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Population Dynamics of *Dactylella oviparasitica* and *Heterodera schachtii*: Toward a Decision Model for Sugar Beet Planting

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Abstract: A series of experiments were performed to examine the population dynamics of the sugarbeet cyst nematode, *Heterodera schachtii*, and the nematophagus fungus *Dactylella oviparasitica*. After two nematode generations, the population densities of *H. schachtii* were measured in relation to various initial infestation densities of both *D. oviparasitica* and *H. schachtii*. In general, higher initial population densities of *D. oviparasitica* were associated with lower final population densities of *H. schachtii*. Regression models showed that the initial densities of *D. oviparasitica* were only significant when predicting the final densities of *H. schachtii* J2 and eggs as well as fungal egg parasitism, while the initial densities of J2 were significant for all final *H. schachtii* population density measurements. We also showed that the densities of *H. schachtii*-associated *D. oviparasitica* fluctuate greatly, with rRNA gene numbers going from zero in most field-soil-collected cysts to an average of 4.24×10^8 in mature females isolated directly from root surfaces. Finally, phylogenetic analysis of rRNA genes suggested that *D. oviparasitica* belongs to a clade of nematophagous fungi that includes Arkansas Fungus strain L (ARF-L) and that these fungi are widely distributed. We anticipate that these findings will provide foundational data facilitating the development of more effective decision models for sugar beet planting.

Key words: Dactylella oviparasitica, *Heterodera schachtii*, nematophagous, sugarbeet cyst nematode, suppressive soil, Arkansas Fungus (ARF).

The sugarbeet cyst nematode (*Heterodera schachtii*) is an economically important plant-parasitic nematode that affects a wide range of crop plants including sugar beet, broccoli, cabbage, cauliflower, spinach, Brussels sprouts, rapini, radish, mustard, kale, canola, Swiss chard, etc. (Whitehead, 1998). In California, this nematode is widespread in the Imperial Valley (Caswell and Thomason, 1985) where sugar beet production is most concentrated. To reduce crop damage caused by *H. schachtii*, representatives of the local sugar beet factory, growers, the County Agricultural Commissioner and nematologists from the University of California designed a cropping scheme based on a cyst nematode dump-sample survey (Roberts and Thomason, 1981). A dump sample is a 500-cm^3 representative soil sample collected from sugar beets harvested from an approximately 5-acre area. Fields are considered infested if three or more cysts are found in a sample. Sugar beets cannot be planted in non-infested fields more than two consecutive years and not more than four out of ten years. Sugar beets can be grown only once every four years in infested fields. This nematode management program has been used effectively for half a century. The success of this program is due to the natural decline of *H. schachtii* in the absence of host plants; for example, in the Imperial Valley, annual population decline rates of more than 50% have been reported. In addition, egg densities in four different fields dropped below the detection level during the fourth year under continuous non-host alfalfa (Roberts et al., 1981). The authors suggested that egg parasitism by *Fusarium oxysporum*, *Acremonium strictum* and other fungi reported in Nigh et al. (1980) may be a major cause of *H. schachtii* egg destruction and consequently contribute to the decline of the nematode population.

The nematophagous fungus *Dactylella oviparasitica* was previously identified in an *H. schachtii*-suppressive field soil (9E) at the Agricultural Experimental Station, University of California, Riverside (Westphal and Becker, 2001). Molecular population studies and Koch's postulates investigations showed that *D. oviparasitica* was the primary suppressive agent in this soil (Yin et al., 2003; Olatinwo et al., 2006c). This fungus was earlier described as a parasite of root-knot nematode eggs (Stirling and Mankau, 1978). In field experiments, the addition of *D. oviparasitica* strain 50 to conducive soil reduced *H. schachtii* population densities to a similar level to that found in the suppressive 9E soil (Olatinwo et al., 2006b). In addition, *D. oviparasitica* was capable of suppressing *H. schachtii* in soils with a variety of chemical and physical characteristics (Olatinwo, 2006a). In preliminary surveys, we found that *D. oviparasitica* is widespread in Californian soils (unpublished data). We

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hypothesize that *D. oviparasitica* affects *H. schachtii* populations throughout the sugar beet growing regions, and that utilizing *D. oviparasitica* population densities in planting decision models will make nematode management programs more effective.

Toward this goal, we performed a series of experiments to (i) determine whether final population densities of *H. schachtii* could be predicted by the initial levels of the nematode and the fungus, (ii) compare *D. oviparasitica* population densities in *H. schachtii* cysts collected from field soil and in females collected from roots and, (iii) examine the phylogenetic relationships among *D. oviparasitica* and its close relatives.

Materials and Methods

Greenhouse experiments: The objective of these pot-based greenhouse experiments was to determine whether final population densities of *H. schachtii* could be predicted by the initial population densities of the *H. schachtii* and *D. oviparasitica*. The treatments consisted of a factorial design of four densities of *H. schachtii* (25, 50, 100 and 200 J2/100-cm^3 soil) and four densities of *D. oviparasitica* strain 50 (Table 1), all added to fumigated 9E soil. Control treatments were fumigated and non-fumigated 9E soil infested with *H. schachtii* but not *D. oviparasitica*. All pots were planted with Swiss chard and then assessed after two nematode generations. Two replicate trials were performed, each arranged in a randomized complete block design with five replicates per treatment.

The soil used in this study was a Hanford fine sandy loam (60.8% sand, 29.8% silt and 9.4% clay, 0.7% OM, pH 7.8) obtained from field 9E, located at the Agricultural Experiment Station, University of California, Riverside. This field has been suppressive to *H. schachtii* for about three decades (Borneman and Becker, 2007). The soil was sieved through a 12-mm metal mesh sieve and amended with 20% steam-pasteurized plaster sand to improve physical characteristics before use in root box and greenhouse experiments.

Fungal inocula was prepared by growing *Dactylella oviparasitica* strain 50 (Yin et al., 2003) on potato dextrose agar medium (PDA) at 23 ± 2°C for 21 days prior to being amended to soil in the greenhouse experiments (described below). The fungus and culture medium were blended (Sunbeam 6 Speed Blender Model 4142, Sunbeam Products Inc., Boca Raton, FL) with 25-ml of sterile water for 30 sec. One milliliter of the fungal suspensions was used to determine the colony forming units (CFU) from a dilution series plated on PDA.

Nematode inocula was prepared by infesting sugar beet (*Beta vulgaris* L.) seedlings with second-stage juveniles (J2) of *H. schachtii*. They were maintained in greenhouse pot cultures for approximately 3 months. Cysts were extracted from the soil using a Fenwick flotation can method (Caswell et al., 1985). Soil samples

Table 1. Plant growth parameters and *Heterodera schachtii* population densities after two nematode generations in relation to various initial amounts of *Dactylella oviparasitica* and *H. schachtii*.

Treatments[a]								
	Population densities at beginning of trials		Values measured at the end of the greenhouse trials, two nematode generations after *H. schachtii* infestation[b]					
			Plant weights (g)		Counts in 350-g soil			
Treatment number	*Dactylella oviparasitica* CFU/1,600-cm^3[c]	*H. schachtii* J2/100-cm^3 soil	Dry shoot	Fresh root	Cysts	Eggs	J2/100-cm^3 soil	Parasitized eggs (%)
1	3.3 x 10^7	25	23.5	176.9	71	194	61	25.7
2	3.3 x 10^7	50	19.7	125.0	83	560	121	19.0
3	3.3 x 10^7	100	22.4	137.5	81	614	160	19.7
4	3.3 x 10^7	200	20.6	117.2	118	1,518	300	14.5
5	3.3 x 10^6	25	19.5	152.2	62	274	111	19.6
6	3.3 x 10^6	50	22.1	152.0	70	506	132	15.9
7	3.3 x 10^6	100	20.5	146.2	74	737	220	15.7
8	3.3 x 10^6	200	19.1	122.3	94	859	340	12.5
9	3.3 x 10^5	25	22.3	166.2	67	353	110	18.1
10	3.3 x 10^5	50	19.0	126.4	97	1,101	408	14.8
11	3.3 x 10^5	100	18.9	136.8	87	891	318	10.5
12	3.3 x 10^5	200	19.3	146.6	134	1,791	478	9.4
13	3.3 x 10^4	25	20.5	172.0	79	623	142	14.8
14	3.3 x 10^4	50	21.1	119.5	88	618	227	15.6
15	3.3 x 10^4	100	21.4	136.9	87	1,090	330	12.3
16	3.3 x 10^4	200	17.7	110.7	101	1,195	468	9.8
17	0	100	5.8	8.3	103	359	11	21.5
18	0	100	18.8	138.9	73	600	253	5.8

[a] Treatments (1-18) were a factorial design of 4 CFU levels of *Dactylella oviparasitica* strain 50 (10^7, 10^6, 10^5, and 10^4 CFU/1,600-cm^3 soil) and 4 population levels of *Heterodera schachtii* (25, 50, 100 and 200 J2/100-cm^3 soil); 9E soil (Treatment 17) and fumigated 9E soil without *D. oviparasitica* amendment (Treatment 18) were the positive and negative controls, respectively.

[b] Results from two trials were similar and were combined. Values in the table are the means of 10 replicates pots.

[c] CFU densities presented in the table are the average from both trials.

were placed on modified Baermann funnels (Flegg and Hooper, 1970) containing 4 mM $ZnCl_2$ to stimulate juvenile hatching. Hatched J2 of *H. schachtii* were collected daily and stored in aerated water at 15 ± 1°C for approximately 48 hours before soil infestation. The numbers of J2 in the suspensions were determined under a stereo microscope at 40X magnification.

Twenty-five milliliters of the fungal suspensions for each dilution were added into plastic bags containing 1600-cm^3 of methyl iodide-fumigated 9E soil (Becker et al., 1998). Soils were thoroughly mixed by tumbling the plastic bags, and then transferred to 15-cm-diameter pulp pots. Several Swiss chard seeds (*Beta vulgaris* L. cv. Large White Ribbed, Lockhart Seeds Inc., Stockton, CA) were planted in each pot. Four weeks after seeding, pots were infested with the designated levels of nematodes (see above) by pipetting aqueous suspensions of freshly hatched J2 into the three holes (~5-cm deep and 1.5-cm wide) in the soil near the base of each plant. The control pots (fumigated and non-fumigated 9E soil) were infested with 100 J2/100-cm^3 soil. The plants were maintained in a greenhouse under ambient light at 25 ± 3°C. Soil temperature was monitored for degree-day determination using HOBO Temperature Data Loggers (Onset Computer Corporation, Bourne, MA) buried in an additional pot. After emergence, the seedlings were thinned to one per pot and fertilized with 16 g of slow-release fertilizer (Osmocote 14-14-14, Scotts Co., Marysville, OH). Two replicate greenhouse trials were conducted, separated in time and space, between the months of February and June of 2008.

The greenhouse trials were terminated 13 weeks after nematode inoculation, which corresponded to ~946 degree-days for trial 1, and 957 degree-days for trial 2, calculated by base temperature of 8°C for *H. schachtii* (Curi and Zmoray, 1966). The plants were cut at soil level and the root systems were removed from soil. Shoot and root weights were determined. Cysts were extracted from 350-g sub-samples of soil using a Fenwick flotation method (Caswell et al., 1985). Cysts were counted and then broken in a tissue homogenizer to enumerate the eggs. Parasitism was assessed by examination of 100 randomly selected eggs per sample under light microscopy (Olatinwo, 2006a). Another 100-cm^3 sub-sample of soil was processed with a centrifuge flotation technique for extracting *H. schachtii* J2 (Jenkins, 1964). Eggs, J2 and parasitized eggs were observed and enumerated under an inverted microscope at 40-100X magnification.

Six linear regression analyses were conducted, one for each dependent variable, and regressed with the initial inoculation densities of *D. oviparasitica* and *H. schachtii*. These analyses did not include control treatments 17 and 18 from Table 1. For the associated hypothesis tests to be valid, identical and independent normally distributed residuals were needed. To achieve this, Box-Cox power transformations were performed on both the dependent and independent variables in some cases.

9E field soil and root box experiments: The purpose of these experiments was to compare *D. oviparasitica* population densities in *H. schachtii* cysts collected from the 9E field soil and in females collected from roots grown in the 9E soil. Prior to performing these experiments, the 9E field had not been cropped to a host of *H. schachtii* for approximately 2 years.

For the field soil experiment, field 9E was divided into 16 sections of equal size. Approximately 20 soil cores from each section were collected with an Oakfield sampler (2.5-cm x 10-cm). The samples from each section were pooled, thoroughly mixed and passed through a 12-mm metal mesh sieve. Cysts were extracted from the soil using a Fenwick flotation can method (Caswell et al., 1985). DNA was extracted from the cysts and *D. oviparasitica* rRNA genes PCR amplified as described below.

For the root box experiments, 9E soil from the field experiment describe above was added to root boxes (27-cm x 23.5-cm x 2.5-cm) and seeded with Swiss chard (*Beta vulgaris* subsp. *cicla* 'Large White Ribbed'; Lockhart Seeds Inc., Stockton, CA) as the host crop; root boxes enable the collection of females from root surfaces because one side of the box is transparent and removable. The soil was infested 3 weeks after planting with approximately 1,000 J2 of *H. schachtii* per root box. Young and mature females were collected from the root surface and stored at -80°C for DNA extraction.

DNA was extracted from 200-mg samples of soil, fungi, *H. schachtii* females and *H. schachtii* cysts using the FastDNA Spin Kit for Soil (Qbiogene, Carlsbad, CA) as described by the manufacturer; a 90 sec bead-beating step (5.5 setting) was used for soil and hyphae and 150 sec for cysts using a FastPrep Instrument (Qbiogene). DNA extracts were further purified by subjecting them to electrophoresis on 1% agarose gel, and isolating the DNA above 3Kb using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) without exposing the DNA to UV or ethidium bromide.

Dactylella oviparasitica was quantified using qPCR. Sequence-selective PCR primers targeting the rRNA ITS region of *D. oviparasitica* 50 were designed using the PRISE software (Fu et al., 2008). These primers were DacITSF5 (GGGCTTGTCTGGGTTT) and Dac50ITSR5 (GTGCTGTTACAACCTATAAAT), and amplified a 100-bp fragment. Twenty-five-µl reactions contained the following reagents: 50 mM Tris (pH 8.3), 500 µg/ml BSA, 2.5 mM $MgCl_2$, 250 mM of each dNTP, 400 nM of each primer, 1-µl DNA template, 2-µl 10X SYBR Green I (Invitrogen, Carlsbad, CA) and 1.25 U *Taq* DNA polymerase. Thermal cycling parameters were: 94°C for 5 min; 42 cycles of 94°C for 20 sec, 63°C for 30 sec, and 72°C for 30 sec; followed by 72°C for 10 min. Real-time PCR assays were performed in Bio-Rad iCycler MyiQ Real-Time Detection System (Bio-Rad Laboratories Inc.).

Phylogenetic analyses: PCR was used to obtain rRNA gene sequences of *D. oviparasitica* and the Arkansas

Fungus strain L (ARF-L) for phylogenetic analysis. PCR primers were ITS1Fuser (GGGAAAGUCTTGGTCAT TTAGAGGAAGTAA) (Gardes and Bruns, 1993) and ITS4user (TCCTCCGCTTATTGATATGC) (White et al., 1990) for the internal transcribed spacer region (ITS) and LR0R (ACCCGCTGAACTTAAGC) and LR5 (TC CTGAGGGAAACTTCG) (Vilgalys and Hester, 1990) for the large subunit (LSU) rRNA gene. Thermal cycling conditions were: 94°C for 5 min, followed by X cycles of 94°C for 20 sec, 52°C for 20 sec, 72°C for Y sec, and a final incubation at 72°C for 5 min; for ITS, X = 40 and Y = 40, for LSU, X = 35 and Y = 50. For the ITS region, 10-µl amplification reactions were performed in 10-µL glass capillary tubes using a RapidCycler (Idaho Technologies, Salt Lake City, UT) containing the following reagents: 50 mM Tris (pH 8.3), 500 µg/ml bovine serum albumin (BSA), 2.5 mM $MgCl_2$, 250 µM of each deoxynucleotide triphosphate (dNTP), 400 nM of each primer, 1-µl DNA template, and 0.5 U *Taq* DNA polymerase. For the LSU region, PCRs were performed in an MJ Research PTC-200 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA) in 25-µl amplification reactions containing the reagents described above. Amplification products were isolated, cloned and sequenced as previously described (Bent et al., 2009). Sequence identities were determined by analyses using BLAST (NCBI) (Altschul et al., 1997).

Phylogenetic trees were constructed using the LSU and ITS rRNA gene sequences obtained in this study, and their closest relatives determined by analyses using BLAST. Sequences were aligned using the ClustalW algorithm (Thompson et al., 1994). The alignments were 847-910 bp (LSU) and 334-418 bp (ITS), after highly variable regions were removed. Phylogenetic trees were generated using the Geneious Tree Builder (Geneious Pro 5.1.7, Biomatters Ltd, Auckland, New Zealand) using the UPGMA method (Sneath and Sokay, 1973) and the Tamura-Nei genetic distance model (Tamura and Nei, 1993) with 1000 bootstrap samplings (Efron, 1979). GenBank accession numbers for rRNA gene sequences identified in this study were: *D. oviparasitica* strain 50 (ITS, JQ638668; LSU, JQ638669), *D. oviparasitica* strain 60 (ITS, JQ638670), ARF-L (ITS, JQ638671; LSU, JQ638672).

Results

Relationships between the population densities of D. oviparasitica and H. schachtii: In the greenhouse trials, at approximately 950 degree-days after *H. schachtii* infestation, initial population densities of *D. oviparasitica* and *H. schachtii* were regressed with final densities of *H. schachtii* using the combined data from two trials (Table 1). Initial population densities of *D. oviparasitica* were significantly associated with final population densities of *H. schachtii* J2 and eggs, and *H. schachtii* egg parasitism, but not with the population densities of *H. schachtii* cysts (Table 2). Initial population densities of *H. schachtii* J2 were significant for all *H. schachtii* variables measured at harvest (Table 2). In the models that included only the initial J2 densities, there was a significant negative relationship when the two plant measurements were the dependent variables; in other words, as the initial J2 densities increased, the final plant weights decreased (regression models not shown; fresh roots ($R^2 = 0.026$, $P = 0.033$), dry shoots ($R^2 = 0.014$, $P = 0.032$). In the other model that included only the initial J2 densities, the final densities of cysts exhibited a significantly positive relationship with initial J2 densities (Table 2).

The fitted models that included both the initial population densities of *D. oviparasitica* and *H. schachtii* J2 are shown in response surface plots (Figs. 1–2). The

Table 2. A summary of the model for each dependent variable and the transformations used in the linear regression analyses.

Regression model	Coefficient	Coefficient estimate	Coefficient standard error	P-value	R^2
$\log(fJ2) = \beta_0 + \beta_1\log(iJ2) + \beta_2\log(iDac)$	β_0	2.91	0.41	0.000	0.208
	β_1	0.64	0.09	0.000	
	β_2	−0.09	0.03	0.001	
$(fegg)^{0.2} = \beta_0 + \beta_1(iJ2)^{0.2} + \beta_2(iDac)^{0.2}$	β_0	1.72	0.42	0.000	0.158
	β_1	0.85	0.17	0.000	
	β_2	−0.07	0.03	0.018	
$\log(par/(1\text{-}par)) = \beta_0 + \beta_1\log(iJ2) + \beta_2\log(iDac)$	β_0	−1.07	0.25	0.001	0.285
	β_1	−0.29	0.05	0.000	
	β_2	0.08	0.02	0.000	
$\log(fcys) = \beta_0 + \beta_1 iJ2$	β_0	4.18	0.06	0.000	0.071
	β_1	0.00	0.00	0.000	

iJ2 = Initial numbers of *H. schachtii* J2/100-cm^3 soil.
iDac = Initial amount of *D. oviparasitica* (CFU/cm^3 soil).
fJ2 = Final numbers of *H. schachtii* J2/100-cm^3 soil.
fegg = Final numbers of *H. schachtii* eggs/350-g soil.
par = Parasitism (%).
fcys = Final numbers of *H. schachtii* cysts/350-g soil.

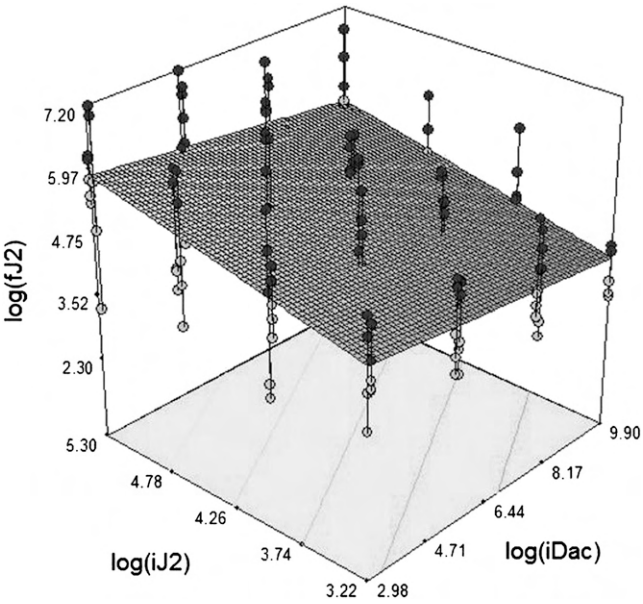

FIG. 1. Response surface plot predicting final *Heterodera schachtii* J2 densities. iJ2 represents the initial numbers of J2/100-cm^3 soil, iDac is the initial amount of *Dactylella oviparasitica* (CFU/ cm^3 soil) and fJ2 is the final numbers of J2/100-cm^3 soil. The dark points above the plane are greater than the predicted value and the lighter points below the plane are smaller than the predicted value.

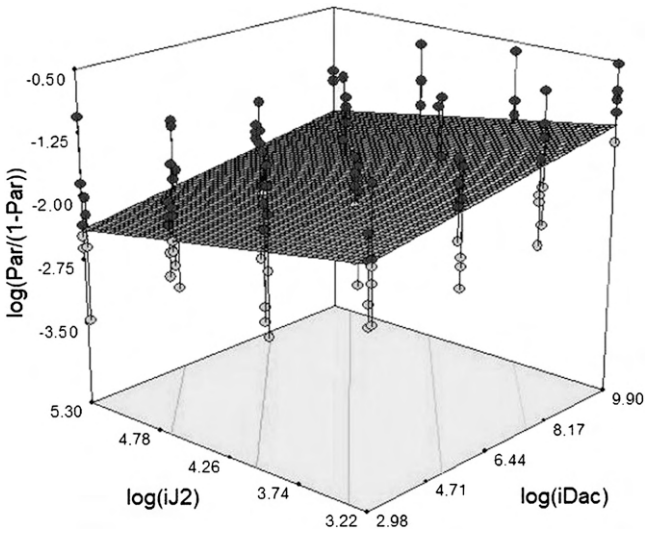

FIG. 2. Response surface plots predicting fungal egg parasitism. iJ2 represents the initial numbers of *Heterodera schachtii* J2/100-cm^3 soil, iDac is the initial amount of *Dactylella oviparasitica* (CFU/ cm^3 soil) and Par is the percentage of parasitism. The dark points above the plane are greater than the predicted value and the lighter points below the plane are smaller than the predicted value.

darker points in the plot are observed values that are above the predicted response surface while the lighter points are the observed values that are below the predicted response surface. The plots show the effect that a change in one of the independent variables has on the predicted value of the dependent variable. For example, when predicting the final densities of J2 (Fig. 1) or eggs (data not shown), these values increase as the initial density of J2 increases, and vice versa. In the case of predicting fungal egg parasitism, the reverse is true (Fig. 2).

Dactylella oviparasitica in H. schachtii cysts: Using a sequence-selective qPCR assay, the population densities of *D. oviparasitica* were enumerated in *H. schachtii* cysts collected from the suppressive 9E field soil. *Dactylella oviparasitica* was detected in only 2 of 16 sections of field 9E, and the amounts measured in these regions were very low: 18.3 and 5.15 rRNA genes per cyst. Conversely, *D. oviparasitica* was detected in all *H. schachtii* females obtained from Swiss chard roots grown in root boxes containing these same soils. The main difference between field soil and root box samples was that the *H. schachtii* obtained from Swiss chard roots grown in the root boxes were considerably earlier in development. In addition, the amounts of *D. oviparasitica* in the root box-derived females were much higher than those in cysts recovered from field soil, with average rRNA genes per mature female being 6.14 x 10^8 and 2.34 x 10^8 after one and two nematode generations, respectively, and average rRNA genes per young female being 7.35 x 10^2 and 2.10 x 10^5 after one and two nematode generations, respectively.

Relationship of Dactylella oviparasitica strain 50 to ARF-L: Phylogenetic analysis of *D. oviparasitica* and its closest relatives showed that they form a clade of fungi comprised of at least one other nematophagous fungus – Arkansas Fungus strain L (ARF-L). An rRNA ITS analysis showed that these two fungi belong to an assemblage of organisms (see DO Clade) with broad geographical distribution, including Austria, China, France, Germany, United States, and Norway (Fig. 3). Analysis of the LSU rRNA gene confirmed the association between *D. oviparasitica* 50 and ARF-L (Fig. 4); LSU sequences for the other *D. oviparasitica*-clade members (DO Clade, Fig. 3) from the ITS tree were not in GenBank (NCBI), and thus were not included in the LSU tree. Analysis of the 5.8S rRNA gene showed that *D. oviparasitica* strain 50 and ARF-L exhibited 97% sequence identity, indicating that they are distinct members of this clade.

DISCUSSION

We posit that planting decision models for sugar beets will be enhanced by incorporating population densities of *D. oviparasitica* and related fungi. Factors supporting this statement include *D. oviparasitica*'s wide distribution in sugar beet growing regions and its abilities to reduce *H. schachtii* populations in field conditions and in soils with different chemical and physical characteristics (Olatinwo et al., 2006a,b,c). In this report, results from our greenhouse experiments showed an inverse relationship between the amounts of *D. oviparasitica* and *H. schachtii*. To develop effective planting decision models, this line of investigation should be expanded to include field-based experiments delineating *D. oviparasitica-H.*

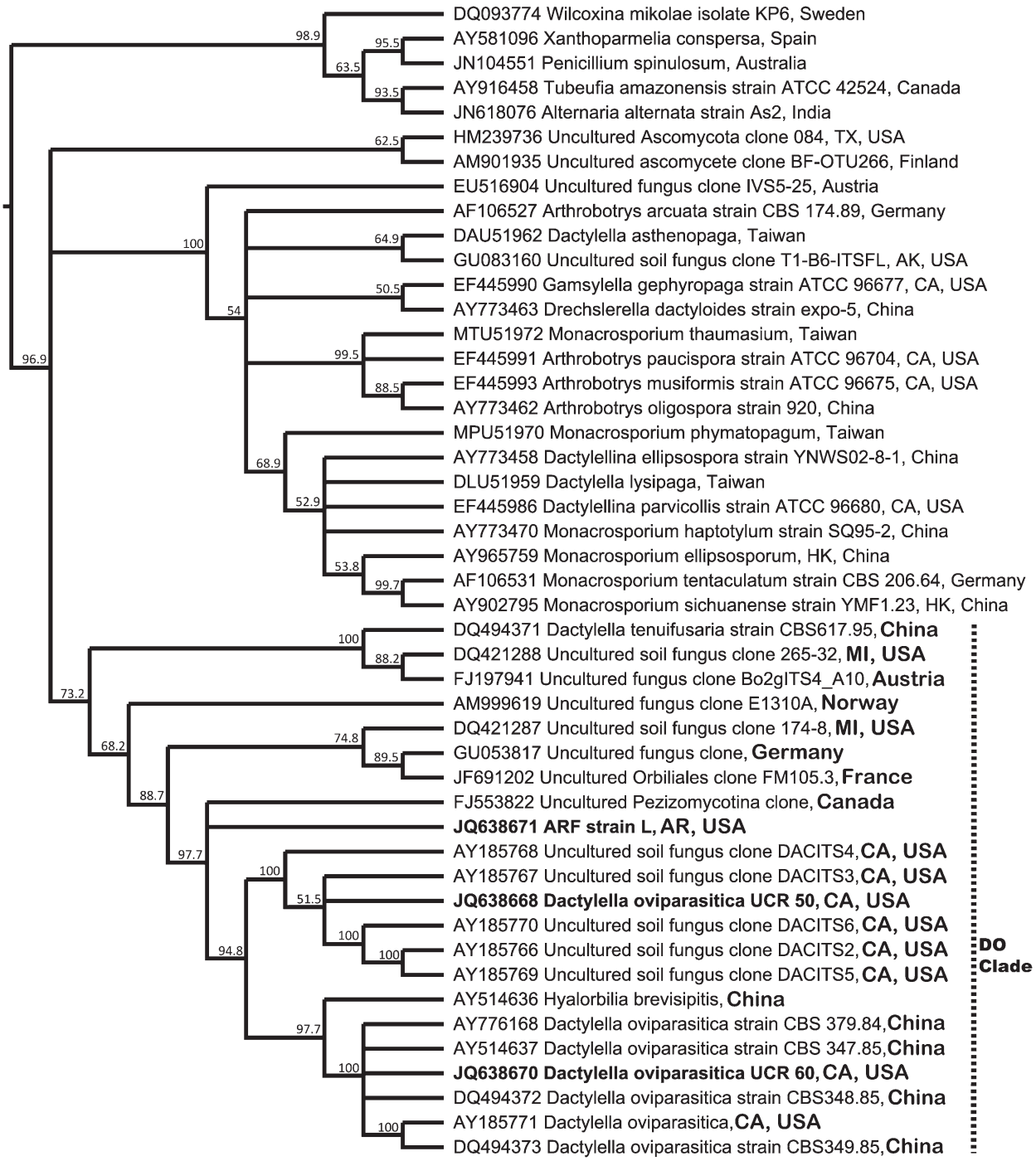

FIG. 3. Phylogenetic analysis of *Dactylella oviparasitica* and related fungi using the rRNA internal transcribed spacer (ITS) region. The analysis includes 47 sequences between 847-910 bp in length. The tree was constructed using the UPGMA method and the Tamura-Nei genetic distance model with 1000 bootstrap samplings.

schachtii associations in relation to cropping regimens and time. For example, if two soils with high amounts of *H. schachtii* are compared, with one containing high amounts of *D. oviparasitica* while the other contains low amounts, we would predict that the soil with higher *D. oviparasitica* populations would have lower *H. schachtii* populations within two nematode generations if both were planted with a host crop. Future investigations should test such predictions.

Implementing more effective planting decision models also will require biologically meaningful measurements of *D. oviparasitica* population densities. In this study, we detected huge differences in the amounts of *D. oviparasitica* in field-collected cysts and root box-obtained *H. schachtii* females. We speculate that parasitism of *D. oviparasitica* is initiated at the late juvenile stages or at the young female stage when the developing nematode first breaks through the root surface,

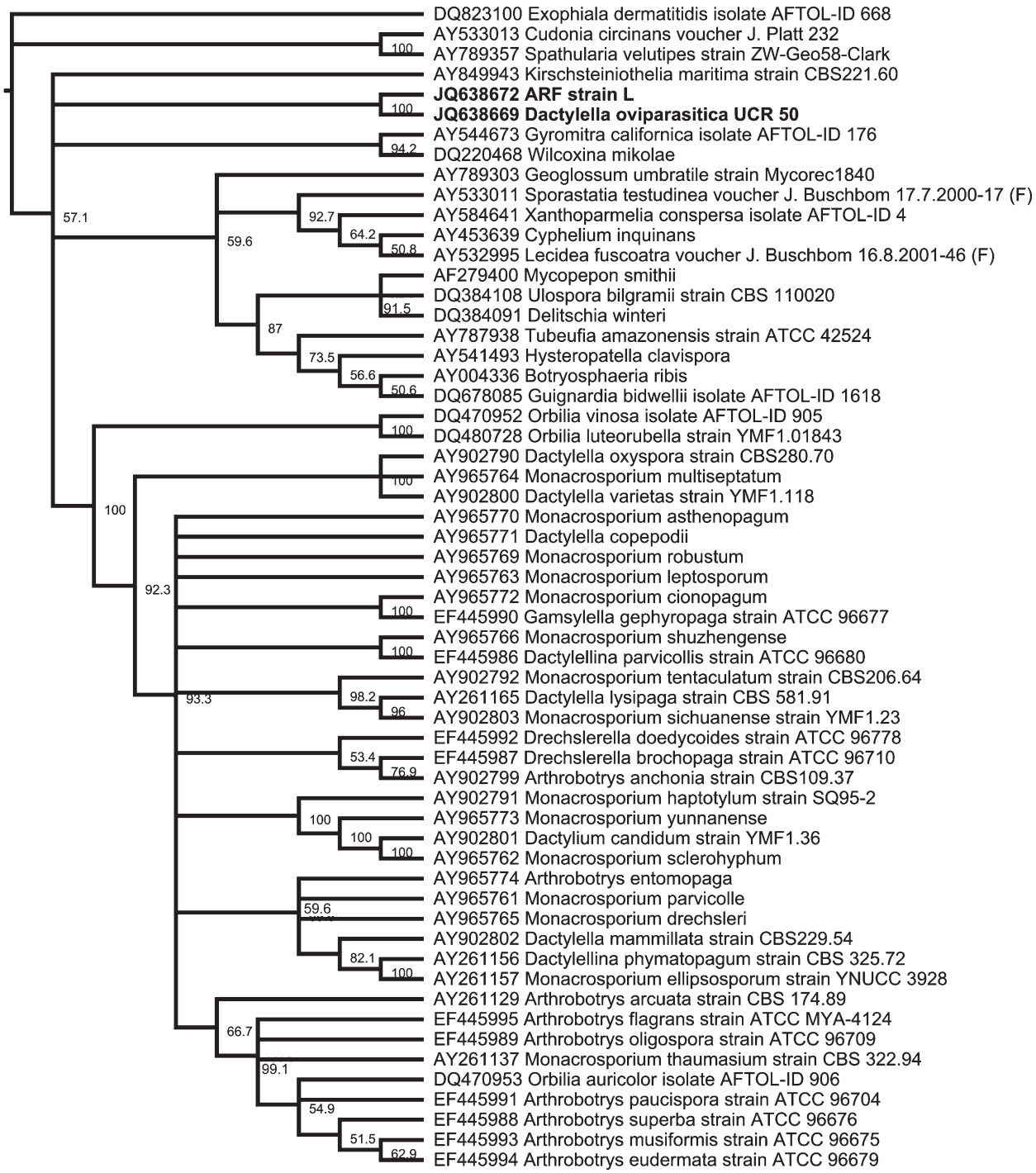

FIG. 4. Phylogenetic analysis of *Dactylella oviparasitica* and related fungi using the rRNA large subunit (LSU) gene. The analysis includes 58 sequences between 334-418 bp in length. The tree was constructed using the UPGMA method and the Tamura-Nei genetic distance model with 1000 bootstrap samplings.

exposing its posterior end to the rhizosphere. In the cysts, other microorganisms compete with *D. oviparasitica* and eventually replace this fungus, resulting in a drop of its detectable rRNA gene levels. Consequently, we find *D. oviparasitica* at very low levels in cysts from the suppressive field soil. In the context of taking measurements for a cropping decision model, these results suggest that *D. oviparasitica* populations will need to be measured in a host plant bioassay in which sedentary juveniles and females serve as semi-selective baits.

Finally, the shared characteristics of *D. oviparasitica* and ARF-L suggest that other members of this assemblage (DO Clade, Fig. 3) may have similar nematophagous capabilities. *Dactylella oviparasitica* strain 50 shares many similarities with ARF-L, which was identified as a parasite of the soybean cyst nematode (*Heterodera glycines*) by

University of Arkansas researchers in the 1990s (Kim and Riggs, 1991). Both *D. oviparasitica* and ARF-L are capable of parasitizing species of root-knot nematodes and cyst nematodes (Kim and Riggs, 1991; Stirling, 1991). Variation in parasitism rates of different ARF strains have been reported on soybean cyst nematodes (Timper and Riggs, 1998). Both *D. oviparasitica* strain 50 and ARF-L are filamentous, non-sporulating fungi with thin hyphae. Both fungi infected white females of *H. schachtii*, yet neither was able to parasitize viable eggs *in vitro* (Smith Becker et al, 2011). Future research should assess the abilities of the different fungal subtypes (DO Clade, Fig. 3) to parasitize and reduce populations of various cyst and root-knot nematodes. As was done in this study for *D. oviparasitica* strain 50, sequence-selective qPCR assays could be developed and used to enumerate the population densities of the subtypes, which could then be incorporated into planting models. More broadly, given that *D. oviparasitica* and related organisms comprise a clade of fungi containing effective biological control agents targeting several economically important nematodes, and that similar fungi have been identified on several continents, this general approach may prove to be useful for a wide range of crops in other geographical locations.

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