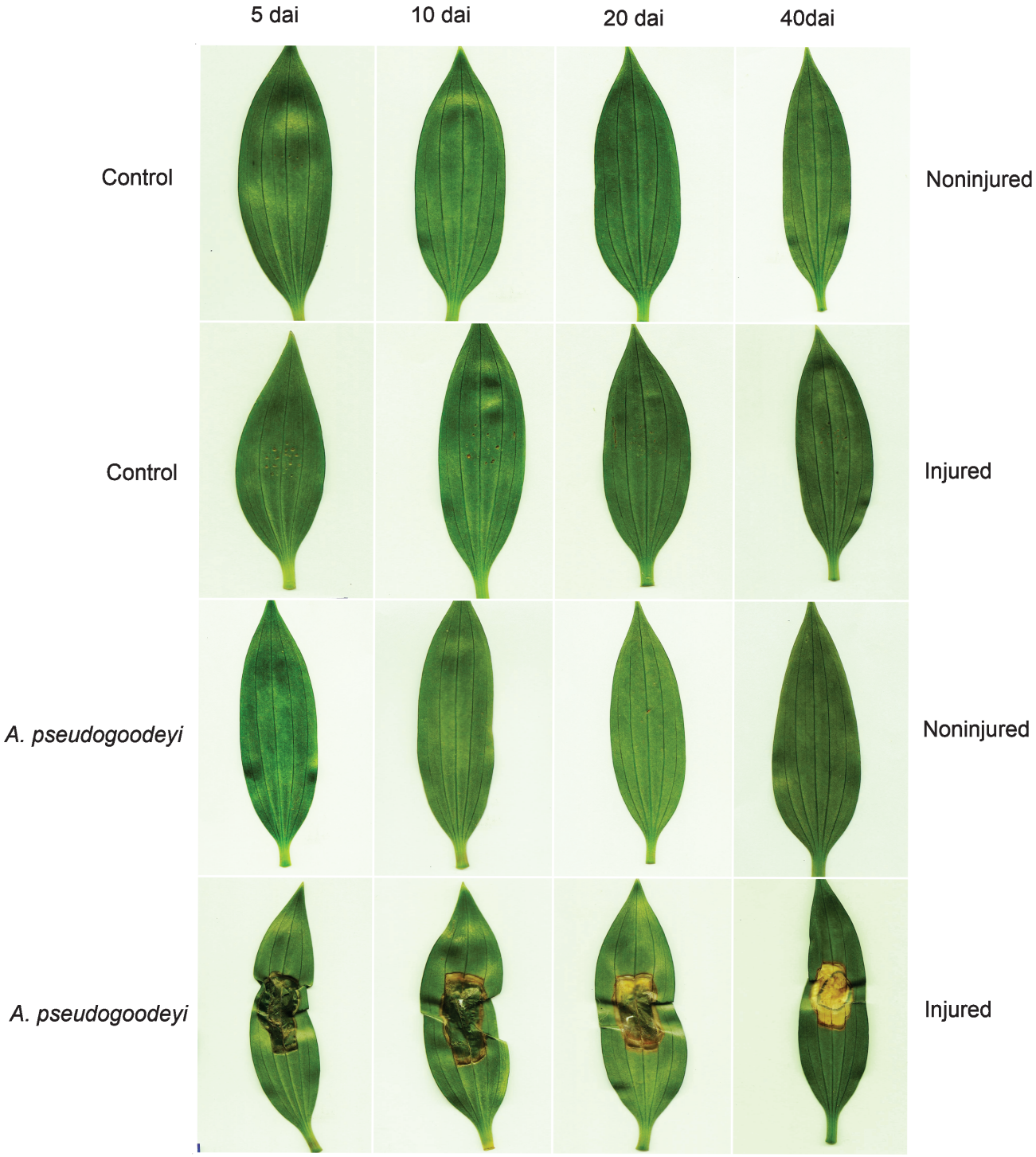

Fig. 4. Symptoms observed at 5, 10, 20 and 40 days after inoculation on Oriental Lily leaves caused by *Aphelenchoides pseudogoodeyi*. The inoculation was performed on the abaxial surface of injured and uninjured leaves.

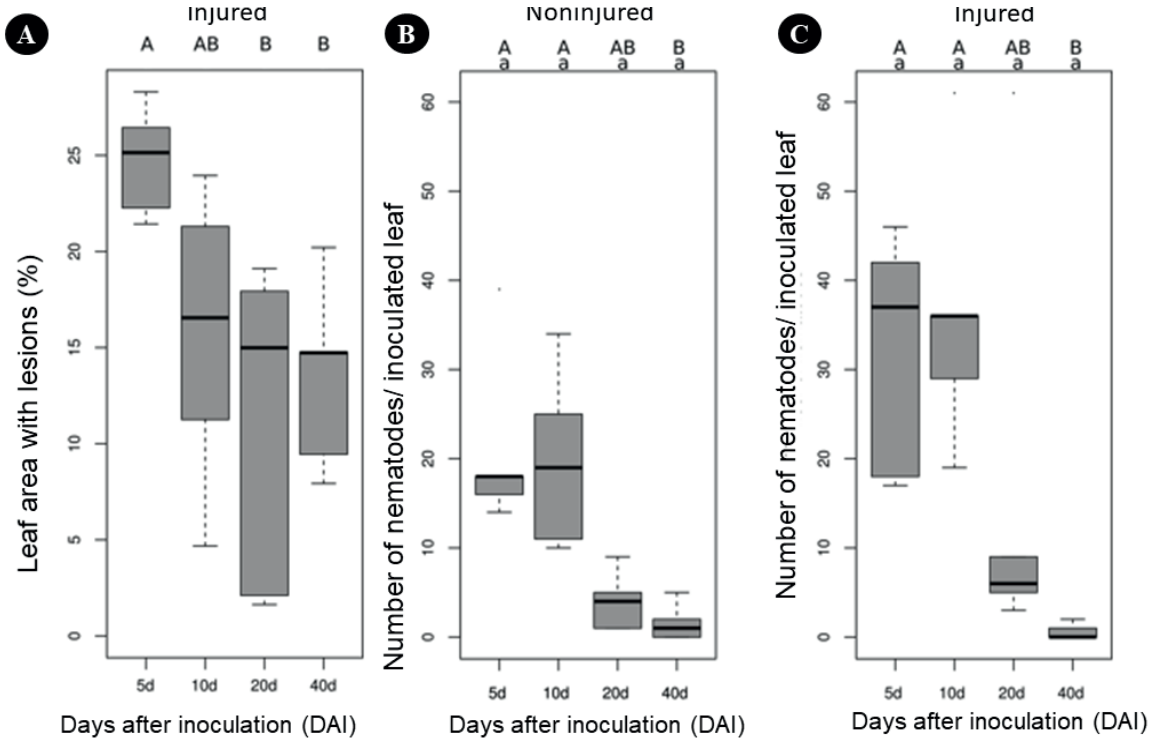

Fig. 5. (A) Symptom severity (expressed as percentage of leaf area with lesions); (B) the final population of *Aphelenchoides pseudogoodeyi* after extraction from uninjured leaves and (C) final population of *A. pseudogoodeyi* after extraction on injured leaves of Oriental Lily inoculated on abaxial surface at 5, 10, 20 and 40 days after inoculation. Different upper-case letters indicate statistical difference between inoculation times according to the Kruskal-Wallis test with 5 % significance (A, B, C). Lower case letters indicate statistical difference between the inoculation methods, with and without injury, according to the Kruskal-Wallis test, with 5 % significance (B, C). The horizontal line inside the box represents the median, while the lower and upper horizontal lines of the box indicate the first quartile and third quartile, respectively.

Also, nematode population gradually decreased over time and the lowest mean of nematodes was recovered at 40 DAI (Figs. 5B-C). However, severity difference was observed between inoculated leaves with and without injuries, at the times evaluated (Fig. 4), considering that symptoms were observed only when *A. pseudogoodeyi* was inoculated on injured leaves (Fig. 5A). The severity of symptoms caused by *A. pseudogoodeyi* on injured leaves of Oriental Lily by mean percentage of affected leaf 5, 10, 20 and 40 DAI, were 24.716 ± 2.560, 15.548 ± 6.945, 11.154 ± 7.699 and 13.416 ± 4.370 (mean ± SD), respectively.

The RF of *A. pseudogoodeyi* was lower than 1 in all evaluation times. At the inoculum concentration used, the RF of *A. pseudogoodeyi* decreased significantly as inoculation days increased. At 5, 10, 20 and 40 DAI, the RF means on injured Lily leaves were 0.016 ± 0.006; 0.018 ± 0.007; 0.008 ± 0.011 and 0.000 ± 0.000, respectively (Table 1). On uninjured leaves, the mean reproduction factor rates at 5, 10, 20 and 40 DAI were 0.010 ± 0.004, 0.009 ± 0.004, 0.002 ± 0.001 and 0.000 ± 0.000, respectively.

Histopathology of A. pseudogoodeyi on A. nidus leaves

There were anatomical alterations in the transversal sections of injured leaves inoculated with *A. pseudogoodeyi,* which differed from the other treatments, as shown in Figure 6. Leaf sections of the uninjured control (Fig. 6A) and those inoculated with *A. pseudogoodeyi* without injury (data not shown) did not present anatomical alterations when compared to each other. Those presented an undifferentiated mesophyll and stoma distributed on the abaxial surface of the epidermis. In the control injured sections, the only anatomical alterations noted were caused by mechanical injury (Fig. 6B). However, those inoculated with *A. pseudogoodeyi* on injured leaves showed a series of cellular disorders, mainly in the mesophyll region (Figs. 6C-G). At 1 DAI, it was possible to observe a large space near the vascular bundle caused by cell rupture (Fig. 6C) and the presence of nematodes into the mesophyll region (Figs. 6D-D1), which was also noted at 3 DAI (Fig. 6E). At 3 DAI, the cells of both the epidermis and the mesophyll regions presented signs of cell death and atrophied appearance, culminating with an apparently reduced leaf blade thickness (Fig. 6F). At 5 DAI, these symptoms were intensified and it was possible to observe a total destruction of the mesophyll and collapse of the cells, with the formation of large intercellular spaces (Fig. 6G), which was also found at 7 DAI (data not shown). There was an increased level of starch in the chloroplasts in all treatments using the injured leaf inoculation method (Fig. 6H1 vs. Figs. 6H2-K). However, when *A. pseudogoodeyi* was present, this starch increase was more pronounced, especially at 3 and 5 DAI (Figs. 6J-K).

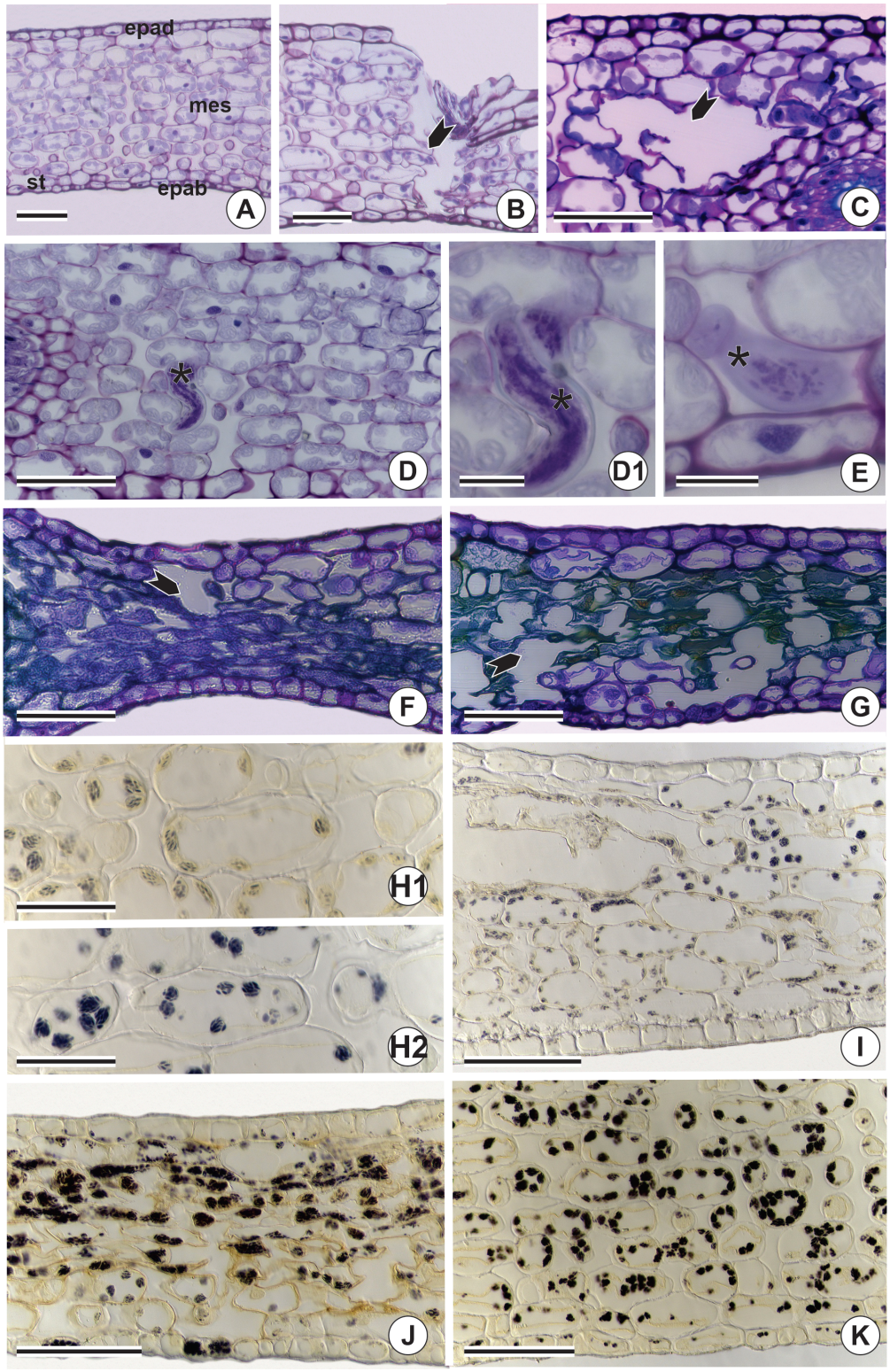

Fig. 6. Anatomical alterations on Bird's-Nest Fern leaves inoculated with *Aphelenchoides pseudogoodeyi*. The transversal sections were stained with toluidine blue (A-G) or Lugol's reagent (H1-K). Sections of control uninjured (A; H1) and injured (B; H2) leaves, at 5 DAI. Sections of injured leaves inoculated with *A. pseudogoodeyi* at: 1 DAI (C-D1; I); 3 DAI (E-F; J) and 5 DAI (G; K). Epad= epidermis of adaxial surface; epab= epidermis of abaxial surface; st= stomata; mes= mesophyll; asterisk= nematode; arrow= formation of intercellular spaces. Bars: A-D, F and G: 100 µm; H1-K: 50 µm and E: 25 µm.

Discussion

Pathogenicity refers to the ability of a microorganism to cause disease or damage to a plant (Agrios, 1988; Trudgill, 1991) and it can be defined as meeting Koch's postulates (Shapiro-Ilan *et al*., 2005). Therefore, the pathogenicity of a given species of nematode will be determined based on its ability to cause symptoms or damage to a potential host. In addition, a resistant plant is a host plant which is able to prevent or delay the development or multiplication of the nematode in its tissues (Trudgill, 1991; Roberts, 2002). Such plant attribute is often measured by the nematode reproduction factor (Oostenbrink, 1966). Tolerance, which is measured as the amount of injury caused by nematodes on a host plant, relates to the ability of plant to withstand or recover from the injury caused by the nematode and yield well (Trudgill, 1991).

Among the *Aphelenchoides* species, only *A. arachidis, A. besseyi, A. bicaudatus, A. blastophthorus, A. dalianensis, A. ensete, A. fragariae, A. nechaleos, A. paranechaleos, A. ritzemabosi, A. saprophilus, A. sphaerocephalus* and *A. subtenuis* have shown to be pathogenic to plants (Sanchez-Monge *et al*., 2015). Other species of the genus, even when associated with plants, have been assumed to be strictly mycophagous, and few studies have actually evaluated their pathogenic potential (Zhuo *et al*., 2010; Sanchez-Monge *et al*., 2015). In the present study, *A. pseudogoodeyi* was pathogenic as it was able to cause symptoms on Bird's-Nest Fern and Oriental Lily leaves. However, the presence of injuries was essential for *A. pseudogoodeyi* to be able to cause those symptoms in both plant species. This indicates that *A. pseudogoodeyi* is not able to penetrate actively into the foliar tissue nor through the stomata of these plants and should be considered as an opportunistic pathogen, mycetophagous and a facultative plant-parasite under stressful conditions.

Foliar nematodes, such as *A. fragariae*, can enter leaf tissue through stomata or wounds (Jagdale & Grewal, 2006) and feed on mesophyll cells (Wallace, 1959), causing characteristic lesions that begin as slightly chlorotic and then become necrotic (Zhen & Agudelo, 2012). The symptoms induced by *A. pseudogoodeyi* on the injured leaves of the Bird's-Nest Fern and Oriental Lily were characterized by brownish, dry lesions and necrotic borders restricted to the injured region, remarkably, with the collapse of the foliar mesophyll. The mechanical wound on the leaves allowed *A. pseudogoodeyi* to enter and feed in the foliar tissues, and consequently led to the formation of necrotic lesions. Similar results were observed by Zhao *et al*. (2013) when investigating the viability of *Arabidopsis thaliana* as a potential host of *Bursaphelenchus xylophilus* and *B. mucronatus*. The authors reported that the artificial wound in the plant had a great impact on the infection by the nematodes, especially when the wound was carried out on the petiole of the first leaf of *A. thaliana*. Zhen and Agudelo (2012) also reported that the previous injury in leaves of cultivars of *Hosta* spp. favoured the infection by *A. fragariae*, with greater severity and reproduction factor, compared to that observed in inoculated plants without injury.

In contrast, Oliveira *et al*. (2019) reported that Florida population of *A. pseudogoodeyi* was not able to induce symptoms in alfalfa, Gerbera daisy or strawberry. However, these authors did not induce injuries prior to nematode inoculation, as performed in the present study. Conversely, when *A. pseudogoodeyi* was locally inoculated into uninjured soybean leaves by Oliveira *et al*. (2019), the nematode penetrated and migrated into the mesophyll, inducing symptoms similar to those reported in our study. Nevertheless, the population levels observed in soybean were very low. This indicates that this nematode can behave as a facultative plant-parasite under stressful conditions (Oliveira *et al*., 2019). However, it is necessary to verify if such behavioural variation occurs under field conditions, as *A. pseudogoodeyi* is frequently reported in association with crops in mixed populations with the facultative plant-parasitic nematode *A. besseyi* (de Jesus *et al*., 2016; Oliveira *et al*., 2019).

Recent research has shown that many plant-parasitic nematodes produce degrading enzymes of plant cell wall components, which act at different stages of the pathogenesis (Jaubert *et al*., 2002; Bohlman & Sobczak, 2014; Rai *et al*., 2015; Danchin *et al*., 2017). Therefore, considering the symptoms on leaves, *A. pseudogoodeyi* is likely to produce some type of plant cell wall degrading enzyme. However, further studies are necessary to clarify the production of cell wall degrading enzymes by *A. pseudogoodeyi*.

Aphelenchoides pseudogoodeyi was unable to establish and reproduce on Bird's-Nest Fern and Oriental Lily (RF < 1), which characterizes these species as poor-host or resistant plants (Oostenbrink, 1966). In addition, nematode eggs were not found in the suspensions obtained from the leaves with *A. pseudogoodeyi* lesions. Thus, *A. pseudogoodeyi* was not able to reproduce in any of the ornamental plants, despite having induced localized symptoms, as reported by Oliveira *et al*. (2019). Zhao *et al*. (2013) demonstrated that *B. xylophilus* was able to infect *A. thaliana* plants, but did not reproduce in the tissues. Similarly, *Heterodera glycines* was able to invade and induce the formation of syncytium in *A. thaliana* roots, but was not able to complete its life cycle or reproduce (Grundler *et al*., 1997). Such reactions in the nematode-plant interaction are typical of resistant plants (Trudgill, 1991; Starr *et al*., 2013).

Our study provides evidence that *A. pseudogoodeyi*, often found in association with cultivated plants, was able to induce localized necrotic lesions in the presence of injuries in Bird's-Nest Fern and Oriental Lily. Despite penetrating into the tissue, the nematode was unable to either establish a compatible relationship or reproduce in the tissues of such plants. The penetration of *A. pseudogoodeyi* into the leaf mesophyll may have led to recognition that triggered the plant immune responses, which resulted in a hypersensitivity reaction (HR)-cell death. However, this hypothesis needs to be tested. HR is a type of programmed cell death induced by avirulent pathogens that plays a central role in plant immunity to plant-parasitic nematodes (Sato *et al*., 2019). Immune responses

to plant-parasitic nematodes have been characterized for sedentary endoparasite nematodes. For example, in *Meloidogyne* spp., HR is considered the most common post-penetration resistance mechanism in incompatible interactions (Anwar & McKenry, 2000), but little is known about how these responses are induced by foliar nematodes in resistant plants. Concomitantly, the extensive damage caused by *A. pseudogoodeyi*, especially in Oriental Lily (Figs. 4; 5A), may have been the result of the intolerance of this plant to the nematode attack. Similar reaction is reported in *Coffea arabica* cv. Mundo Novo, a poor-host for *Pratylenchus brachyurus* (RF <1), but this plant is strongly intolerant to attack of this nematode, which leads to extensive destruction of the root system of plants (Oliveira *et al*., 1999). Resistance and tolerance are independent attributes of the plant but in some cases the resistance responses can decrease the plant's tolerance to nematode attack (Trudgill, 1991), as exemplified above.

In conclusion, our research demonstrated that *A. pseudogoodeyi* did not infect Bird's-Nest Fern and Oriental Lily plants without the aid of injury. Conversely, injury was necessary to show that *A. pseudogoodeyi* is an opportunistic pathogen and causes symptoms on both ornamental plants. However, these species are poor-host plants for *A. pseudogoodeyi*. Complementary studies are needed to determine if the progression of symptoms is related to the resistance and intolerance of the plants studied and clarify the nature of this interaction. Furthermore, other ornamental plants or cultivars of the species already tested should be evaluated in the future.

Conflict of Interest

The authors declare no conflict of interest.

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