Aphelenchoides hylurgi as a Carrier of White, Hypovirulent *Cryphonectria parasitica* and its Possible Role in Hypovirulence Spread on Blight-Controlled American Chestnut Trees

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Abstract: Individual nematodes were isolated from American chestnut blight-controlled cankers to determine if they were carriers of biocontrol (hypovirulent) isolates of the chestnut blight fungus, *Cryphonectria parasitica*. These hypovirulent isolates have a white fungal colony phenotype due to infection by the virus CHV1. Of 1,620 individual *Aphelenchoides hylurgi* isolated, 29.4% carried propagules of the blight fungus and 8.2% of these yielded white hypovirulent isolates. In attraction and movement tests in Petri plates, *A. hylurgi* moved 2 cm over 24 hr to mycelial discs of white hypovirulent *C. parasitica* and pigmented *C. parasitica* strains in nearly equal numbers. After 2 days of nematode movement to fungal colonies on agar in Petri plates and 21 days of nematode growth, large numbers of *A. hylurgi* were extracted from both white hypovirulent and pigmented *C. parasitica* strain colonies. Lower numbers of *A. hylurgi* were extracted from excised young American chestnut blight cankers that were inoculated with *A. hylurgi* and incubated for 22 days. *A. hylurgi* inoculated on the surface of an excised American chestnut canker moved within 24 hr to the small, spore-bearing *C. parasitica* reproductive structures (stromata) on the canker surface. The results indicate that *A. hylurgi* may play a role in the spread of hypovirulence on American chestnut trees.
Key words: biocontrol, chestnut blight, fungivorus nematodes, hypovirulence spread, white hypovirulent strains.

Chestnut blight, caused by the fungus *Cryphonectria parasitica* (Murr.) Barr, devastated the American chestnut tree (*Castanea dentata* (Marsh.) Borkh.) in the eastern United States during the first part of the 20th century, killing an estimated 3.5 billion timber American chestnut trees (Roane et al., 1986). Among several American chestnut restoration approaches, progress has been made in controlling this disease through interbreeding trees having partial resistance from the few large American chestnut trees that survived the blight (Griffin, 2000). Integration of this approach with biocontrol fungal inoculation of blight cankers, using hypovirulent or low-virulence strains of the blight fungus, has resulted in a high level of blight control in Virginia for 27 years (Hogan and Griffin, 2008). The hypovirulent strains found effective on the inoculated trees are infected with a virus, *Chryphonectria hypovirus 1* (CHV1) that originated in hypovirulent strains from Italy (Elliston, 1985; Griffin et al., 2004). This hypovirus reduces the virulence of the blight fungus and can be transmitted among blight fungus strains. It also changes the fungal colony phenotype *in vitro* from yellow-orange pigmented to white (Anagnostakis and Day, 1979; Elliston, 1985). Research has shown that Italian hypovirulence has spread throughout the American chestnut trees since hypovirulent strain inoculation in 1982-83 (Robbins and Griffin, 1999; Hogan and Griffin, 2002), but the means by which hypovirulence has spread has not been elucidated.

The host-finding and feeding of fungivorous *Aphelenchus* and *Aphelenchoides* may have ecological effects on the fungus (Bae and Knudsen, 2001; Bakhtiar et al., 2001; Hasna et al., 2007). Fungivorus nematodes may acquire fungal propagules (spores, mycelial fragments, etc.) during feeding and carry these propagules to new locations as the nematodes seek out new areas of the fungus mycelium on which to feed in nearby locations.

Surveys in Virginia and neighboring states have indicated that tree stem tissues commonly yield nematodes upon extraction and these nematodes can be grown *in vitro* on fungi in Petri plates (J. Eisenback, unpublished). These observations suggest the possibility that blight cankers on American chestnut tree stems, having a large fungal mycelium, may be colonized by fungivorus nematodes. These nematodes may acquire propagules of the blight fungus as they feed and the nematodes may be attracted to and move to new locations having fungal food sources. This investigation was undertaken to determine if nematodes are present in blight-controlled cankers on American chestnut trees, if any identified nematodes found are carriers of white hypovirulent isolates of the blight fungus, and if they are attracted to, move towards, and grow on the mycelium of white hypovirulent and normal-pigmented isolates of the blight fungus.

MATERIALS AND METHODS

Trees studied: The American chestnut trees studied were located in the Lesesne State Forest, Virginia, USA. The trees were grafted in 1980 using blight-susceptible American chestnut rootstocks and scions from large, surviving American chestnuts with partial blight resistance. Blight cankers on the trees were inoculated in 1982 and 1983 with a mixture of hypovirulent *C. parasitica* strains of American and European origin, including white hypovirulent Italian strains Ep 47, Ep 49, and Ep 51. Thereafter, the trees exhibited a very high level of blight control (Robbins and Griffin, 1999). Blight cankers on the trees were highly superficial, with a layer of healthy phloem tissue between the infected

bark tissues and the vascular cambium. Low densities of stromata reproductive structures of the blight fungus were produced on the outer bark surface of the superficial cankers. The blight-controlled cankers throughout the trees were infected with both white hypovirulent and the normal yellow-orange pigmented virulent isolates of the blight fungus, although the pigmented isolates predominated (Hogan and Griffin, 2002). Stromata reproductive structures produced on the canker surfaces also were mainly associated with pigmented isolates although some (5.2 %) were associated with white hypovirulent isolates (Hogan and Griffin, 2008). Samples of this canker-surface bark were collected for use in the present study.

Fungi: The white Italian hypovirulent strains, Ep 49#5 and EP51W, were subcultures of the Italian white hypovirulent strains used to inoculate blight cankers on the trees in 1982 and 1983 and had a 99.1-99.7% CHV1 nucleotide sequence identity to CHV1 in the white hypovirulent isolates recovered from blight cankers throughout the trees 12 to 18 years later (Griffin et al., 2004). The normal pigmented strain used in the experiments here, THBP#9, was isolated from canker tissues from the test trees at the start of this study. All fungal strains were maintained on acidified potato-dextrose agar (APDA) Petri plates (Hogan and Griffin, 2002).

Nematodes: Nematodes were extracted from the canker-bark tissues on the test trees by the Baermann funnel technique and by the canker-bark-plate method (see below). Representative individual nematodes extracted from cankers were identified as *Aphenlenchoides hylurgi* Massey, 1974 from morphological characteristics and measurements of 30 females. Nematode cultures for inoculation were made from aliquots of nematode suspensions pipetted onto 1-2 week-old APDA cultures of *C. parasitica*. After approximately 3-4 weeks of nematode growth on the fungal colonies at room temperature, the fungal cultures were cut into small pieces and the nematodes were extracted by the Baerman funnel technique. Nematode densities in the collection vials were determined using a counting chamber and microscope; water dilutions were made to obtain the desired nematode density for inoculation and the final nematode density was confirmed by one or more counts before use.

Canker-bark-plate method for recovery of individual nematodes: Preliminary tests indicated that individual nematodes could best be isolated, with minimum bark and nematode disturbance, by placing about 3 g of canker bark tissue in the bottom of a flat-bottom, glass Petri dish (9 cm diameter) and gently adding a small amount of water to cover the dish bottom. Individual nematodes that moved into the water around the canker bark after 24 hr were gently recovered using a fine needle under a dissecting microscope. The nematode was placed on an APDA plate and incubated at 27°C for 3-4 days until fungal colonies developed. Fungal colonies were subcultured to another APDA plate for further evaluation of *C. parasitica* colony phenotype. Canker-bark tissues were obtained from three American chestnut trees exhibiting a high level of blight control. The weight and surface area of the canker bark samples were estimated.

Nematode attraction, movement, and growth tests: Water-agar (1%) Petri plates (9 cm diameter) were used for nematode attraction and movement tests. Nematode inoculum of a desired density was prepared as indicated above so that 0.1 ml of water contained 285 nematodes. In the first trials with one target fungus, a droplet of inoculum was placed on the agar surface on one side of a Petri dish and a 1-cm diameter agar disk of pigmented *C. parasitica* THBP#9 was positioned 2 cm from the droplet on the other side. The disk was obtained from a 1-2 week-old colony growing on an APDA plate. In the second set of trials with two target fungi, one pigmented THBP#9 and one white hypovirulent Ep49#5, the procedure was the same except that the two discs were 2 cm away from the inoculum droplet and 2 cm away from each other. For both types of trials, a nematode movement period of 24 hr was used, after which counts of the nematodes on each disc were made. Five repeats of the experiments were conducted for both types of trials at room temperature (27°C) with five replications in each experiment. Mycelial discs were placed in a Baermann funnel and nematodes were extracted and quantified with a counting chamber and an inverted brightfield light microscope.

For nematode attraction, movement, and growth tests combined, a 9-cm Petri plate with three equal sections (Y-Plate, VWR, West Chester, PA) was used. One section was filled with water agar (1%) and the other two sections were filled with APDA. The test fungal strains were grown in the two APDA sections, with both sections containing either pigmented or white hypovirulent strains, until the growth covered the sections. Then, a 0.1 ml droplet of nematode inoculum was placed 2 cm from the edges of both APDA sections. After 2 days of nematode attraction and movement, each of the fungal colonies was removed from the Y-Plates and placed in separate, full-size (9-cm diameter) APDA Petri plates for nematode population growth over 21 days at room temperature (27°C). After this time, the fungal colonies were cut into pieces, nematodes extracted by the Baerman funnel technique, and nematode counts made as indicated above. Strains of *C. parasitica* used in these tests were white hypovirulent Ep49#5, Ep51W, and pigmented THBP#9 with three replications per fungal strain.

Tests on nematode attraction and movement on a chestnut blight canker surface were made with an excised blight canker from a young (~10-yr old) American chestnut tree stem growing in Blacksburg, VA. The canker was removed from the tree stem with a saw and placed in a high-humidity moist chamber in the laboratory. Both ends of the stem section were

TABLE 1.   Recovery of *Aphelenchoides hylurgi* from blight-controlled American chestnut canker tissues and recovery of pigmented and white hypovirulent phenotype isolates of *Cryphonectria parasitica* from *A. hylurgi* in canker tissues.

	Number	%
Nematodes recovered from canker tissues	1,620[a]	-
C. parasitica isolates recovered from nematodes	476	29.4 [b]
Pigmented *C. parasitica* isolates recovered from nematodes	437	26.9 [b]
White hypovirulent phenotype isolates recovered from nematodes	39	2.4 [b]
White hypovirulent phenotype isolates as a percent of total *C. parasitica* isolates	-	8.2

[a] Individual nematodes recovered from water in 120 blight-canker-bark plates with a fine needle and plated on acidified potato-dextrose agar Petri plates.
[b] Percent of 1,620 nematodes yielding indicated fungal phenotype.

covered with parafilm to retard water loss. At this time, a 1-cm diameter water agar disc containing a 0.1 ml nematode inoculum droplet was placed on the canker surface. Inoculum was prepared as indicated above. After a 24 hr. incubation at room temperature, 24 stromata on the canker surface in each of four quadrants (each 1.0 x 0.5 cm) surrounding the inoculum disc were excised from the canker with the aid of a dissecting microscope. Each stroma was placed in a separate well of a 96-well plate and a small amount of water was added to allow nematode movement out of the mycelium. Nematodes were clearly visible in the water 24 hr later. For reference, 198 stromata from two cankers not having any nematode inoculum were evaluated in the same manner. Baermann funnel extraction and nematode counting, as indicated above, were used to determine the number of nematodes remaining on the inoculum disc.

Nematode growth tests were similarly conducted on excised blight cankers obtained as indicated above. Following placement of the inoculum discs on the surface of two cankers, the cankers were incubated in a moist chamber for 22 days at room temperature (27°C) to allow nematode population growth. Two reference cankers not receiving any nematode inoculum

were placed in a separate moist camber for 22 days. Canker area was determined for each American chestnut stem. For extraction of the cankers, the entire canker was removed from the American chestnut stem with a large knife and the canker tissues cut into small pieces before Baermann funnel extraction and nematode counting as indicated above.

RESULTS

Isolation of A. hylurgi *from blight-controlled canker tissues and recovery of* C. parasitica *from the nematode:* Individual nematodes were readily isolated from the canker bark tissues using the canker-bark-plate technique and were then transferred to APDA Petri plates. Upon Petri plate incubation, many of these nematodes yielded colonies of *C. parasitica* (Table 1). Of 1,620 individual nematodes isolated, 476 yielded *C. parastica* colonies and the majority of these were pigmented colonies. Significantly, 8.2% of the *C. parasitica* colonies had the white hypovirulent phenotype. All canker-bark samples collected in the field yielded nematodes, although the range per sample plate and per gram of canker bark were highly variable (Table 2). Overall, a mean of 5 nematodes/g canker-bark tissue were recovered. The estimate of the number of nematodes recovered per unit canker-bark surface area was 2 nematodes/cm^2. Similar yields of nematodes per grams of canker bark were made from these canker bark samples by extraction with the Baermann funnel technique (data not shown).

Attraction and movement of A. hylurgi *to mycelium of pigmented and white, hypovirulent strains of* C. parasitica: Over 24 hr, almost one-third of the inoculated nematodes moved a distance of 2 cm to the mycelial disc of pigmented strain, THBP#9 in trial A (Fig. 1). Microscopic examination of the agar surface of supplementary Petri plates with a dissecting microscope indicated that two nematodes on one plate had reached the fungal mycelial disc on the plate within 1 hr and that most nematodes not only took longer time intervals, but appeared to take meandering paths to the mycelial discs. Some nematodes were widely scattered over the

TABLE 2.   Population densities of *Aphelenchoides hylurgi* in blight-controlled American chestnut canker tissues infected with white hypovirulent and pigmented isolates of *Cryphonectria parasitica*[a].

Canker-bark sample code	No. of canker-bark plates assayed	Mean number of nematodes per plate[b]	Mean canker-bark weight per plate (g) [b]	Mean number of nematodes per g canker bark
RM 7-17	12	21 ± 7	2.74 ± 0.23	8
RM 1	8	4 ± 2	2.94 ± 0.23	1
TH 10-9	4	1 ± 1	2.65 ± 0.16	1
RM 10-23	15	9 ± 4	3.30 ± 0.18	3
LD 11-13	22	12 ± 2	2.55 ± 0.12	5
RM 8-27	6	27 ± 7	4.00 ± 0.30	7
TH 4-8	21	20 ± 4	2.97 ± 0.13	7
Total/means	88	14 ± 2	2.94 ± 0.08	5[c] (n=88)

[a] Based on the number of nematodes recovered in canker-bark-plate assays.
[b] Variation is expressed as standard error.
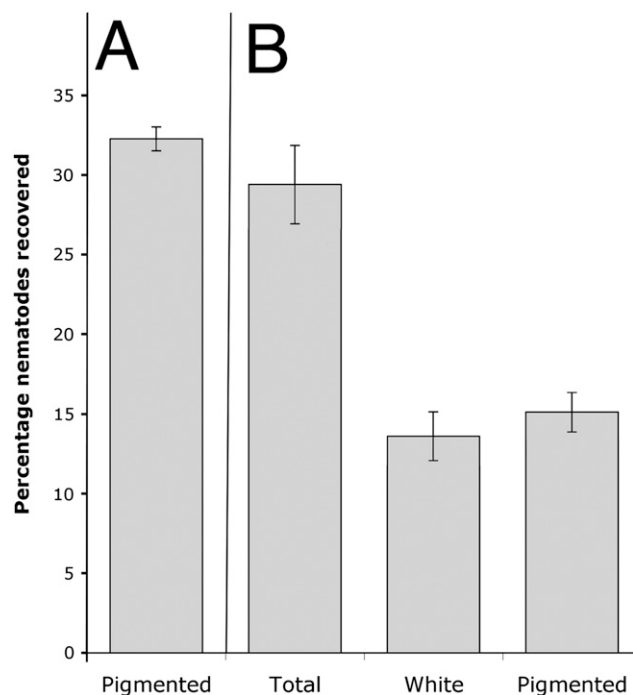[c] Equals 2 nematodes per cm^2 canker-bark surface.

FIG. 1. Attraction and movement over 24 hr of *Aphelenchoides hylurgi* from a nematode suspension droplet on a water agar Petri plate to a mycelial disc of a pigmented *Cryphonectria parasitica* (THBP#9) in trial A or to mycelial discs of both pigmented (THBP#9) and white hypovirulent *C. parasitica* (Ep 49#5) in trial B. Inoculum levels were 286 and 285 nematodes per Petri plate for trial A and trial B, respectively. Movement distance was 2 cm and mycelial disc diameter was 1 cm. Data indicated for percentage of inoculated nematodes recovered from mycelial discs are the means of five experiments for both trial A and trial B. Variation is expressed as standard error. In trial B, no significant difference was found between the mean values for white hypovirulent and pigmented *C. parasitica* strains as determined by a t-test ($P \leq 0.05$).

surface of the Petri plates during the 24 hr. The area of the target fungal mycelial disc was 1.2% of the area of the Petri plate. When pigmented and white hypovirulent mycelial targets were used in trial B, similar levels of nematodes were recovered from both fungal types (Fig. 1). No difference was found between the mean values of the number of nematodes recovered from pigmented versus white hypovirulent mycelial discs, as determined by a t-test ($P \leq 0.05$). Although there were two mycelial targets in trial B compared to one in trial A, the combined number of nematodes recovered in trial B was not greater than found in trial A.

Growth of A. hylurgi *on pigmented and white hypovirulent* C. parasitica *colonies*: Following nematode attraction and movement to *C. parasitica* colonies for 2 days in the Y-Plates with a plastic ridge barrier between the nematode inoculum section and the fungal colony section, large *A. hylurgi* populations developed on both white hypovirulent and pigmented colonies of *C. parasitica*, after 21 days of incubation, though populations were highly variable (Table 3). Mean nematode numbers extracted per colony for the fungal strains were not

different from each other as determined by the Kruskal-Wallis test ($P \leq 0.05$). The mean value for all *C. parasitica* strains was 121,283 nematodes per colony. For comparison, the natural log (L_n) values used by others workers are presented for these populations in Table 3.

Attraction and movement of A. hylurgi *to stromata reproductive structures of* C. parasitica *on an excised, natural canker and nematode growth in canker tissues*: Over 24 hr, nematodes moved from the inoculum disc to the small stromata reproductive structures of *C. parasitica* on the canker surface and also to other parts of the canker surface (Table 4). These canker surface areas are composed of smooth bark as well as cracks in the canker bark into which nematodes could move. Below these cracks are mycelial fans of the blight fungus, which are the structures responsible for killing chestnut bark tissues and are potential food sources for the nematodes. Gentle washing of the canker surface was used to remove nematodes, but only a portion of the nematode inoculum was recovered from the three-dimensional blight canker. About 20% of the nematodes were recovered from the inoculum disc.

After 22 days of incubation, moderate populations (12,142) of *A. hylurgi* were extracted from the excised natural canker tissues that were inoculated with the nematode (Table 5). In contrast, only low populations (412) of nematodes were extracted from excised natural canker tissues not inoculated with *A. hylurgi*. On a per-unit-canker-area basis (cm^2), the different values for inoculated versus not inoculated may be more meaningful, as the canker areas for both treatments were not the same (Table 5).

DISCUSSION

Aphelenchoides hylurgi is present in blight-controlled cankers on the American chestnut trees studied and *A. hylurgi* is a carrier of white hypovirulent isolates of the chestnut blight fungus. Pigmented isolates were frequently carried by the nematode, but the frequency of

TABLE 3. Attraction, movement, and growth of *Aphelenchoides hylurgi* on three-sectioned Petri plates containing a nematode inoculum suspension droplet on water agar in one section and colonies of either white hypovirulent (W) or pigmented (P) strains of *Cryphonectria parasitica* in the remaining sections[a].

Fungal strain	Mean nematodes/colony[b]	Ln (Mean)
Ep49#5 (W)	85,333 ± 24,062	11.35
Ep51W (W)	233,600 ± 90,000	12.36
THBP#9 (P)	45,915 ± 9,528	10.71

[a] Nematode attraction/movement period and distance were 2 days and 2 cm respectively and nematode-fungal growth period on acidified potato-dextrose agar was 21 days after transfer of fungal colony section to a 9-cm diameter Petri plate at 2 days. Inoculum = 200 nematodes per 0.1 ml droplet per plate. Numbers are the average of three replications.

[b] Mean nematode numbers extracted per colony for the fungal strains were not different from each other as determined by the Kruskal-Wallis test ($P \leq 0.05$). Mean for all fungal strains was 121,283 nematodes per colony. Variation is expressed as standard error.

TABLE 4.   Movement of *Aphelenchoides hylurgi* over 24 hr from a water agar disc on a natural chestnut blight canker to canker stromata of *Cryphonectria parasitica* and the blight canker surface of an excised, young American chestnut stem.

Nematode recovery location	Number of nematodes recovered	Per cent of nematode inoculum recovered[a]
Stromata on canker	16[b]	5.9
Canker surface	28	10.4
Agar disc	56	20.7

[a] Inoculum droplet (0.1 ml) containing 270 nematodes was placed on a 1-cm diameter water agar disc.
[b] Nematodes were recovered from one or more excised stromata reproductive structures (@ 1 mm diameter) in each of three of the four canker quadrants (1.0 x 0.5 cm) surrounding the inoculated agar disc. Number of stromata assayed per quadrant was 24. Eleven of 96 stromata assayed were positive for nematodes. All 192 stromata assayed from two other cankers not having *A. hylurgi* inoculum were negative for nematodes. The canker was gently washed with water to recover canker-surface nematodes.

white hypovirulent isolates in the *C. parasitica* population carried was low (8.2%). However, this value is very close to the percentage (5.2%) of *C. parasitica* stromata on the canker bark surface of the same trees that yielded white hypovirulent isolates in a previous study (Hogan and Griffin, 2008). These low values may nevertheless be important to the spread of hypovirulence on the trees as white hypovirulent isolates were present in 85.7% of the cankers on the trees and were abundant to a height of 8.25 m (Hogan and Griffin, 2002). As the hypovirus suppresses asexual sporulation (stromata) in the blight fungus (Elliston, 1985), the low frequencies of white hypovirulent isolates indicated are not surprising, even though the frequency of white hypovirulent isolates in the canker mycelium is considerably higher (30-40% of *C. parasitica* isolates).

Researchers have suggested for many years that the asexual spores in the blight fungus stomata on the canker surface are the most likely source of hypovirulent strain propagules that may be spread on chestnut stems and cankers by potential disseminating agents, such as insects, mites, and water (Anagnostakis, 1982; Heald, 1926; Nannelli and Turchetti, 1999; Russin et al., 1984; Scibilia and Shain, 1989). Significantly, *A. hylurgi* moved to the asexual spore-producing stromata within 24 hr in the present study following inoculation of the canker with the nematode. The three-dimensional nature of the canker and stromata in this experiment extends the attraction and movement test evaluations

beyond the two-dimensional horizontal (Spence et al., 2008), which mainly characterizes the Petri-plate data. Based on our observations of the blight-controlled cankers studied at Lesesne over many years, carpenter ants (Anagnostakis, 1982) and rain water (Heald, 1926) are potential disseminating agents of hypovirulence. Mites have not been found to carry the white hypovirulent isolates thus far (G. J. Griffin, unpublished). Compared to ants, the nematode association with cankers appears to be more constant, but nematodes likely require rainy days or nights and a moist environment to allow for significant movement on the blight canker surfaces. Within an expanding discrete canker or over coalesced cankers on these trees, nematodes potentially would have a continuous food source to follow during short-range host finding. Long-range (scale of several cm or longer) host finding (Spence et al., 2008) or nematode movement to distant discrete cankers may proceed differently, perhaps in association with other carriers. As indicated, white hypovirulent isolates are present throughout the trees and the spread of hypovirulence on the trees is to a large extent in an upward direction. Water-aided dissemination would more likely be in a downward direction, but nematode and ant movement potentially could be in any of three directions, including vertically into cracks and stromata in the canker. Following dissemination of white hypovirulent propagules, hypovirulent *C. parasitica* colonies may be initiated in or near an existing pigmented-isolate blight canker (Hogan and Griffin, 2002).

The short-range (2 cm) horizontal *in vitro* nematode attraction and movement data indicate that *A. hylurgi* is not preferentially attracted to either pigmented or white hypovirulent strains. This is an important finding as the biomass of hypovirus-free pigmented mycelium in the cankers is greater than for white hypovirulent mycelium, especially in young cankers. Thus, the pigmented mycelium may present a large, "equally attractive" food target for *A. hylurgi* that may be carrying propagules of white hypovirulent isolates. Also, for *A. hylurgi* to acquire propagules of white hypovirulent isolates, the latter may likewise be equally attractive to the nematode. The data and microscopic observations indicated that some nematodes were less efficient in fungal host finding than others. This short-range *in vitro* attraction and movement model is consistent with

TABLE 5.   Number of nematodes extracted after 22 days incubation from excised young American chestnut tree blight cankers that were either inoculated or not inoculated with *Aphelenchoides hylurgi*.

	Mean canker area (cm^2)	Mean number of nematodes extracted	Mean number of nematodes extracted per cm^2
Natural blight cankers inoculated with *A. hylurgi* [a]	107 ± 2	12,142[b] ± 5,172	112[b] ± 46
Natural blight cankers not inoculated with *A. hylurgi*	127 ± 1	412[b] ± 112	3[b] ± 1

[a] Inoculum droplet (0.1 ml) containing 270 nematodes was placed on a 1-cm diameter water agar disc on the canker surface.
[b] Mean number of nematodes extracted after 22 days incubation in a moist chamber. Values indicated are the means of two canker replications for each treatment. Variation is expressed as standard error.

the spatial scale and patterns of white hypovirulent and pigmented isolates found in lattice-plot sampling (each lattice cell = 2.54 x 2.54 cm) of blight-controlled cankers at Lesesne (Griffin, 1999). Some "intermediate-pigmented" isolates also are infected with the hypovirus and thus propagules of these isolates could also be carried to the hypovirus-free pigmented mycelium areas in cankers (Hogan and Griffin, 2008). The transmission of the hypovirus from infected mycelium to adjacent uninfected, pigmented mycelium has been extensive on the trees, as the hypovirus has been found in a large number (45) of genotypes (vegetative compatibility types) of the blight fungus on trees, which were not present on the trees or infected initially with the hypovirus at the time of hypovirulent strain inoculation in 1982-83 (Hogan and Griffin, 2002). Once infected with the hypovirus, the mycelium exists as a reservoir for its further dissemination and transmission.

Aphelenchoides spp. feed on the mycelium of *Verticillium, Trichoderma, Rhzoctonia, Pyrenocheata, Pochonia, Aspergillus, Fusarium, Cladosporium, Penicillium, Botrytis, Pestalotia, Diaporthe, Gleosporium,* and *Monilia* (Hasna et al., 2007; Ikonen, 2001; Perper and Petriello, 1977; Ruess et al., 2000). Of these, *Pestalotia* and *Diaporthe* ranked as the top two hosts for population growth among 28 fungi tested against four species of *Aphelenchoides* (Perper and Petriello, 1977). The genus *Diaporthe* is very closely related to the chestnut blight fungus and it is in this genus that the blight fungus was first classified in 1906 (Roane et al., 1986). In a direct comparison of nematode population levels, all natural logarithm values (Ln) of nematode populations found here, after 21 days of growth on *C. parasitica,* fall within the range found by Hasna et al. (2007) after 21 days growth of *Aphelenchoides* sp. on five fungi, with that of Ep 51W being equal to the highest growth found in the latter study. Thus, the chestnut blight fungus appears in general to be a very good host for *A. hylurgi* population growth, although population growth in our combined attraction, movement, and growth studies was variable. Similar variability was observed in this study among the Petri plate *A. hylurgi* populations grown on *C. parasitica* for inoculum purposes after 3-4 weeks incubation. It is not known how much the vigor of the mycelium is affected by nematode feeding or if the nematode acquires the hypovirus while feeding.

The results suggest that *A. hylurgi* may play a role in the spread of hypovirulence on the American chestnut trees studied. The importance of *A. hylurgi* population density in hypovirulence spread is not known, nor whether the mycelium in all blight cankers is colonized by nematodes. The Lesesne canker nematode population densities were highly variable among bark samples in this study. However, the Lesesne blight cankers and the excised Blacksburg blight cankers examined here had mean nematode population densities in the same range on a per-unit-canker-area basis, even though the Lesesne cankers were much older and the bark was thicker. Inoculation of *A. hylurgi* on the Blacksburg cankers resulted in a much higher nematode population density on a per-unit-canker-area basis, after 22 days incubation, than no inoculation. This suggests *A. hylurgi* inoculum density (Hasna et al., 2007) and/or aggressiveness may be important to nematode attraction, movement, and growth on cankers. On a per-unit-area basis, all blight canker nematode populations were lower than populations extracted from fungal Petri plate cultures, which had a high fungal biomass per unit area. Further research is required to determine if *A. hylurgi* is present in chestnut blight cankers in other geographic regions within the natural range of the American chestnut, which includes the Appalachian Mountains and neighboring areas.

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