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Research

Hyalorbilia oviparasitica Clade Detected in Field Soils Cropped to Sugar Beets and Enriched in the Presence of *Heterodera schachtii* and a Host Crop

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Abstract

This study endeavored to test two hypotheses: (i) *Hyalorbilia oviparasitica* and related species (*H. oviparasitica* clade) will be detected in sugar beet fields in California's Imperial Valley and (ii) the population densities of these fungi will increase in the presence of their nematode host (*Heterodera schachtii*) and a host plant of this nematode. The *H. oviparasitica* clade includes potent hyperparasites of sedentary stages of plant-parasitic nematodes, which have the ability to substantially suppress the population densities of several endoparasitic nematode species. In this study, 21 of 25 Imperial Valley soils cropped to sugar beets harbored members of the *H. oviparasitica* clade. The population densities of these fungi increased, on average, approximately 10,000-fold in the presence of *H. schachtii* and its host Swiss Chard over one nematode generation. An rRNA ITS sequence analysis showed that members of the *H. oviparasitica* clade were the dominant group of fungi associated with *H. schachtii* females derived from these soils. These results provide evidence supporting the hypotheses.

Keywords: *Hyalorbilia oviparasitica*, *Dactylella oviparasitica*, *Brassica oleracea*, *Hyalorbilia* aff. *multiguttulata*, *Heterodera schachtii*, sugarbeet cyst nematode, suppressive soils, biological control

Funding

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This study endeavored to test two hypotheses: (i) *Hyalorbilia oviparasitica* (G.R. Stirling & Mankau) E. Weber & Baral (Baral et al. 2018) and related species (*H. oviparasitica* clade) will be detected in sugar beet fields in California's Imperial Valley and (ii) the population densities of these fungi will increase in the presence of their nematode host (*Heterodera schachtii*) and a host plant of this nematode. Members of the *H. oviparasitica* clade are expected to be found in sugar beet fields in the Imperial Valley, because (i) earlier research detected these fungi associated with cyst nematodes in other locations (Kim and Riggs 1995; Timper and Riggs 1998; Westphal and Becker 2001a; Yin et al. 2003), (ii) *H. schachtii* is the primary pathogen of sugar beets in this region (Roberts and Thomason 1981), and (iii) these fungi have a widespread geographical

distribution (Yang et al. 2012). The populations of these fungi are expected to increase in the presence of both the fungal and nematode hosts because the host crop will enable the proliferation of the nematode, which should facilitate an increase in the fungi.

The original *H. oviparasitica* clade strain, named *Dactylella oviparasitica* DoUCR50, was assigned other names due to advances in taxonomy, including the anamorphic *Brachyphoris oviparasitica* (Chen et al. 2007), the teleomorphic *Hyalorbilia oviparasitica* (Baral et al. 2018) and most recently *Hyalorbilia* aff. *multiguttulata* (H. O. Baral, *personal communication*). In the remaining portions of this paper, *H.* aff. *multiguttulata* DoUCR50 refers to that strain, and the *H. oviparasitica* clade refers to that strain and related fungi.

The sugarbeet cyst nematode (*Heterodera schachtii*) has been reported to be among the most damaging plant-parasitic nematodes in the world (Jones et al. 2013; Sasser and Freckman 1987). In addition to parasitizing beets (*Beta vulgaris*) (Harveson et al. 2009), it causes considerable damage on a wide range of important vegetables including broccoli, Brussels sprouts, cabbage, and cauliflower, among others, all cultivars of *Brassica oleracea* (Johnson 1998).

To mitigate crop losses due to *H. schachtii*, the primary management tool in California's sugar beet production over the past 50 years has been crop rotation (Roberts and Thomason 1981). However, specialized production systems and a limited choice of economically profitable nonhost plants make it difficult and costly to employ the long crop rotation periods that are needed to reduce *H. schachtii* populations (Cooke and Thomason 1979). Resistant cultivars that maintain the industry-desired characteristics of today's preferred crops are not always available for many *H. schachtii* host crops, although there is a push to increase the number of available sugar beet cultivars with cyst nematode resistance, as well as tolerance to the high temperatures of California's Imperial Valley (Panella et al. 2014; Richardson 2018). U.S. sugar beet growers outside of California have often relied on nematicides and soil fumigants. However, most of these products are no longer available due to environmental and health concerns as well as for economic reasons (Becker 2014). Metam sodium is currently the only registered fumigant/nematicide registered as a preplant treatment for sugar beets in California (Becker et al. 2016). However, its efficacy against cyst nematodes is often inconsistent, and it is rarely used in sugar beet production. In addition, it poses potential hazards for human exposure because of its off-site movement and its ability to form hazardous ground-level ozone. In sum, there is a need for additional economically feasible management options to control *H. schachtii*.

The *H. oviparasitica* clade includes effective hyperparasites of sedentary stages of endoparasitic nematodes, which have the ability to substantially suppress the population densities of several cyst nematode species in one cropping cycle (Kim and Riggs 1995; Olatinwo et al. 2006c; Timper and Riggs 1998). In a *H. schachtii*-suppressive soil located at Agricultural Operations, University of California Riverside (Westphal and Becker 1999), a nematophagous strain of a *Hyalorbilia* sp. was first detected and identified (Westphal and Becker 2001a; Yin et al. 2003). Another strain, DoUCR50, isolated from *H. schachtii* females from the same field, became the subject of several investigations demonstrating its effectiveness in parasitizing and suppressing nematode populations (Olatinwo et al. 2006a; Smith Becker et al. 2013). Amendment with DoUCR50 showed a ~30-fold reduction in *H. schachtii* second-stage juveniles (J2) after two nematode generations, which was the same level of population suppression as that produced by the suppressive soil from which DoUCR50 was isolated (Olatinwo et al. 2006c). DoUCR50 amendments also produced similar levels of population reductions in several soil types tested (Olatinwo et al. 2006a) and in a field microplot study (Olatinwo et al. 2006b). Meanwhile, an unidentified fungal strain ARF, isolated from *Heterodera glycines* eggs and derived from a soybean cyst nematode-suppressive soil (Kim and Riggs 1995), showed morphological similarities to DoUCR50. This ARF strain had the ability to markedly reduce *H. glycines* population densities and to parasitize eggs as well as immature and mature females (Kim and Riggs 1995; Timper and Riggs 1998). Eventually, ARF was identified as another species of the *H. oviparasitica* clade (Yang et al. 2012).

The study described in this paper is an expansion of prior studies, which focused on one strain belonging to the *H. oviparasitica* clade (DoUCR50) as described above. Soils from 25 fields cropped to sugar beets in California's Imperial Valley were examined to determine if they harbored indigenous populations of these fungi. To accomplish this, a sequence-selective quantitative polymerase chain reaction (qPCR) assay was used. This assay was also used to determine whether the population densities of these fungi were enriched by the presence of both the fungal host (*H. schachtii*) and one of the nematode's hosts (Swiss chard). In addition, an Illumina-based fungal ribosomal RNA (rRNA) internal transcribed spacer (ITS) assay was used to characterize the types of fungi associated with *H. schachtii* females derived from these soils.

MATERIALS AND METHODS

Acquisition and processing of the soil samples

The Spreckels Sugar Company (Brawley, CA) provided dump soil samples from 25 sugar beet fields in the Imperial Valley, CA, in 2013. Large debris was removed from the soils using a 0.5-cm sieve. The soils were air dried in paper bags at room temperature and then stored at 15°C in the dark. Subsamples of these soils were stored at –80°C for subsequent molecular analyses. Prior to their use in the greenhouse baiting experiments, the soils were amended with 40% (v/v) steam-pasteurized (70°C for 3 h) plaster sand to improve their physical characteristics to facilitate aeration and water drainage.

Nematode inoculum preparation

Nematode inoculum was reared by infesting pasteurized sandy loam with *H. schachtii* J2 and growing Swiss chard (*Beta vulgaris* subsp. *vulgaris* cv. Large White Ribbed, Lockhart Seeds, Stockton, CA) in greenhouse pot cultures for approximately 3 months. Cysts were extracted from the soil using a Fenwick flotation can method (Caswell and Thomason 1985). The cysts were placed on Baermann funnels (Flegg and Hooper 1970) containing 4 mM $ZnCl_2$ to stimulate J2 hatching. The J2 were collected daily and stored in aerated water at 15 ± 1°C for approximately 48 h before soil infestation. The numbers of J2 in the suspensions were determined using a stereo microscope at 40× magnification.

Greenhouse baiting experiment

A subsample of each dump soil was filled into 150-cm^3 seedling pots. All pots were planted with two seeds of untreated Swiss chard cv. Large White Ribbed. The plants were maintained in a University of California, Riverside greenhouse in a complete randomized design at 24 ± 2°C under ambient light. Two weeks after emergence, the seedlings were thinned to one per pot and fertilized with slow-release fertilizer (Osmocote 14-14-14, Scotts, Marysville, OH). Four weeks after seeding, each pot was

infested with approximately 2,500 *H. schachtii* J2 by pipetting aqueous suspensions of freshly hatched J2 into two holes next to the seedling. Soil temperature was monitored for degree-day determination using HOBO Temperature Data Loggers (Onset Computer Corporation, Bourne, MA) buried in an additional pot. After 4 weeks, 10-cm^3 soil samples were collected from each pot, and 15 white *H. schachtii* females were picked from the roots using forceps. Both the soils and females were stored at –80°C for further analysis. The greenhouse baiting experiment was performed twice in time and space.

DNA isolation from soil and *H. schachtii* females

DNA was isolated from each soil (250 mg per isolation) and *H. schachtii* females (15 per isolation) derived from each soil using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) and a 210-s beat-beating step using a Mini-Beadbeater-16 (BioSpec, Bartlesville, OK).

Sequence-selective nested qPCR of fungi belonging to the *H. oviparasitica* clade

Fungi belonging to the *H. oviparasitica* clade were enumerated using a sequence-selective nested TaqMan qPCR assay. It should be noted that using a nested assay makes this assay less quantitative, but this two-step process was needed to detect these fungi in a greater number of the soil samples.

The first PCR used universal fungal primers that amplify portions of the small and large subunit rRNA as well as the 5.8S rRNA gene and the ITS1 and ITS2 regions as follows. PCRs were performed using a DNA Engine thermal cycler (Bio-Rad, Hercules, CA) and 20-µl reactions containing 50 mM Tris (pH 8.3), 500 µg/ml of bovine serum albumin (BSA), 2.5 mM $MgCl_2$, 250 µM of each deoxynucleotide triphosphate (dNTP), 400 nM of each of the PCR primers, 2 µl of DNA template, and 0.2 units JumpStart Taq DNA polymerase (Sigma-Aldrich, St. Louis, MO). PCR primers were ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). Thermal cycling parameters were 94°C for 5 min; 35 cycles of 94°C for 20 s, 52°C for 20 s, and 72°C for 40 s; followed by 72°C for 10 min. The PCR primers at their working dilution produced fewer amplicons with each freeze-thaw cycle, and so after purchasing the primers, they were aliquoted at their working dilution in DNA LoBind tubes (Eppendorf North America, Hauppauge, NY), dried in a SpeedVac (Thermo Scientific Savant, Waltham, MA), stored at –20°C, and used only once after resuspending them.

The second PCR was a TaqMan assay that targeted the rRNA ITS region using primers and a probe selective for members of the *H. oviparasitica* clade, and that used DNA templates that were 1:10 dilutions of the first PCRs. qPCRs were performed using a CFX384 Touch Real-Time PCR Detection System (Bio-Rad) and 10-µl reactions containing 50 mM Tris (pH 8.3), 500 µg/ml of BSA, 2.5 mM $MgCl_2$, 250 µM of each dNTP, 400 nM of each of the PCR primers, 250 nM of the probe, 1 µl of DNA template, and 0.1 units JumpStart Taq DNA polymerase (Sigma-Aldrich). PCR primers were DO ITS 2017 F1 (TGACTATAAAACTTTCAAC) and DO ITS 2017 R1 (GGTTTTACTGACGCT), which amplify a ~373-bp fragment. The probe was DO ITS 2017 P1 (TTCCGARAGGCACGTC), which was labeled with 6-carboxyfluorescein on the 5′ end and with the ZEN and Iowa Black FQ quenchers on the 3′ end (Integrated DNA Technologies, Coralville, IA). Thermal cycling parameters were 94°C for 5 min; 40 cycles of 94°C for 20 s, 53°C for 30 s, and 72°C for 30 s. Two replicate qPCRs were performed, each having three technical replicates per sample.

Illumina fungal rRNA ITS library construction and sequencing

An Illumina fungal rRNA ITS library was constructed as follows. PCRs were performed using a DNA Engine thermal cycler (Bio-Rad) and 25-µl reactions containing Phusion High-Fidelity DNA Polymerase Mix (New England Biolabs, Ipswich, MA) supplemented with 500 µg/ml of BSA, 1 mM $MgCl_2$, 250 µM of each dNTP, 400 nM of each primer, and 2.5 µl of DNA template. The PCR primers gITS7 (GTGARTCATCGARTCTTTG) and ITS4 (TCCTCCGCTTATTGATATGC) targeted the ITS2 region of the ribosomal rRNA gene operon (Ihrmark et al. 2012; White et al. 1990), with the reverse primers including 12-base barcodes, and both primers including the Illumina sequences needed for cluster formation. Thermal cycling parameters were 94°C for 5 min; 35 cycles of 94°C for 20 s, 56°C for 20 s, and 72°C for 30 s; followed by 72°C for 10 min. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA sequencing (single-end 300 base) was performed using an Illumina MiSeq.

Illumina fungal rRNA ITS sequence data processing

The UPARSE pipeline was used for demultiplexing, length trimming, quality filtering, and operational taxonomic unit (OTU) picking using default parameters or recommended guidelines that were initially described in Edgar (2013) and that have been updated at https://www.drive5.com/usearch/manual/uparse_pipeline.html. Briefly, after demultiplexing and using the recommended 1.0 expected error threshold, sequences were trimmed to a uniform length of 297 bases. Sequences were then dereplicated and clustered into zero-radius OTUs using the UNOISE3 algorithm (Edgar 2016), which detects and removes chimeric sequences. An OTU table was then generated using the otutab command. Taxonomic assignments of the fungal OTUs, or amplicon sequence variants (ASVs), were performed using the RDP Classifier version 2.12 (Wang et al. 2007), trained on the ver8_99_s_02.02.2019 release of the UNITE database (Kõljalg et al. 2013), and ASVs having nonfungal assignments were removed.

The fungal rRNA ITS sequences have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the BioProject accession number PRJNA614529.

Illumina fungal rRNA ITS sequence analyses

QIIME (Caporaso et al. 2010) was used to create abundance tables of the phylotypes at various taxonomic levels. Correlation analyses were performed using Prism (GraphPad, La Jolla, CA). Prism was also used to make the correlation plots as well as the fungal phyla and species plots.

Phylogenetic tree construction

The fungal rRNA ITS sequences used to construct the phylogenetic tree were from the *Hyalorbilia* clade and related groups described in Baral et al. (2018), prior studies examining nematophagous fungi (Olatinwo et al. 2006c; Stirling and Mankau 1978; Timper and Riggs 1998; Yin et al. 2003), and the Illumina rRNA ITS sequences with high identity to *H. oviparasitica* from this study (Sugar Beet ASVs) and from a related study examining soils frequently cropped to *Brassica oleracea* species (*Brassica* ASVs) (Chen et al. 2020).

The phylogenetic tree was constructed by performing the following analyses using GenomeNet (https://www.genome.jp/). Sequences

were aligned using the CLUSTAL W algorithm (Thompson et al. 1994). The final alignment had a length of 279 nucleotides, and the sequences ranged from 232 to 261 nucleotides. Alignment and phylogenetic reconstructions were performed using the function "build" of ETE3 v3.0.0b32 (Huerta-Cepas et al. 2016) as implemented on the GenomeNet (https://www.genome.jp/tools/ete/). Maximum-likelihood trees were inferred using RAxML v8.1.20, which was run with model GTRGAMMA and the default parameters (Stamatakis 2014). Branch supports were computed out of 100 bootstrapped trees. The tree figure was created using the Interactive Tree of Life (Letunic and Bork 2007) (https://itol.embl.de/).

The Illumina fungal rRNA ITS sequences from this study with high identity to those from the *H. oviparasitica* clade, and that were included in this phylogenetic analysis, have been deposited in the NCBI under the accession numbers MT248320, MT248321, MT248322, MT248323, and MT248324.

RESULTS AND DISCUSSION

This study endeavored to test two hypotheses: (i) *H. oviparasitica* and related species (*H. oviparasitica* clade) will be detected in sugar beet fields in California's Imperial Valley and (ii) the population densities of these fungi will increase in the presence of their nematode host (*H. schachtii*) and a host plant of this nematode.

Soils cropped to sugar beets in the Imperial Valley harbored indigenous populations of fungi belonging to the *H. oviparasitica* clade

To test the first hypothesis, a sequence-selective qPCR assay was used to determine whether soils cropped to sugar beets in the Imperial Valley harbored indigenous populations of fungi belonging to the *H. oviparasitica* clade. An analysis of 25 soils detected these fungi in only seven of them (Fig. 1A, before the baiting experiment). However, when these soils were subjected to a baiting experiment, in which soils were planted with a *H. schachtii* host crop and infested with sugarbeet cyst nematodes (J2) and then examined after one nematode generation, 21 of the 25 soils had detectible levels of these fungi (Fig. 1A, after one nematode generation in the baiting experiment). These results provided evidence supporting the first hypothesis.

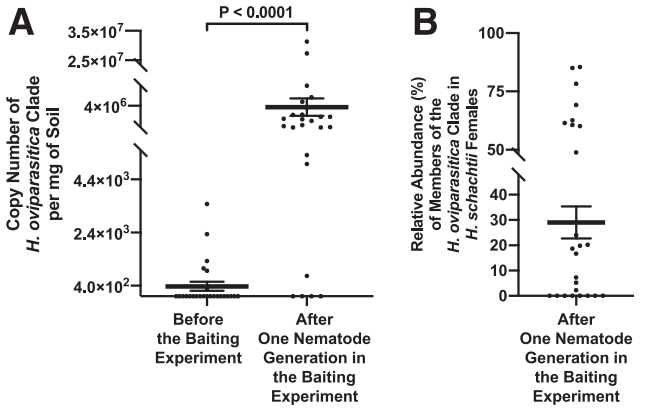

FIGURE 1
Population densities of fungi belonging to the *Hyalorbilia oviparasitica* clade in soils previously cropped to sugar beets and the *Heterodera schachtii* females derived from those soils. **A,** A sequence-selective nested qPCR assay was used to measure the population densities of these fungi in the soils before and after one nematode generation in the presence of both *H. schachtii* and a *H. schachtii* host crop (the baiting experiment). Differences were assessed by a two-tailed *t* test ($n = 25$ soils). **B,** An Illumina-based fungal rRNA ITS sequence analysis was used to determine the relative abundance of these fungi in *H. schachtii* females collected from the roots of a *H. schachtii* host plant grown in these soils. Mean values are thick horizontal lines, error bars are standard error, and the samples are the dots.

The amount of fungi belonging to the *H. oviparasitica* clade in soil dramatically increased over one nematode generation

The qPCR analyses also showed that the population densities of members of the *H. oviparasitica* clade in soil dramatically increased over one nematode generation in the presence of both the fungal host (*H. schachtii*) and one of the nematode's hosts (Swiss chard) (Fig. 1A). The average copy number of these fungi per milligram of soil before the baiting experiment was 377.8, whereas after the baiting experiment the value increased to 3,768,944, an almost 10,000-fold average increase, with the range being 0 to 31,307,313. These results provided evidence supporting the second hypothesis.

An analysis of the fungal rRNA ITS sequences associated with *H. schachtii* females, which were picked from the roots of Swiss chard grown in these soils (the baiting experiment), showed that 17 of the 25 samples had detectible levels of fungi belonging to the *H. oviparasitica* clade (Fig. 1B).

To determine whether there was a relationship between the population densities of members of the *H. oviparasitica* clade in the soils before and after one nematode generation in the baiting experiment, a correlation analysis was performed using the values generated by the sequence-selective qPCR assay. As described above, only seven of the 25 soils had detectable levels of fungi before performing the baiting experiment. For these seven soils, there was a positive relationship between the population densities of these fungi in the soils before and after the baiting experiment (Fig. 2).

The abundant fungi in *H. schachtii* females derived from soils cropped to sugar beets

To examine the relative abundance of the fungal phyla and species associated with *H. schachtii* females derived from soils recently cropped to sugar beets, taxonomic plots were constructed using data generated by an Illumina-based fungal rRNA ITS sequence assay (Fig. 3). This analysis showed that most of the fungi belonged to the *Ascomycota* phylum (94.17%) (Fig. 3A). At the species level, the most abundant taxa were facultative nematophagous fungi belonging to the *H. oviparasitica* clade (Fig. 3B).

H. oviparasitica and related species belong to the class *Orbiliomycetes* (Eriksson et al. 2003). Most fungi belonging to the *H. oviparasitica* clade are nematophagous, but they are not known to produce traps. Instead, these fungi attack primarily sedentary stages of endoparasitic nematodes such as third- and fourth-stage sedentary juveniles and females, as well as eggs (Smith Becker et al. 2013; Stirling and Mankau 1978; Timper et al. 1999). *Arthrobotrys* spp. are nematode-trapping fungi that use specialized adhesive networks to capture their mobile prey, but they are not known to parasitize sedentary stages. *Fusarium* spp. are common soil fungi, mostly occurring as saprotrophs, but some species are important plant pathogens. Both *F. solani* and *F. oxysporum* have also been reported to be female and egg parasites of cyst nematodes (Carris et al. 1989; Chen and Chen 2002; Gao et al. 2008; Nigh et al. 1980; Ownley Gintis et al. 1982, 1983; Westphal and Becker 2001a). *Acremonium strictum* and *F. oxysporum* were previously isolated from *H. schachtii* eggs obtained from sugar beet fields in the Imperial Valley (Nigh

et al. 1980). In laboratory studies, both fungi infected young female nematodes through natural openings, and then they parasitized and destroyed nondifferentiated eggs.

Although there are reports in the literature describing fungi that are able to parasitize one or more life stages of cyst nematodes, the methods used in this study did not enable the differentiation of microbes parasitizing nematodes versus those simply being associated with the nematodes. Contributing to this potential problem, anamorphic stages of common *Ascomycetes* that produce abundant conidia are likely to produce a large number of PCR amplicons. In addition, some fungi may only become nematophagous after encountering a nematode that is somehow made vulnerable by other soil organisms (Stirling 2014). On the other hand, several nematophagous fungal species, including *Fusarium* spp. and fungi belonging to the *H. oviparasitica* clade, had high relative abundances in the *H. schachtii* females produced by this study.

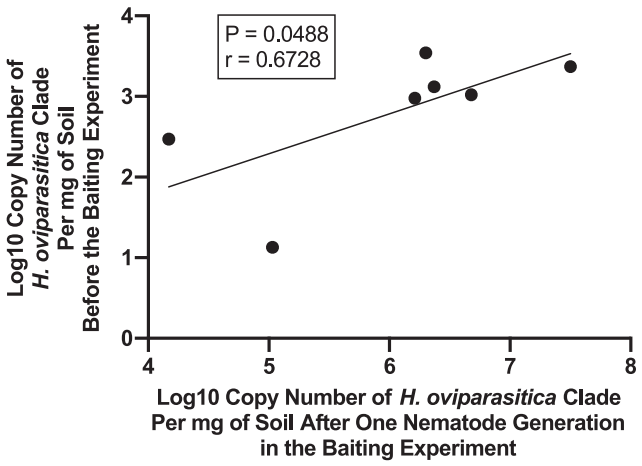

FIGURE 2
Relationship between the population densities of members of the *Hyalorbilia oviparasitica* clade in the soils before and after one nematode generation in the baiting experiment. The amounts of members of the *H. oviparasitica* clade (Log10 copy numbers/mg of soil) were measured by using a sequence-selective nested qPCR assay. The correlation coefficient (*r*) and probability value (*P*) are shown (*n* = 7).

A phylogenetic analysis of the *H. oviparasitica* clade sequences identified by this study

Finally, a phylogenetic analysis of rRNA ITS sequences was performed to examine the relationships between the members of the *H. oviparasitica* clade identified by this study and several related nematophagous fungi (Fig. 4). This analysis shows a coherent assemblage of organisms and phylotypes (the *H. oviparasitica* clade) composed of four nematophagous fungi (green text), sequences from this study (red text), sequences from a similar study examining soils cropped to *Brassica* species (*Brassica*) (Chen et al. 2020), and *H. brevistipitata*.

It is likely that all of the fungal rRNA ITS sequences obtained from the two culture-independent studies, designated by either Sugar Beet or *Brassica* and an ASV number, come from nematophagous fungi because of their placement in the *H. oviparasitica* clade (Fig. 4) and because they were found in the analyses of *H. schachtii* females. It also appears that these fungi are not crop specific, because the sequences from these two studies are intermingled in the *H. oviparasitica* clade. This is consistent with prior research that showed that DoUCR50 was attracted to emerging nematode females but not to plant roots (Smith Becker et al. 2013). In addition, because members of these fungi have been detected on at least four of the seven continents (Fig. 4), indigenous members of the *H. oviparasitica* clade could be suppressing cyst nematode populations worldwide.

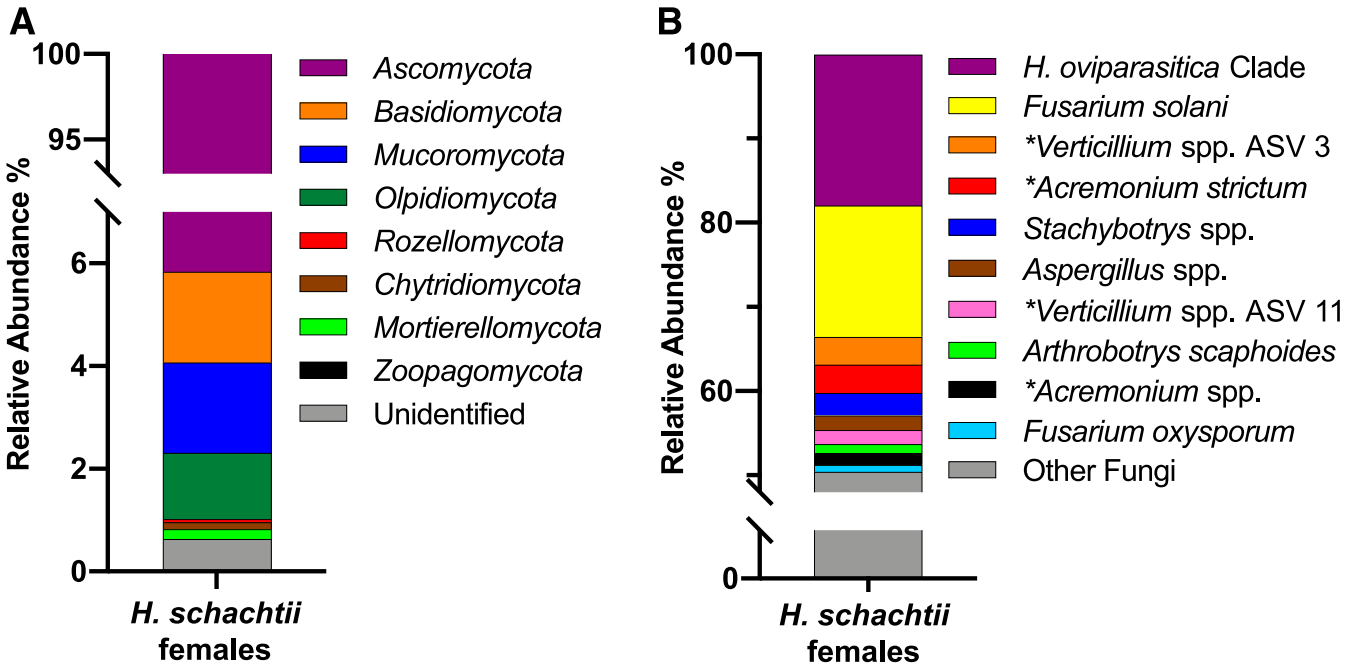

FIGURE 3
Fungal taxa associated with *Heterodera schachtii* females collected from the roots of Swiss chard, a *H. schachtii* host plant, grown in soils cropped to sugar beets. Average relative abundance values of fungal phyla (**A**) and species (**B**) from *H. schachtii* females derived from 25 soils were determined by an analysis of fungal rRNA ITS sequences. Asterisks (*) indicate sequences that had more than one best match, and therefore the taxonomic assignments are not definitive. Other fungi are all of the taxa with less than 0.015% relative abundance combined.

The cropping decision model for sugar beet cultivation in California

The results presented in this study may also have relevance for *H. schachtii* management in Imperial Valley sugar beet production. The Imperial Valley is currently the primary sugar beet growing region in California. Its most economically important pathogen is *H. schachtii*, which is managed by a crop rotation scheme. This scheme was developed and implemented in the early 1960s by nematologist I. J. Thomason from the University of California, Riverside, representatives from the local sugar factory, sugar beet growers, and the County Agricultural

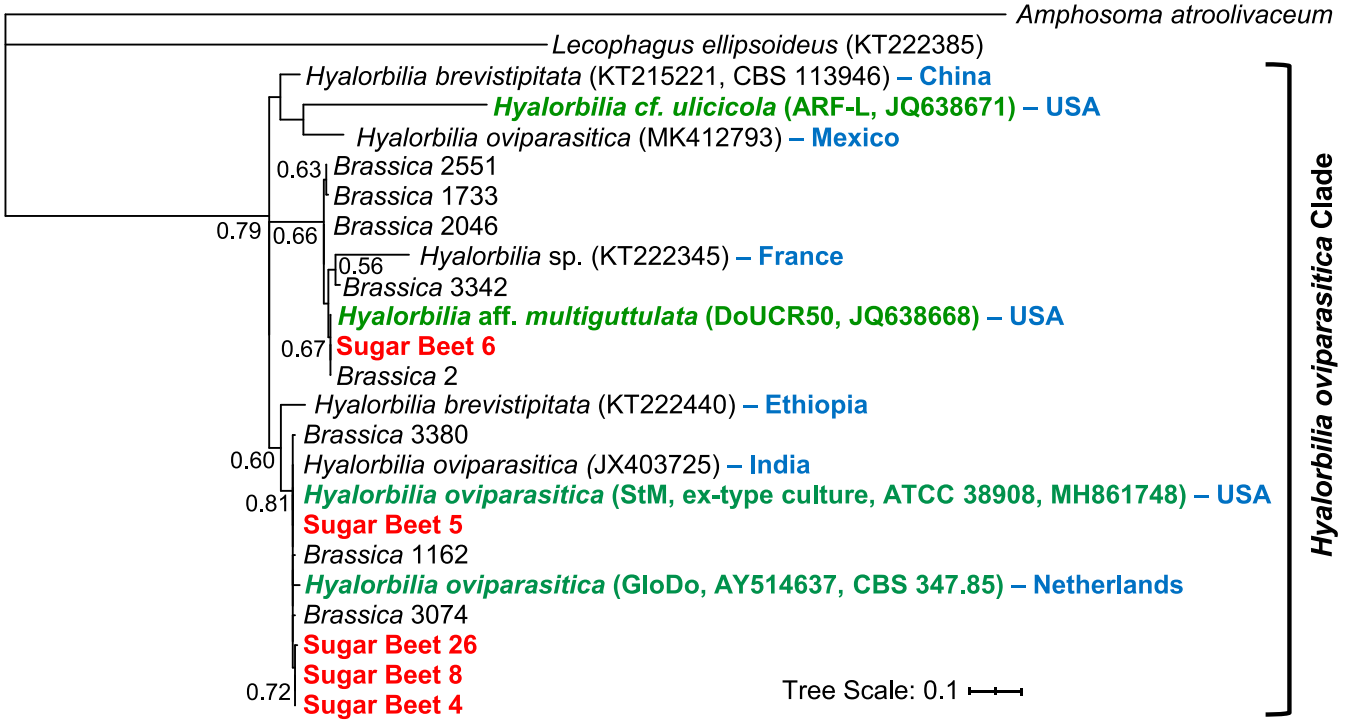

FIGURE 4
A phylogenetic analysis of the *Hyalorbilia oviparasitica* clade sequences identified in this study and related nematophagous fungi shows their worldwide distribution. The tree was constructed by performing a maximum likelihood analysis of fungal rRNA ITS sequences obtained from four cultured nematophagous fungi, related phylotypes obtained by culture-independent analyses, and members of *Hyalorbilia*. The fungal phylotypes obtained by culture-independent analyses of *Heterodera schachtii* females derived from soils cropped to (i) sugar beets (this study) and (ii) *Brassica* species (Chen et al. 2020) are indicated by Sugar Beet followed by an ASV number and by *Brassica* followed by an ASV number, respectively. The outgroup is *Amphosoma atroolivaceum*. Bootstrap values (out of 100) are shown on the branches if ≥ 0.50. Branch lengths are the expected number of substitutions per nucleotide. Additional info for *H. oviparasitica* StM is CBS 379.84, and for *A. atroolivaceum* it is KT222387.

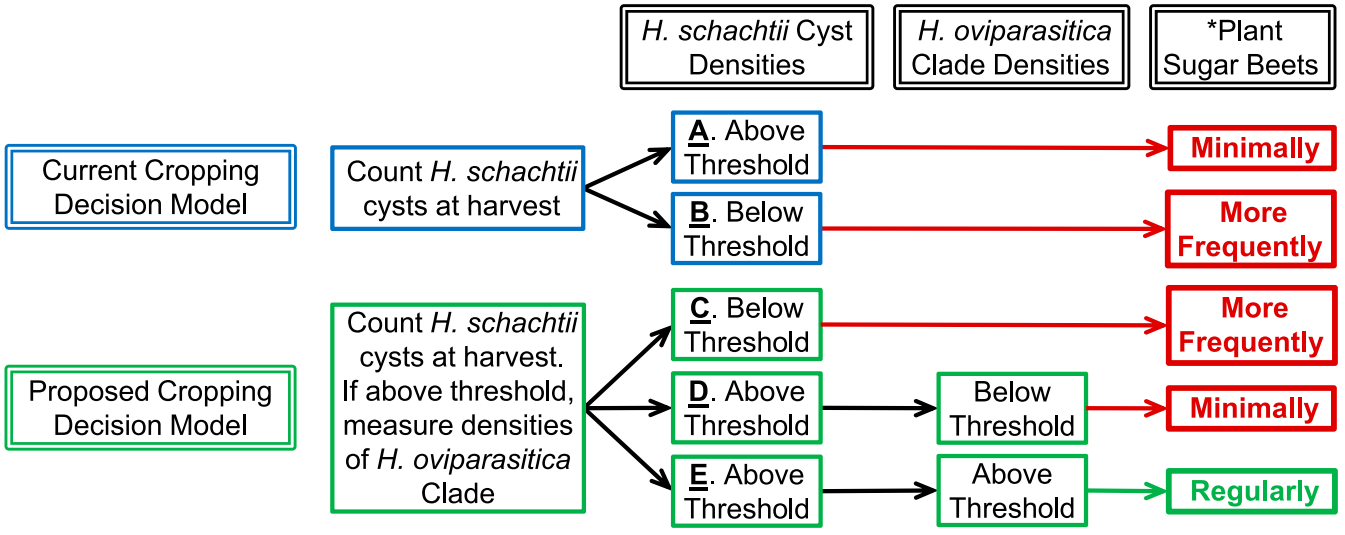

FIGURE 5
Comparison of the current and newly proposed cropping decision models to manage *Heterodera schachtii* for sugar beet production in the Imperial Valley of California. * = Plant sugar beets or another *H. schachtii* host crop.

Commissioner. One of the key components of this approach is the collection and analysis of dump soil samples (Altman and Thomason 1971; Thomason 1972, 1978). Upon delivery, sugar beets are transported into the sugar factory via conveyor belts that shake off soil adhering to the crop. For every truckload of sugar beets, a portion (473 cm^3) of this soil is collected. These dump soil samples come from a harvested field of approximately 2 ha in size, providing a simple method to collect a soil sample that is representative of the harvested area. *H. schachtii* cysts in these dump soil samples are extracted and counted.

These *H. schachtii* cyst counts are then used to make cropping decisions that are contractually enforced by the sugar factory as follows (Fig. 5). Fields are considered infested with *H. schachtii* if the number of nematode cysts in a dump soil sample exceeds the threshold value (>3 cysts per 473 cm^3 of soil). For infested fields, host crops including sugar beets can only be grown once every 4 years (Fig. 5A). For fields below the infestation threshold, host crops including sugar beets can be grown no more than two consecutive years and less than a total of 4 out of 10 years (Fig. 5B) (Roberts and Thomason 1981).

This nematode management scheme has been effectively used for over a half-century. The success of this program is due to the natural decline of *H. schachtii* populations in the absence of host plants. In the Imperial Valley, annual rates of *H. schachtii* decline of more than 50% have been reported with nonhost crops. Furthermore, egg densities in four different fields dropped below the detection level during the fourth year under continuous nonhost alfalfa (Roberts et al. 1981). These authors suggested that egg parasitism by *Fusarium oxysporum*, *Acremonium strictum*, and other fungi reported by Nigh et al. (1980) may be contributing to the decline of the nematode populations via the destruction of *H. schachtii* eggs. In addition, Zheng and Ferris (1991) found that 40 to 50% of *H. schachtii* eggs are nondormant. These eggs will therefore hatch without any environmental stimulus such as host root exudates. In the absence of hosts, this will contribute to the decline of the *H. schachtii* populations.

Although this system has been remarkably successful at managing *H. schachtii* populations in the Imperial Valley, it considerably limits the overall production of sugar beets. This is because even in the fields below the *H. schachtii* threshold value, sugar beets can only be grown in 3 of 10 years.

A possible change to the sugar beet cropping decision model

To enable sugar beets to be planted more often in the Imperial Valley, one modification to the existing cropping decision model could be considered, which will involve manipulating native populations of members of the *H. oviparasitica* clade. However, more data will be needed before implementation of this model could be considered.

In this new cropping decision model, if the population densities of both *H. schachtii* and members of the *H. oviparasitica* clade are above their threshold values (Fig. 5D), then a tolerant cultivar of sugar beets could be planted (Heinrichs 2011). This action would be expected to result in both a normal crop yield and the development of a *H. schachtii*-suppressive soil by the time the sugar beets are harvested. This is because fungi belonging to the *H. oviparasitica* clade are potent hyperparasites of sedentary stages of endoparasitic nematodes that can dramatically suppress the populations of several cyst nematode species in one cropping cycle (Kim and Riggs 1995; Olatinwo et al. 2006c; Timper and Riggs 1998).

Evidence supporting the feasibility of the revised cropping decision model is presented as follows. First, normal sugar beet yields from the first cropping cycle are expected because despite the high initial *H. schachtii* population densities, the plants will thrive due to their genetic tolerance to the nematodes. Second, low *H. schachtii* population densities are expected when the first crop of sugar beets is harvested, because these soils will have developed *H. schachtii* suppressiveness. This expectation is based on prior research that has shown that DoUCR50 amendments produced dramatically lower *H. schachtii* population densities after two nematode generations in the presence of a *H. schachtii* host crop (Olatinwo et al. 2006a, 2006b, 2006c). Finally, this suppressiveness should keep *H. schachtii* population densities low in all subsequent cropping cycles as long as these soils are either left fallow or cropped to (i) a *H. schachtii* host crop, (ii) a resistant or tolerant *H. schachtii* host crop, or (iii) one cycle of a non-*H. schachtii* host crop. This expectation is based on prior research as follows. First, host crops of *H. schachtii*, including resistant and tolerant ones, as well as fallow periods, maintained a soil's sugarbeet cyst nematode-suppressiveness, whereas more than one cycle of the nonhost crop wheat led to a decrease in the suppressiveness (Westphal and Becker 2001b). Second, a single amendment of DoUCR50 to a nonsuppressive soil at the time of planting a *H. schachtii* host crop reduced *H. schachtii* population densities to levels observed in the naturally suppressive 9E field soil after two nematode generations. These low levels were maintained over two cropping cycles and at least six *H. schachtii* generations (Olatinwo et al. 2006b). Third, other *H. schachtii* host crops such as Swiss chard were able to produce DoUCR50-induced suppressiveness against *H. schachtii* (Olatinwo et al. 2006c). Furthermore, because several agronomically important and economically desirable vegetable crops are hosts of *H. schachtii* (Dixon 2007; Rimmer et al. 2007), crop rotations that maintain the *H. schachtii* suppressiveness could be implemented to reduce the population densities of other pathogens that may build up under sugar beet monoculture. Overall, if additional data are obtained that support the effectiveness of the proposed cropping decision model, its adoption could enable growers to increase their productivity and economic viability.

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