Identification and Distribution of Fungal Parasites of *Heterodera schachtii* Eggs in California

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ABSTRACT

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Acremonium strictum and/or *Fusarium oxysporum* were isolated from eggs within *Heterodera schachtii* young females and cysts in 20 of 25 fields infested with the nematode. Investigation of *H. schachtii* egg parasitization in greenhouse cultures indicated the presence of the nematode and one or both fungi in 26 of 29 soils tested. Both fungi parasitized eggs on agar and in soil when either mycelial or conidial inocula were used. The fungi also parasitized eggs of other nematodes on agar and both fungi grew saprophytically in *H. schachtii* eggs killed by heat. *A. strictum* and *F. oxysporum* invade the female of *H. schachtii* through natural openings. There was no destruction of the cuticle or disruption of subsequent development of the cyst wall. In the greenhouse, *A. strictum* was weakly parasitic on seven of nine plants tested, including sugar beet. The amount of parasitism exhibited by isolates of both fungi in laboratory and in greenhouse studies suggested that the fungi may be useful in the control of *H. schachtii* besides being important as plant pathogens in some crops.

Additional key words: biological control.

Plant pathologists are becoming more aware of the potential contribution of naturally-occurring biological control agents in the control of pathogenic nematodes. Two recent studies have suggested that low populations of two major nematode pests were attributable to biological control. An *Entomophthora*-like fungus parasitizing *Heterodera avenae* Woll. females has been found (8) and recently *Dactylella oviparasitica* Stirling, an egg parasite of *Meloidogyne*, has been described (15). In 1978, Tribe (17) published a review of the pathogens of cyst nematodes and indicated that two major female parasites and three major egg parasites were widespread in European populations of the nematodes, but not in the few United States samples that had been examined.

Recently, *Fusarium oxysporum* Schlecht and *F. solani* (Mart) Appel & Wor. were reported associated with *Globodera rostochiensis* (Woll.) Stone eggs in a population with large numbers of dead eggs and larvae within the cysts (6). An unknown fungus was suspected as the causal organism rather than the two *Fusarium* spp. isolates. *Fusarium* spp., including *F. oxysporum*, and *Acremonium bacillosporium* Gams were among the ovicidal fungi reported by Lysek (10) to parasitize *Ascaris* eggs.

Thomason (*unpublished*) found low populations of *Heterodera schachtii* Schmidt in fields planted for several consecutive years to hosts under conditions apparently suitable for the nematode. Fungal parasitism of cyst nematode eggs in field soil used in greenhouse cultures prompted a search for pathogens of the eggs of the nematode in California. Fields in beet-growing areas of the state known to have populations of the nematode were sampled to determine the presence of egg parasites. Results of the survey and methods for isolation and culture of fungal egg pathogens are described in this paper.

Experiments described in this paper were designed to determine whether any *Acremonium strictum* Gams or *F. oxysporum* Schlecht were actively parasitizing *H. schachtii* eggs rather than living saprophytically in old and dead eggs and to determine invasion and proliferation of the fungi in females and their subsequent penetration into eggs. Since both fungi are known to be plant parasites, a plant host range study was conducted to determine the plant parasitic ability of isolates from nematode eggs. Aspects of the biology and ecology of the two fungi in relation to their potential as biological control agents of *H. schachtii* also are discussed.

MATERIALS AND METHODS

Collection of field samples. Thirty-two fields with a history of sugar beets in five beet-growing areas of California were sampled for *H. schachtii* in 1978. Fields were located in the Imperial Valley, the Oxnard Plain, Riverside County, the Salinas Valley, and San Joaquin County. Soil types included sandy loam, sandy clay loam, silty clay loam, silty clay, and clay. The distribution of *H. schachtii* in these areas was recently described (3). Histories of the fields varied from continuous cropping to sugar beets for 5–8 yr to being out of beets for 10 yr. Soil samples 1–30 cm deep were taken in a zig-zag pattern throughout a field with a 2.5-cm-diameter Oakfield sampling tube. Samples were composited, mixed well, and two 600-g subsamples were taken for egg extraction.

Egg extraction procedures. Soil samples were soaked in water for 24–48 hr to increase dispersal of soil. Cysts were extracted from the soil by using a modified Fenwick flotation can. Organic material was separated from cysts on a 850-μm sieve and the cysts were collected on a 150-μm sieve. Cysts were washed onto filter paper and counted by handpicking into water all cysts from each sample with the aid of a dissecting microscope. Eggs were released from cysts by blending the cysts in a homogenizer for 30 sec, care being taken not to break the egg shells when crushing cysts and separating egg masses.

Assessment of fungal parasites in eggs. A modification of Kerry's method (8) was used. Egg suspensions were passed through a 250-μm sieve to remove broken cyst walls and other debris. The eggs were collected on a 25-μm sieve, washed several times with sterile distilled water, and transferred to a measuring cylinder. Volume was made up to 5, 10, or 20 ml of sterile distilled water containing 1 ml of 0.05% streptomycin sulfate and 1 ml of 0.1% penicillin solutions/total 10 ml water. Choice of suspension volume depended on estimated number of eggs per sample. After the suspensions were agitated, a 1.0-ml sample was removed for counting, and a 0.5-ml sample was pipetted onto five 5-cm-diameter plates containing 0.8% distilled-water agar. The plates were incubated at 25 C for 24–48 hr and 100 eggs per plates examined at random for growth of fungal hyphae (Figs. 1–4). To aid in the identification of fungal parasites, 10 eggs from every 100 examined were transferred to plates containing weak potato-dextrose agar

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(PDA: 4.0 g potatoes, 20.0 g dextrose, and 15 g agar per liter of water) to allow fungi to grow and sporulate. Single-spore isolates on PDA were made of several egg-isolates of *A. strictum* and *F. oxysporum* to determine cultural variation and the wild type.

Bioassay. Two 1,500-g subsamples were taken from soil composites of each field and placed in 15-cm-diameter clay pots. A 3-wk-old sugar beet seedling (*Beta vulgaris* L. 'USH 10') was planted in each pot and placed in a greenhouse where air temperatures varied from 24 to 30 C. Ninety days later, soil from the pots was processed as previously described to extract cysts. Females and young cysts were washed from the beet roots by forcefully spraying the roots with water and collecting the nematodes on a 850-μm sieve. The cysts from the soil and roots were combined and a 100-cyst subsample was taken from each pot. Eggs were released and suspended in water and fungal parasites were assessed as described before.

There was little variation in any of the single-spore isolates of either *A. strictum* or *F. oxysporum*. Characteristics of the wild types of both fungi were within the ranges of descriptions made by the respective authorities. The isolates used in later experiments were chosen because they came from fields of varied histories and locations and not because of any particular growth characteristics. The *A. strictum* isolates were: isolate 1 from a Riverside field with a history of five consecutive sugar beet crops; isolate 2 from a field out of beets for 3 yr; isolate 3 from a field on the Oxnard Plain that had been in cruciferous crops for at least 10 seasons; and isolates 4 and 5 from Shasta daisy (*Chrysanthemum maximum* Ramond) (provided by A. R. Chase, University of California, Riverside). The *F. oxysporum* isolates were: isolate 1 from a field in the Salinas Valley that had been in sugar beets for 5 yr; isolate 2 from a field in the Salinas Valley in rotation with sugar beets and just out of sugar beets; and isolate 3 from a field in the Imperial Valley in alfalfa for 2 yr.

Parasitization of eggs. The five isolates of *A. strictum* and three isolates of *F. oxysporum* were grown for 3 days on PDA in petri plates. Plugs 5 mm in diameter were taken from the outer edge of the colonies and placed on the center of eight distilled-water agar (WA) plates 5 cm in diameter. Two days later, three young females of *H. schachtii* selected from growth chamber-grown sugar beets in sterilized soil were added to each plate. This method of culturing *H. schachtii* provided young females free of parasitizing fungi. Controls were young females on WA plates without any fungus.

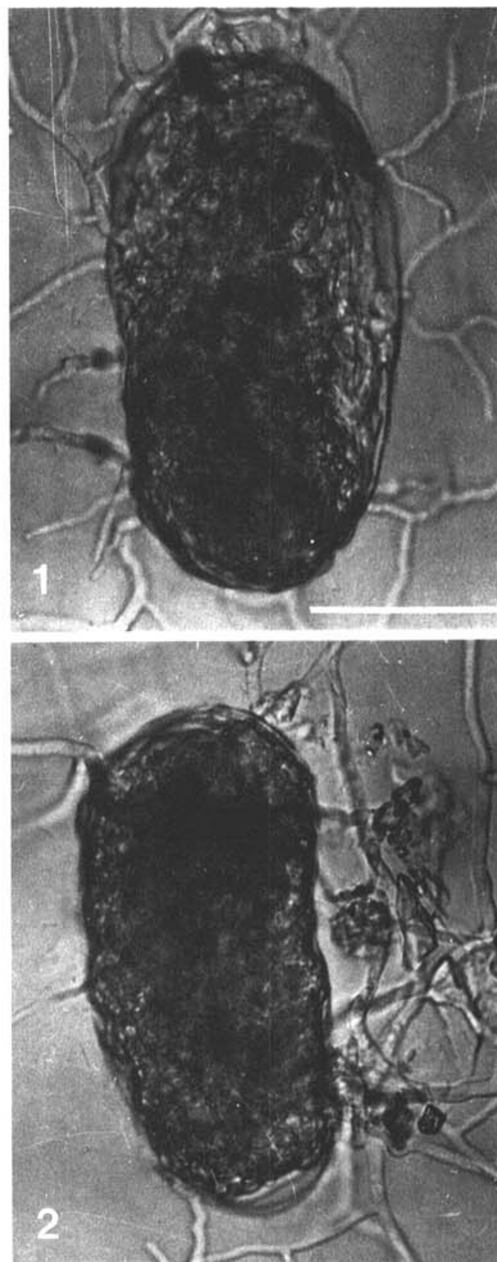
The plates were maintained in a laboratory at ~25 C for an additional 10 days. The females from each of six plates in each treatment were removed and crushed and the eggs were extracted and plated on WA. Hatched juveniles were counted on the WA plates after the females had been removed.

Parasitized and unparasitized eggs were counted by using a dissecting microscope. Some eggs were placed in drops of water on slides and observed at a magnification of ×100 to determine whether the fungus was inside the egg or merely associated with it superficially. Individual eggs were transferred to weak PDA for reisolation and identification of the fungus from parasitized eggs. Eggs from control plates were transferred to assure that they were not infected.

Eggs of *H. cacti* Filipjev & Stek, *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, *Tylenchulus semipenetrans* Cobb, and *Ascaris suum* Goeze were placed on WA plates of *F. oxysporum* and *A. strictum* and parasitism determined as described. Reisolations were made on PDA.

Infestation of dead eggs on WA. *A. strictum* and *F. oxysporum* were grown on PDA and placed on WA as described. Young females of *H. schachtii* collected from sugar beets were heated in water at 60 C for 5 min or were untreated. Three females were placed on each of six WA plates per treatment and infestation was assessed after 10 days. Control treatments were heat-killed and living females were plated on WA without fungus.

Parasitization of eggs in autoclaved soil. Mycelia of isolate 1 of *A. strictum* and *F. oxysporum* were obtained from the outer edge of 3-day-old colonies of each fungus on PDA. Thirty-five plugs were macerated in potato-dextrose broth (PDB) with a Waring blender. Suspensions of known quantities of the mycelium in PDB



Figs. 1–2. 1, *Fusarium oxysporum* growing on water agar from Heterodera *schachtii* egg collected in the field. **2,** *Acremonium strictum* growing on water agar from egg collected in the field. Bar represents 25 μm.

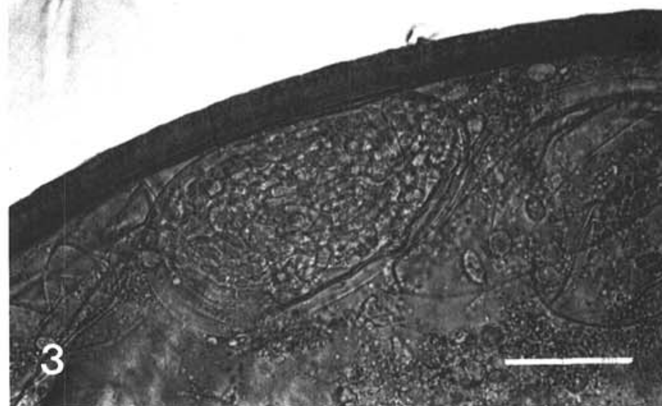


Fig. 3. Fungal hyphae within eggs and body of *Heterodera schachtii* cyst with normally-developed thick cyst wall. Bar represents 25 μm.

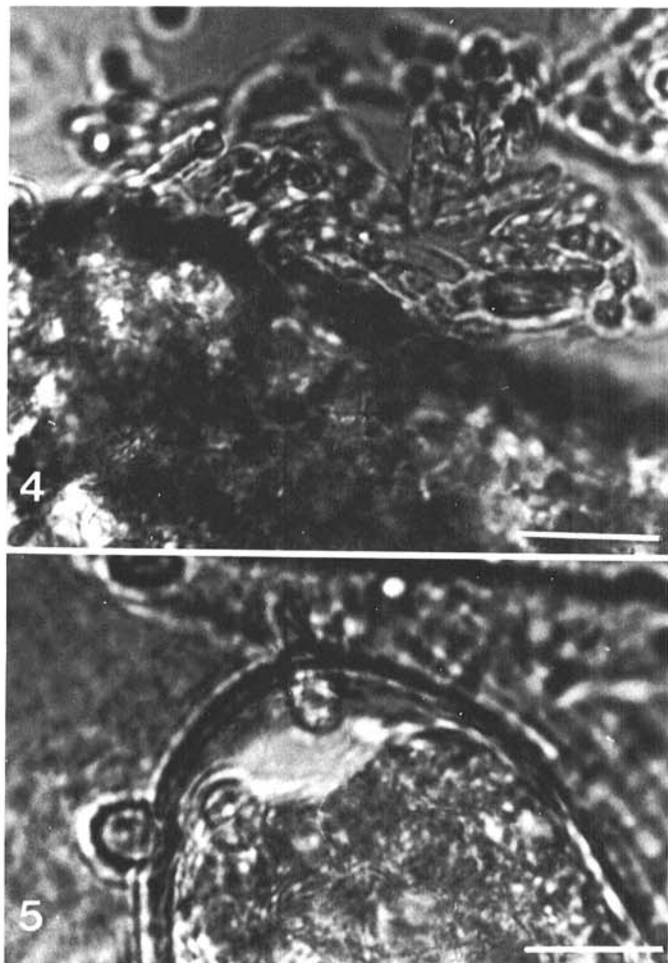
were pipetted into an autoclaved, dry, sandy loam soil, and the soil moisture content was adjusted to 8% (about 75% of field capacity). Soil was amended with 1% cornmeal (w/w) before autoclaving. The dry-weight equivalent of the mycelium was obtained by oven-drying a sample of each isolate and comparing it with an oven-dried sample from 35 PDA plugs macerated in PDB. Each fungal suspension was mixed thoroughly into the soil, and 10-g subsamples were placed in each of eight sterilized glass vials. Four young females from growth chamber-grown sugar beets were placed in the soil and the caps were loosely fitted to allow gaseous exchange. The vials were maintained at 25 C for 25 days. Females were then removed and eggs counted. Few juveniles hatched in this system so no counts of hatched juveniles were made.

Conidia of isolates 1 of both *A. strictum* and *F. oxysporum* were obtained by washing the surface of 7-day-old PDA cultures with sterile water. Conidia of *A. strictum* and macroconidia of *F. oxysporum* were counted by using a hemocytometer, and dilutions made with PDB to prepare 1.0-ml subsamples of 10^5 conidia ml. These subsamples were thoroughly mixed with 80 g of autoclaved, cornmeal-amended, dry sandy loam soil for each fungus to give 10^5 conidia per gram dry soil samples. Soil moisture was adjusted to 8%. Ten grams of soil was placed in each of eight glass vials. Four young females from growth chamber-grown sugar beets were placed in the soil and the caps loosely fitted to allow gaseous exchange. Vials were kept at 25 C for 25 days before parasitism was assessed.

Development of parasitism within *H. schachtii* females and individual eggs. Females of *H. schachtii* were added to mycelium of one of the two fungi (isolates *A. strictum* 1 and *F. oxysporum* 1) on WA plates, using techniques described earlier. Plates were incubated in the laboratory at 25 C, and females and eggs were examined periodically by squashing the females in water on slides and observing them microscopically. Conidial suspensions of *A. strictum* and *F. oxysporum* were made with sterile water and pipetted onto WA plates. Twenty-four hours later, *H. schachtii* egg suspensions were pipetted onto these plates and incubated in the laboratory at 25 C. The plates were examined periodically to observe conidial germination, growth of hyphae, and hyphal contact with eggs. Individual eggs were identified and their stages of development were noted to determine parasitism of eggs in different developmental stages. The plates were observed for 10 days. To determine chitinase activity of *A. strictum* and *F. oxysporum,* the fungi were grown on agar containing colloidal chitin prepared as described by Lingappa and Lockwood (9).

Plant host range. Seedlings of each plant cultivar tested were germinated in steamed UC mix soil (11) in 10.0 cm-diameter clay pots. Plants were thinned to four plants per pot and replicated five pots per cultivar. Fourteen days after germination, 10-ml suspensions of 5×10^5 *A. strictum* conidia per pot were poured around the plants. Control plants were drenched with sterile water. For *F. oxysporum*, seeds were germinated in steamed vermiculite. One week after germination, roots of 20 seedlings of each plant tested were dipped in a 1.1×10^6 conidia per milliliter suspension of macroconidia for 15 min, and the seedlings were transplanted into steamed UC mix soil in 10.0-cm-diameter clay pots. Four seedlings were planted in each of five pots. Twenty milliliters of the prepared conidial suspension was used as drench inoculum in each of the pots after transplanting. Control plants were dipped in sterile water and drenched with 20 ml sterile water.

All plants were observed for symptoms of disease 10–14 days after inoculation and weekly for 8 wk. After 8 wk, plants from all treatments were removed from the pots, washed thoroughly in tap water and finally in sterile water. Five root and stem pieces from each plant were plated on weak PDA and observed for growth of *A. strictum* in seven days and *F. oxysporum* in 3 to 4 days. Transfers from root or stem isolates were made to PDA for identification.



Figs. 4–5. 4, A loose network of *Acremonium strictum* hyphae on *Heterodera schachtii* egg surface prior to development of visible hyphae within the egg. **5,** Terminal swellings of *Fusarium oxysporum* on *H. schachtii* egg surface prior to development of visible hyphae within the egg. Bar represents 10 μm.

RESULTS

Field samples. Fungal egg parasites were widespread in *H. schachtii* populations in surveyed fields. Twenty different fungi were isolated from eggs. Only two fungi, *A. strictum* and *F. oxysporum,* occurred with any degree of consistency throughout the sugar beet growing area (Table 1). Other fungi isolated included *Phoma, Chaetomium, Alternaria, Fusarium, Cephalosporium, Cylindrocarpon,* and *Penicillium* spp.

Isolations directly from cysts in field samples indicated that *A. strictum* was rare in populations from southern areas of the state. *F. oxysporum* occurred in four of the five areas sampled. No comparisons of degree of parasitism could be made among the fields because of their varied histories. Both fungi were isolated from eggs collected from field 6, Imperial Valley (Table 1), that had been out of sugar beets for 10 yr.

Bioassay. Greenhouse cultures indicated that the nematode was present in 26 of 29 soils tested (Table 2). *A. strictum* was present in 23 fields and *F. oxysporum* in 20 fields. Under greenhouse conditions, both *A. strictum* and *F. oxysporum* were detected in soil from all four areas. From 1.0–85.3% of the eggs were parasitized. Sensitivity of detection of nematodes and parasites was increased by bioassaying field samples in the greenhouse rather than extracting cysts from field soil and isolating from their eggs. Only rarely were other fungi isolated from diseased eggs. In both field and greenhouse samples, eggs containing juveniles only occasionally were parasitized.

Parasitization of eggs on water agar. All isolates of *A. strictum* and *F. oxysporum* parasitized eggs, but *A. strictum* isolate 4 from Shasta daisy was significantly less parasitic than were the other isolates tested (Table 3). Considering that juveniles apparently are not easily parasitized, the two fungi generally parasitized 80% of the developing eggs. Both fungi were easily reisolated from parasitized eggs. Dead eggs were readily used for a substrate, since both fungi grew from 98% of the eggs treated with heat. Eggs

collected from field samples (Figs. 1–2) were similar in appearance to those parasitized on water agar. None of the other fungi tested parasitized *H. schachtii* eggs. Rarely was there any particular association by these fungi with the egg. *Monacrosporium ellipsosporium*, a fungus capable of trapping second-stage juveniles, was not active because few juveniles hatched from the eggs.

Eggs of five other nematode species tested were parasitized. Parasitization ranged from 21% of *T. semipenetrans* eggs to 92% of *Meloidogyne* spp. eggs. The low levels of parasitization in eggs of *T. semipenetrans* may have been partly due to the large proportion of eggs in the J_2 stage which are not readily parasitized. Sixty-two percent of the *A. suum* and 67% of the *H. cacti* eggs were parasitized.

Parasitization of eggs in soil. In soil, *A. strictum* parasitized significantly more eggs than did *F. oxysporum* (Table 4). The number of eggs readily available to be parasitized by both fungi was about equal. When less than 1.0 mg of dry mycelium per gram of soil was used as inoculum, the percent parasitization of eggs was reduced.

When conidia were added to soil at an inoculum density at 10^5 conidia per gram of soil, the amount of parasitism by *A. strictum* and *F. oxysporum* was the same as when mycelium was used as inoculum. Parasitization was 58% for *A. strictum* and 38% for *F. oxysporum;* with an average of 30% of the eggs available in J_2 stage.

Parasitization of eggs within *H. schachtii* females and in individual eggs. *A. strictum* and *F. oxysporum* invaded females rapidly; hyphae were observed proliferating throughout the mucilage surrounding eggs in females 18–20 hr after females were placed on the fungi. No cuticle damage was observed. In some older females, thick cyst wall developed normally even though the fungi were within the body and eggs of the nematode (Fig. 3).

A loose network of hyphae was formed among the eggs by *A. strictum* before mycelium was seen inside (Fig. 4). Terminal swellings formed on the hyphae when *F. oxysporum* contacted eggs and possibly acted as appressoria (4) (Fig. 5). They often followed the contour of the egg shell and appeared tightly appressed to its surface. Penetration of eggs from these swellings was not observed. Both fungi were able to parasitize eggs that were in pre-J_1 stages of development. Seventy hours after inoculation, some eggs contained visible hyphae, and the number of parasitized eggs increased rapidly with time.

During early stages of parasitism, hyphae completely occupied the contents of the egg. After the contents were consumed, the hyphae became vacuolate, and eventually the hyphal protoplasm disappeared completely. At this stage it was difficult to recognize previously parasitized eggs, since they looked empty. When these eggs were plated on agar, often the fungus would grow from the egg so positive identification of parasitism could be made. Neither fungus demonstrated chitinase activity when grown on agar containing colloidal chitin prepared as described by Lingappa and Lockwood (9).

No resting spores were produced in nematode eggs. Chlamydospores of *F. oxysporum* were observed within female mucilage. Hyphae of *A. strictum* within eggs ranged from 3–7 μm in width, *F. oxysporum* ranged from 3–4 μm in width.

Plant host range. *A. strictum* was able to parasitize seven of nine

TABLE 1. Survey of parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum* in 32 California sugar beet fields

Field	Location	Eggs[a] (no./g soil)	Eggs infected (%)	Isolations (%) *F. oxysporum*	Isolations (%) *A. strictum*
1	Imperial Valley	3.3	20.9	52.6	0
2	Imperial Valley	3.2	6.2	64.7	0
3	Imperial Valley	1.5	11.7	68.4	0
4	Imperial Valley	0.3	0	-[b]	-
5	Imperial Valley	0.2	2.6	0	0
6	Imperial Valley	2.2	1.4	35.7	21.4
7	Imperial Valley	0.2	1.3	-	0
8	Imperial Valley	0	-	-	0
9	Imperial Valley	0	-	-	0
10	Imperial Valley	0	-	-	0
11	Imperial Valley	0	-	-	0
12	Oxnard Plain	0.1	33.3	0	0
13	Oxnard Plain	0.3	22.2	100	0
14	Oxnard Plain	0.1	100	100	0
15	Oxnard Plain	0	-	-	-
16	Oxnard Plain	0	-	-	-
17	Oxnard Plain	0.1	0	-	-
18	Oxnard Plain	3.2	9.4	22.2	0
19	Oxnard Plain	0.8	33.3	37.5	0
20	Oxnard Plain	1.9	15.8	66.7	0
21	Oxnard Plain	0.4	58.3	50	0
22	Oxnard Plain	5.6	1.2	100	0
23	Riverside	6.4	32.3	23.4	12.5
24	Riverside	8.3	45	0	100
25	Spreckels Ranch Salinas	0.6	13.5	83.3	3.3
26	Spreckels Ranch	1.2	16	52.5	15
27	Spreckels Ranch	0.7	10.4	40	18
28	Spreckels Ranch	1.4	13.3	35	7.5
29	Spreckels Ranch	1.5	12.2	50	40
30	Salinas Valley	0	-	-	-
31	Salinas Valley	0.5	81.8	95	0
32	Stockton Area	12.3	40.8	0	100
	% Total fields	78.1	71.9	59.4	28.1

[a] Means from two replicates with numbers of eggs averaging up to 500 eggs per replicate.
[b] – Values not calculable from zero nematode egg count.

TABLE 2. Parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum* in bioassays of soil from 32 California sugar beet fields

Field	Location	Eggs[a] (no./g soil)	Eggs infected (%)	Isolations (%) *F. oxysporum*	Isolations (%) *A. strictum*
1	Imperial Valley	1.6	59.2	4.3	95.7
2	Imperial Valley	16.4	1.4	33.3	66.7
3	Imperial Valley	0.4	73.8	100	0
4	Imperial Valley	11.1	25.9	2.2	97.8
5	Imperial Valley	36.6	7.9	0	100
6	Imperial Valley	7.6	85.3	0.2	99.8
7	Imperial Valley	3.0	75.9	1	99
8	Imperial Valley	14.6	1.4	16.7	83.3
9	Imperial Valley	0	-[b]	-	-
10	Imperial Valley	0	-	-	-
11	Imperial Valley	8.8	41.5	0	100
12	Oxnard Plain	2.8	37.9	12.1	65.7
13	Oxnard Plain	0.2	32.2	0	100
14	Oxnard Plain	6.8	43.4	9.3	90.7
15	Oxnard Plain	0	-	-	-
16	Oxnard Plain	15.6	1.0	100	0
17	Oxnard Plain	...[c]	...	...	...
18	Oxnard Plain	7.7	55.7	4.4	95.6
19	Oxnard Plain	1.2	23.8	16.7	83.3
20	Oxnard Plain	0.6	33.4	0	100
21	Oxnard Plain	9.3	15.5	26.7	73.3
22	Oxnard Plain	5.1	5.9	0	100
23	Riverside	10.8	12.4	0	100
24	Riverside	1.3	37.3	85.7	14.3
25	Spreckels Ranch Salinas	7.4	10.8	41.9	58.1
26	Spreckels Ranch	1.8	16.6	20.3	60.4
27	Spreckels Ranch	5.3	23.4	8.8	78.4
28	Spreckels Ranch	...	...	...	...
29	Spreckels Ranch	3.3	24.8	1.4	64.3
30	Salinas Valley	4.4	42.0	4.3	95.7
31	Salinas Valley	0.5	81.8	100	0
32	Stockton Area	...	...	...	...
	% Total Fields	89.7	89.7	70.0	79.3

[a] Means from two replicates with numbers of eggs averaging up to 500 eggs per replicate.
[b] - Values not calculable from zero nematode egg count.
[c] ... No data, and values not calculable.

plant cultivars tested. Some lesions developed on roots of sugar beet and cauliflower and on roots and stems of Shasta daisy, which is susceptible to *A. strictum* (Table 5). The two monocotyledonous plants were not parasitized. *A. strictum* was readily isolated in 7 days from infected root and stem pieces. There was no detectable infection of any cultivar by *F. oxysporum* using the root-dip and drench methods of inoculation.

DISCUSSION

The survey confirmed initial observations that fungal parasitism of eggs occurs naturally in *H. schachtii* populations in California. In previous reports (13,14,17) on disease or parasitization in *H. schachtii* populations, data were presented in terms of percent cysts diseased or infected. We reported percent parasitized eggs, because current research on population thresholds suggests that it is more quantitative to report numbers of sugar beet cyst nematodes in eggs per gram of soil. Our data should help refine these thresholds by identifying viable eggs per gram of soil.

Most quantitative studies on disease in *Heterodera* spp. have been related to cysts sieved from soils. Previous reports of percent diseased *H. schachtii* cysts extracted from soil ranged from 14.5–17.9% (13) to 63.7% (14). In the most recent study (17), an average of 14.8% of cysts in soil and 17.2% of cysts from rootlets, were diseased. Although these levels of disease are similar, the data relate to different populations with different pathogens. Differences did occur in individual instances. For example, at 26.4%, the level of disease in new brown cysts was high in comparison with the level from old cysts sieved from soil samples. These comparisons do not necessarily reflect accurately the relative frequencies of infested eggs because cysts recorded as diseased may contain many viable, nonparasitized eggs. In our study, the frequency of parasitized eggs generally was higher in egg samples taken from young females and new cysts from roots and soil than in samples from old cysts from soil in the same sample. The presence of both parasitic fungi and relative percentages of infection can be assessed accurately from young, viable populations of the nematode such as those obtained from fields where the nematode is reproducing. Greenhouse culture results tend to be less accurate because they may be influenced by restrictions of plant development within pots, and by temperature and moisture extremes. Subsequent work has shown direct influence by temperatures and moisture on parasitization of *H. schachtii* eggs.

A. strictum and *F. oxysporum* were aggressive parasites of *H. schachtii* eggs on agar and in autoclaved soil infested with mycelia or conidia. Eggs parasitized by both fungi in laboratory conditions appeared similar to infected eggs collected in the field and both fungi were readily reisolated from parasitized eggs. Observations of second-stage juveniles contained in eggs suggest that they are resistant to these fungi. They were seldom infected by *A. strictum* and never by *F. oxysporum*. Similar results were reported for *D. oviparasitica* Stirling, a parasite of *Meloidogyne* spp. eggs (15).

Both *A. strictum* and *F. oxysporum* are considered to be facultative saprophytes as defined by Garrett (5) and appear to be capable of colonizing both living and dead eggs as a growth substrate.

The young females used in these experiments contained eggs in different developmental stages. This made interpretation of some results difficult. As previously stated, few or none of the second-stage juveniles were parasitized. Therefore, the pool of eggs available for parasitism was reduced, at least, to the number of eggs in stages prior to J_2 development. Many times, all eggs within a cyst were either parasitized or contained second-stage juveniles indicating that the fungi had consumed all available and susceptible substrate.

Observations on the proliferation of *A. strictum* and *F. oxysporum* in *H. schachtii* females showed that the fungi enter the young female through natural openings, and invade the eggs rapidly with little or no damage to the female. Females filled with parasitized eggs were not distinguishable from females filled with healthy eggs. Fungus hyphae were seen within eggs 70 hr after the fungal contact. Penetration appeared in part to be mechanical, but enzymatic action may have been involved. *Dactylella oviparasitica* produces chitinase and Stirling and Mankau (16) suggested a combination of mechanical and enzymatic action in its penetration of *Meloidogyne* eggs. When *A. strictum* and *F. oxysporum* were grown on agar containing chitin, there was no suggestion of chitinase production. The amount of chitin in the chitinous layer of egg shells varies greatly (12). The chitin content of *Globodera rostochiensis* eggs is 9% compared with 59% protein (2). Little damage is done to the egg shell after penetration into the egg contents so the shell itself is apparently not used as a source of nutrients by the fungi. Likewise the female cuticle is not attacked and can remain intact and form a cyst wall that protects the remaining eggs within the cyst.

Research concerning egg parasites as biological control agents of nematodes is limited (15,16). An *Entomophthora*-like fungus has received considerable attention and is believed responsible for population declines of *H. avenae* (7,8). Organisms such as nematode-trapping fungi, predacious microarthropods, and predacious nematodes consume some nematodes in soil, but

TABLE 3. Parasitization of *Heterodera schachtii* eggs on water agar by isolates of *Acremonium strictum* and *Fusarium oxysporum* after 10 days

Fungus	Eggs parasitized[y] (%)	Eggs in J_2 stage[z] (%)
A. strictum		
isolate 1	32.1 a	61.7
isolate 2	24.9 ab	74.3
isolate 3	34.6 a	60.5
isolate 4	13.4 b	62.4
isolate 5	23.4 ab	69.8
F. oxysporum		
isolate 1	32.3 a	57.6
isolate 2	25.2 ab	59.8
isolate 3	25.0 ab	61.3
Control	0.0 c	83.3

[y]Means are from six replicates with three females (average total of 963 eggs) per replicate.
[z]Eggs in second juvenile (J_2) stage appear to be resistant to both fungi.

TABLE 4. Parasitization of *Heterodera schachtii* eggs in autoclaved soil infested[x] with *Acremonium strictum* or *Fusarium oxysporum*

Fungus	Eggs parasitized[y] (%)	Eggs in J_2 stage[z] (%)
A. strictum isolate 1	60.1 a	28.7
F. oxysporum isolate 1	36.4 b	29.6
Control	0.0 c	31.3

[x]The amounts of inoculum used for both fungal isolates was 1.0 mg dry mycelium per gram of dry soil.
[y]Means are from six replicates with three females (average total of 790 eggs) per replicate.
[z]Eggs in second juvenile (J_2) stage appear to be resistant to both fungi.

TABLE 5. Plant cultivars parasitized by *Acremonium strictum* following exposure to a drench inoculum containing 10^5 conidia per plant

Host	Cultivar	Root and Stem lesions[x]	Infection[x] (%)
Sugar beet	USH 10	20	100
Broccoli	DeCicco	0	100
Cabbage	Premium Flat Dutch	0	55
Cauliflower	Early Snowball A	+19	95
Alfalfa	Mesa Sirsa	0	85
Barley	UC 566	0	0
Cotton	DP 16	0	15
Wheat	Yecora Rojo	0	0
Shasta daisy	Killian	+20	100

[x]Means of 20 plants 8 wk after inoculation. No vascular discoloration was found in any of the cultivars.

studies on biological control of *Meloidogyne* spp. in Lovell peach orchards suggest limited capacity to reduce populations of root-knot nematodes below economically damaging levels (15).

Of all the stages in the life cycle of *Heterodera* spp., the eggs may be the most vulnerable to parasitism and predation. They are present within females when their swelling bodies first burst through the root cortex and can remain inside until encystment is complete. The development of eggs within the female is variable. In some young females a majority of the eggs contain second-stage juveniles, whereas some cysts contain many eggs that have not reached the eight-celled stage. The cyst, therefore, may provide a long lasting protected food source for egg-parasitic fungi such as *A. strictum* and *F. oxysporum*. Equally important is that the cyst also provides protection for the fungi in the eggs within her. These two fungi were both isolated from eggs in cysts that were presumably at least 10 yr old.

Another important food source for *A. strictum* is host plants. These studies revealed its ability to parasitize a range of plants, including sugar beets and crucifers as well as crops that are in rotation with sugar beets to control the nematode. *A. strictum* was probably only weakly pathogenic, because little damage was observed in these plants. Economic damage to plants appears to be limited to the Killian cultivar of Shasta daisy (1). It is conceivable that fungus inoculum could be increased in susceptible host plants prior to planting a *H. schachtii* host plant and thus increase parasitism of newly produced eggs by *A. strictum*.

The amount of parasitism observed in the laboratory and greenhouse, and the widespread occurrence of both fungi in California *H. schachtii* populations and especially in areas where *H. schachtii* levels are low, suggests that both *A. strictum* and *F. oxysporum* may contribute to the control of the sugar beet cyst nematode in the field.

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