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Parasitoids and Pathogens of Japanese Beetle (Coleoptera: Scarabaeidae) in Southern Michigan

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ABSTRACT The density of Japanese beetle (*Popillia japonica* Newman) and the prevalence of its natural enemies were evaluated in southern Michigan. Third instars were collected in spring and fall and adults during the summer of 1999 and 2000 at 11 golf courses. Larvae were also collected once in fall of 2000 from 24 additional sites including golf courses ($n = 8$), blueberry farms ($n = 7$) and low-maintenance turf ($n = 9$). Larval density in the irrigated rough at the 11 primary golf course sites averaged 9.5/0.1 m^2 (range, 3.7–21.0). At the 24 additional sites, where habitat was more diverse, Japanese beetle larval density averaged 2.9/0.1 m^2 (range, 0.60–14.4). The larval parasitoid *Tiphia vernalis* Rohwer and the adult parasitoid *Istocheta aldrichi* (Mesnil) were absent from all sites in this study. Cephaline gregarines (*Stictospora* sp.) were the most common parasites, infecting 36.1% of all larvae in fall of 2000. The microsporidean *Ovavesicula popilliae* Andreadis was absent at all but two locations near Kalamazoo, MI. The bacterial pathogen *Paenibacillus popilliae* (Dutky) and entomopathogenic nematodes were uncommon, infecting <1% of larvae. Overall, two parasites (*T. vernalis* and *I. aldrichi*) and two pathogen (*O. popilliae*), reported to be widespread and epizootic in some eastern states, were absent or nearly so (*O. popilliae* was found at two of 35 locations) in Michigan. *Stictospora* sp. was found at most locations in Michigan (25/36) where Japanese beetle infestations have been active for more than 20 yr, but was scarce or absent from areas where Japanese beetle has become established in the last 10 yr.

KEY WORDS *Popillia japonica*, biological control, *Ovavesicula*, gregarinidae, *Tiphia*, *Istocheta*

THE JAPANESE BEETLE, *Popillia japonica* Newman is one of the most damaging pests in the eastern United States, feeding on roots of turf and ornamentals as a larva, and defoliating landscape ornamentals and fruit crops as an adult (Fleming 1972, Potter 1998). The cost of chemical control of grubs in turf is increasing as registrations for inexpensive organophosphates and carbamates are being withdrawn under the Food Quality Protection Act. Management of adults is problematic for landscapers and nursery growers, particularly when adults emerge from nearby areas of turf that they have no control over.

Discovered in southern New Jersey in 1916, the Japanese beetle slowly spread across the northeastern and midwestern states over the next 80 yr, becoming established as far west as Kansas and Minnesota in the last few years (USDA 2000, Fleming 1968). Early researchers recognized that while the Japanese beetle was becoming a pest in the United States, it was insignificant in Asia where several natural enemies were found (King and Holloway 1930). Later, it was observed that Japanese beetle populations often peak 5–10 yr after introduction to a new area, followed by a decline over the next 10–15 yr; a pattern consistent with the expected effect of growing populations of natural enemies (Fleming 1968, 1972).

To establish whether or not natural enemies of Japanese beetle are present in Michigan, and to establish baseline data for evaluating natural enemy introductions, we sampled Japanese beetle populations and looked for parasitoids and pathogens in 15 counties of southern Michigan. Our study focused on species of natural enemies already established in the eastern United States: *Istocheta aldrichi* (Mesnil), *Tiphia vernalis* Rohwer, *Paenibacillus popilliae* (Dutky), *Ovavesicula popilliae*Andreadis, *Stictospora* sp., and entomopathogenic nematodes (King 1931, 1950; Hadley and Hawley 1934; Fleming 1968; Andreadis and Hanula 1987; Poinar et al. 1987; Hanula 1990).

Materials and Methods

Larval Sampling. Sampling focused on golf courses, because they tend to support high densities of Japanese beetles in relatively uniform habitats. Following interviews with thirty golf course managers, we selected 11 "primary" research sites scattered from southwestern Michigan where Japanese beetle has been active for at least 20 yr, to areas north of Detroit, where infestations tend to be less than 10 yr old (Fig.

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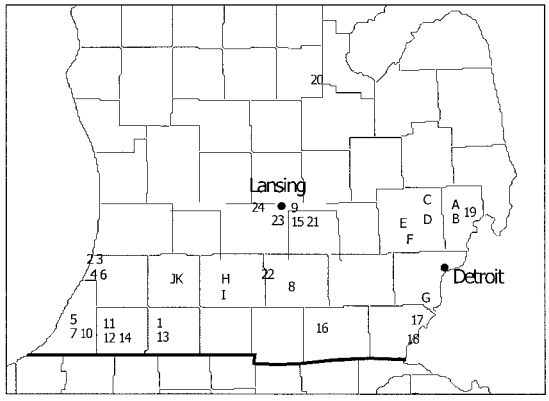

Fig. 1. Locations of sampling sites. Letters are primary sites; numbers are secondary sites

1). Managers at all 11 sites reported Japanese beetle damage in recent years. Samples were collected in May and September of 1999 and 2000 from a single block of insecticide-free, irrigated rough, adjacent to a fairway but separated from the fairway by 3 m. On each sample date we used golf course cup-cutters (10.2 cm diameter) to remove one pair of 10 cm deep soil cores from each of 24 uniformly distributed points (5.0 m apart) in a 15 by 25-m rectangular grid. Scarab larvae were identified to species in the field and a subsample of 30 Japanese beetle larvae were placed in 1.0-liter plastic bags filled with soil for transport in a cooler to the laboratory. Larvae were then held for up to 1 wk at 10°C, or frozen for evaluation of pathogens later in the year.

Nematode Infections. All larvae found in the 48 soil cores (10.2 cm diameter) dug from a sampling grid at each golf course were examined in the field for signs of nematode infection. Individuals with red or straw-yellow discoloration were collected, returned to the laboratory and dissected to confirm the presence of entomopathogenic nematodes (Tanada and Kaya 1993). The random subsample of 30 larvae from each site that were examined for pathogens and parasites, were also checked for the presence of nematodes in the hemolymph.

Fungal Pathogens. Dead larvae with fungal overgrowth were collected and held in the laboratory. *Metarhizium anisopliae* (Metch.) infection was confirmed by checking cadavers for characteristic conidiophores after 1 wk in a moisture chamber (Humber 1997). Healthy larvae were also examined for the presence of fungal hyphal bodies or mycelium in the hemolymph when they were dissected for diagnosing *P. popilliae*, *Ovavesicula*, and *Gregarina* sp. infections. All the larvae collected for pathogen diagnosis were examined for the presence of *Entoderma colletosporium* fungal growth around the spiracles.

Tiphia vernalis Larvae and Pupae. Parasitism by *T. vernalis* was assessed during the previously described grub collections. Japanese beetle larvae were always checked during spring samples to see if a *T. vernalis* larva was attached. In addition, the cup-cutter soil cores (15 cm deep) were broken apart and examined for the presence of characteristic *Tiphia* cocoons while looking for grubs. Because *Tiphia* cocoons remain in the soil from June until the following April or May, they are easily found at sites where they are active (Fleming 1968, Vittum et al. 1999).

Paenibacillus popilliae and Ovavesicula popilliae. The incidence of bacterial and protozoan pathogens was determined by dissecting 30 larvae from each primary site, and if possible, 30 larvae from secondary sites. When <30 were found at a secondary site, all of the larvae that were collected were dissected. Hemolymph smears from fresh or frozen larvae were examined with a phase contrast microscope at 400× for vegetative rods and sporangia of *P. popilliae* (Klein and Jackson 1992; Pettersson et al. 1999). Gregarine infection was determined by removing the midgut and hindgut and scanning the interior gut wall and gut contents at 25× for the presence of gamonts. *Ovavesicula popilliae* infections were determined by the presence of sporophorous vesicles in malpighian tubules. The malpighian tubules were removed and mounted on slides in saline solution before viewing at 400× with a phase contrast microscope (Andreadis and Hanula 1987).

Overall Density of Larvae at Golf Course Sites. To determine if larval density at the sample sites was typical of the density throughout each golf course, more samples were taken from each golf course in September 1999. At that time cup-cutter samples were also taken from six randomly selected fairways at each golf course. Samples were taken by walking along the fairway/rough border and stopping six times, with 20–30 m between stops. At each stop, the sampler walked five m perpendicular to the fairway/rough border into the rough, collecting one sample at the 5-m mark and another one at the 10-m mark. This was repeated for all six stops along the fairway/rough border, for a total of 12 cup-cutter samples per fairway and 72 per golf course. Larvae were counted and identified in this expanded sampling at each golf course, but they were not dissected for pathogens and parasites.

To examine Japanese beetles from other habitats, and to increase the number of sites surveyed for natural enemies, 24 additional "secondary" sites were sampled in Fall 2000 (Fig. 1). A minimum of 10 pairs of cup-cutter cores or 10 pairs of 0.1-m^2 turf squares were pulled from grassy areas within blueberry fields, parks and lawns, and golf course roughs. Larvae were identified and transported to the lab as described.

Adult Japanese Beetle and Adult *T. vernalis* Sampling. Beetles were collected at primary sites to look for *Istocheta* eggs and to check for infection of adults by *Ovavesicula popilliae.* Standard Japanese beetle traps were baited with floral lures (2 phenylethyl propionate, geraniol, and eugenol; Trécé, Salinas, CA). Two traps were placed 50 m apart at each of the primary sites, along the edge of fairways where larvae were sampled. Collections were made during three short periods (3–5 d long) at the beginning, peak, and declining phases of the adult flight (1 July, 15 July, 5

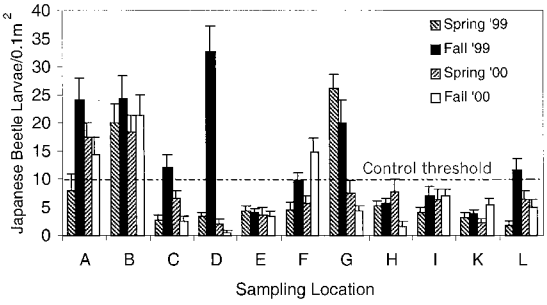

Fig. 2. Mean ± SEM Japanese beetle larval density in irrigated rough at primary sample sites ($n = 24$).

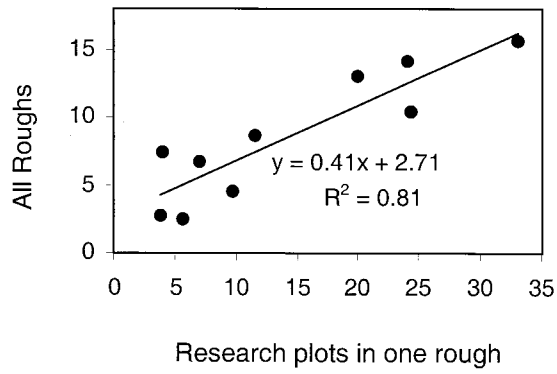

Fig. 3. Japanese beetle larval density in research plots compared with average larval density over entire golf course.

August) in 1999, and at the beginning and declining phase of the flight (7 July, 7 August) in 2000.

Istocheta aldrichi. Parasitism by *I. aldrichi* was evaluated by examining 300 beetles from each trap on each sample date for the presence of *I. aldrichi* eggs on the pronotum (Fleming 1968). In 1999 we examined 900 beetles at each site (total of 9,900) and in 2000, 600 beetles at each site (total of 6,600).

Ovavesicula popilliae and Paenibacillus popilliae. Samples of 30 adults were removed from traps at all 35 locations in July or August of 1999 and 2000. The adults were transported to the laboratory in a cooler where they were dissected within 48 h, or frozen. Dissection and examination procedures for the presence of *Ovavesicula popilliae* and *Paenibacillus popilliae* are as previously described for Japanese beetle larvae.

Sampling for Adult T. vernalis. Surveys for adult *Tiphia* were also conducted once at each primary site during a sunny day in mid-late May in 2000 or 2001. *Tiphia vernalis* adults congregate in the vicinity of honeydew (Gardner 1938), and researchers have identified natural *Tiphia* colonies by attracting adults with sugar solutions (R. MacDonald, personal communication). Approximately 300 ml of a 10% solution of honey was sprayed onto 0.25 m^2 foliage of 20 trees within a 50-m radius of primary sampling sites. After 30 min, sprayed foliage was examined for *T. vernalis* adults.

Results and Discussion

Larval Density. Japanese beetles comprised >95% of all scarab larvae collected from our primary research sites at golf courses. These plots were all located in irrigated portions of the rough within 10 m of the fairway on one hole per golf course. Irrigation made these sample sites suitable for Japanese beetle and not for European chafer (Vittum et al. 1999). Although these sites were also suitable for *Ataenius spretulus* (Haldeman) and *Aphodius granarius (L.)*, our sampling times for Japanese beetle (May, September, and October) did not overlap with the time that *A. spretulus* and *A. granarius* larvae are present in Michigan (July and June, respectively, Smitley et al. 1998).

The density of Japanese beetle larvae averaged 9.5/0.1 m^2 in our plots (range, 3.7–21 larvae/0.1 m^2) (Fig. 2). The density of larvae in our plots at one hole was proportional to the density found in the irrigated rough of six other holes on the golf course ($R^2 = 0.81$), but nearly twice as high, probably because we located our plots where we found a high density of Japanese beetle larvae (Fig. 3). Our data suggest that the density of Japanese beetle populations in irrigated golf course roughs in Michigan is frequently below 10 larvae/0.1 m^2, a threshold below which "injury to well-kept turfgrass is usually not apparent" (Crutchfield and Potter 1995a, 1995b; Vittum et al. 1999).

Secondary sites produced a wider range of scarab larvae species and densities, with an overall average density of 2.9/0.1 m^2 (range, 0.06–14.4, Table 2). Of the three habitats sampled, irrigated golf courses and blueberry fields supported relatively high densities of Japanese beetle (7.1/0.1 m^2) and no other white grub species. By contrast, the low-maintenance turf sites (nonirrigated parks and lawns) hosted lower densities of Japanese beetle (2.0/0.1 m^2) among significant numbers of other scarabaeid larvae (2.9/0.1 m^2) including European chafer, *Rhizotrogus majalis* (Razoumowsky), Asiatic garden beetle, *Maladera castanea* (Arrow), and *Phyllophaga* spp. (Table 2). Low maintenance sites may have supported lower densities of Japanese beetle larvae compared with golf course sites because of the lack of irrigation (Vittum et al. 1999).

Parasitoids. During the collection of >3,400 larvae from 35 sites over a 2-yr period, we did not find any larvae or cocoons of *T. vernalis. Tiphia vernalis* adults were also not observed in spring surveys. *Tiphia vernalis* has been resident in the United States since its introduction in New Jersey in the 1920s (Fleming 1968), and populations have spread as far as central Kentucky (Rogers and D. A. Potter, personal communication) and Ohio (J. Moyseenko, personal communication). The only published data on parasitism by *T. vernalis* was reported in the first 20 yr following the initial introductions. Hadley and Hawley (1934) reported 7–38% parasitism at sites in the eastern United States, and King (1950) reported values between 19 and 61% at a dozen sites in Pennsylvania and New Jersey.

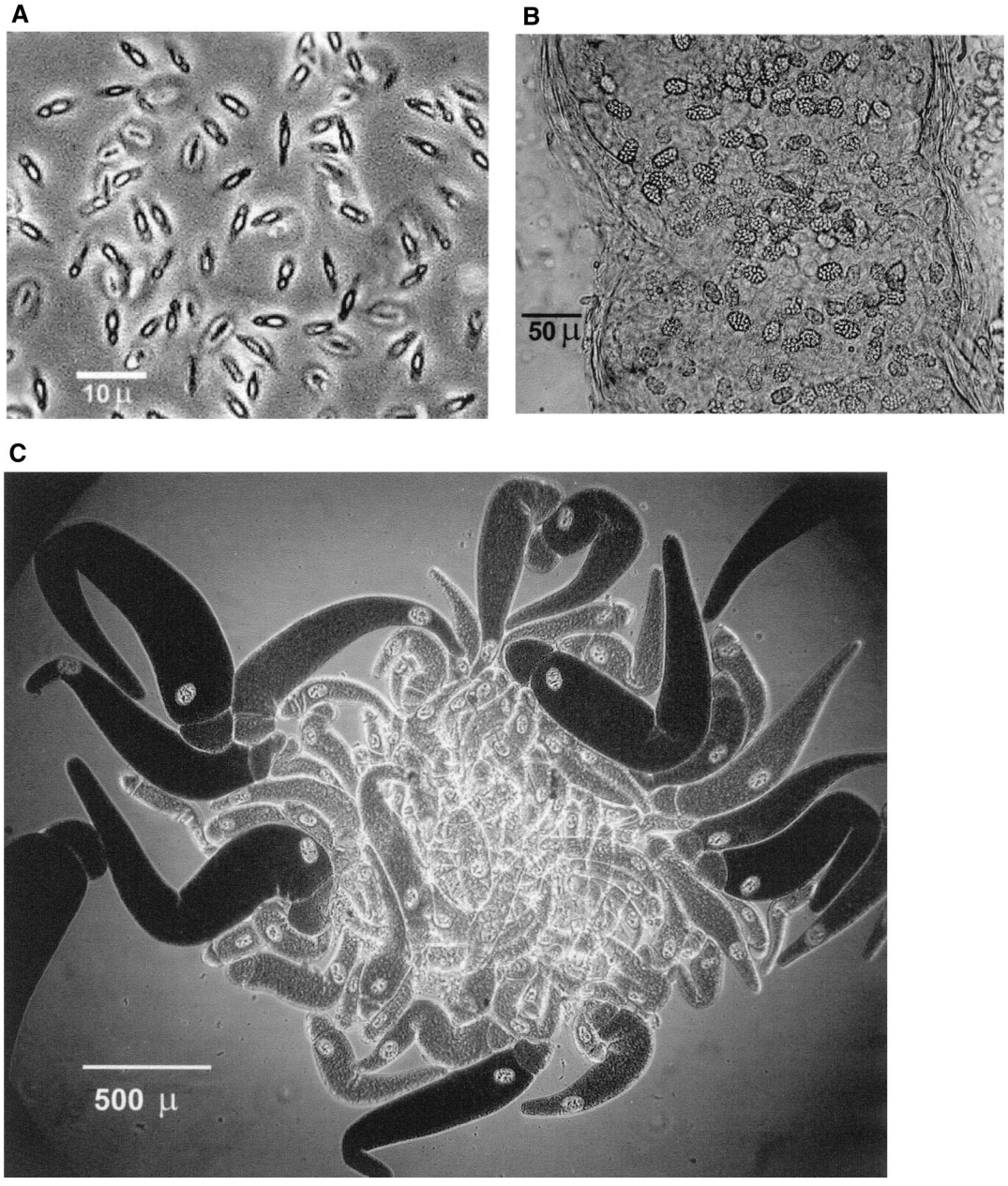

Fig. 4. Pathogens isolated from Japanese beetle in Michigan: (A) Sporangia of *Paenibacillus popilliae.* (B) Sporophorous vesicles of *Ovavesicula popilliae* in a Malpighian tubule. (C) Gamonts of a *Stictospora* sp., cephaline gregarine in the midgut.

Istocheta aldrichi was also not detected among 16,500 beetles examined from the 11 primary sites in Michigan. *Istocheta aldrichi* was reported to parasitize 20–90% of Japanese beetle in Japan (Fleming 1968). Introduced in the eastern United States in the 1930s, *I. aldrichi* has been consistently found in Connecticut in recent years (R. MacDonald, personal communication). Unfortunately, the incidence of *I. aldrichi* has not been reported in journal articles. While collecting *I. aldrichi* in Connecticut in 2000 for introduction into Michigan we found 1.1–5.9% of the adult Japanese beetles in traps to be infested with *I. aldrichi* eggs (unpublished data).

Paenibacillus popilliae. Paenibacillus popilliae (Fig. 4A) infection was detected at four of 11 primary sites, with an overall prevalence of 1.0% (Table 1). Although *P. popilliae* was observed in both fall and spring, infection was not observed on more than one sample date at any one location. *P. popilliae* was detected at just three of 24 secondary sites (0.80% infection, Table

Table 1. Prevalence of Japanese beetle larval pathogens at primary sites

Site	*Paenibacillus popilliae* % infection (n = 30)				Gregarinidae % infection (n = 30)				*Ovavesicula popilliae* % infection (n = 30)				Nematodes % infection (n)			
	Spring 1999	Fall 1999	Spring 2000	Fall 2000	Mean	Fall 1999	Spring 2000	Fall 2000	Mean	Spring 1999	Fall 1999	Spring 2000	Fall 2000	Mean	Spring 1999	Fall 1999	Spring 2000	Fall 2000	Mean
A	0	0	0	0	0.0	3	0	0	0.3	0	0	0	0	0.0	0.0 (180)	0.0 (102)	0.0 (74)	0.0 (61)	0.0
B	0	0	0	0	0.0	0	11	14	8.3	0	0	0	0	0.0	0.0 (74)	0.0 (171)	0.0 (65)	0.0 (75)	0.0
C	0	0	0	0	0.0	0	40	100	47.0	0	0	0	0	0.0	0.0 (15)	0.0 (68)	0.0 (37)	0.0 (14)	0.0
D	9	0	0	0	2.1	0	0	0	0.0	0	0	0	0	0.0	1.3 (74)	0.0 (138)	0.0 (9)	0.0 (2)	0.3
E	0	0	0	0	0.0	0	43	0	14.0	0	0	0	0	0.0	3.9 (78)	0.0 (34)	0.0 (15)	0.0 (14)	1.0
F	0	0	0	0	0.0	0	13	0	4.2	0	0	0	0	0.0	0.0 (19)	0.0 (41)	0.0 (24)	0.0 (62)	0.0
G	0	0	0	0	0.0	100	0	60	53.0	0	0	0	0	0.0	0.0 (54)	0.0 (168)	0.0 (32)	0.0 (18)	0.0
H	0	0	3	0	0.8	0	53	70	41.0	0	0	0	0	0.0	0.0 (68)	0.0 (33)	0.0 (27)	0.0 (6)	0.0
I	28	0	0	0	7.0	15	22	ND	18.0	0	0	0	0	0.0	7.4 (27)	0.0 (45)	0.0 (41)	0.0 (44)	2.5
J	0	0	0	0	0.0	7	23	96	42.0	0	0	40	0	13.0	0.0 (32)	0.0 (28)	0.0 (8)	0.0 (19)	0.0
K	0	0	0	7	1.7	10	40	70	40.0	0	0	0	27	9.0	0.0 (8)	0.0 (49)	0.0 (27)	0.0 (21)	0.0

ND, Not sampled.

2). A statewide survey in Connecticut detected higher levels of *Paenibacillus popilliae.* Hanula and Andreadis (1988) found *P. popilliae* at 16 of 49 sites, for a statewide average of 3.7%, and Hanula (1990) recorded >25% infection at three of four Connecticut sites over a 3-yr period. Hutton and Burbutis (1974) also reported an infection rate of 4.0 % at sites in Delaware. *Paenibacillus popilliae* may have a greater impact on populations of scarab larvae than prevalence data suggest, because infected larvae may be more susceptible to pathogens or insecticides. Cherry and Klein (1997) showed that over 60 d, mortality was eight times higher for *P. popilliae*-infected *Cyclocephala parallela* Casey larvae than for healthy larvae. The incidence of infection on a single sample date fails to account for the portion of infected grubs that disappear from the population over time, Thurston et al. 1994. Nonetheless, our data suggest that at this time *P. popilliae* is relatively insignificant in Michigan, and that its occurrence is episodic rather than chronic.

Protozoa. *Ovavesicula popilliae* (Fig. 4B) was detected at two locations, both within the city limits of Kalamazoo, MI. Adult samples yielded infection rates of 10.4% (15 July 1999) and 3.3% (7 August 2000) at site K. Infected larvae were also found on one of four sample dates at the same sites (J and K, Table 1). No infected larvae or adults were found at the remaining 33 sites.

The distribution of *O. popilliae* has been reported in only one other study. *Ovavesicula popilliae* was found at 69% of Connecticut sites, and infected 25% of all larvae, statewide (Hanula and Andreadis 1988). The relative scarcity of *O. popilliae* in Michigan appears to result from incomplete introduction, rather than incompatibility with local conditions, because it has become epizootic at two locations near Kalamazoo. Japanese beetle established in Connecticut 60 yr ago, whereas local infestations in Michigan occurred only in the last 20 yr (Vittum et al. 1999). *Ovavesicula popilliae* may disperse less readily than its host, or was introduced into North America later than Japanese beetle.

Stictospora sp. (Fig. 4C) was found in Japanese beetle larvae at 10 of 11 primary sites and 16 of 24 secondary sites (Tables 1 and 2; Fig. 4C). Average infection at all sites was 36.5% in fall 2000. All the gregarines we found appear to be one species of Eugregarinida: Gregarinidae, probably the same species reported by Regniere and Brooks (1978) and Hanula and Andreadis (1988). Although this gregarine has been photographed and roughly described, it has not been adequately described at the species level. At this time it can only be classified at the genus level as a *Stictospora* sp. (R. Clopton, personal communication). Hanula and Andreadis (1988) found that the proportion of Japanese beetle larvae infected with *Stictospora* sp. declined from September to December for unknown reasons, making it necessary for us to use infection data from the same month for comparing *Stictospora* sp. activity in Michigan with the same from Connecticut. Of all the Japanese beetle larvae collected from all sites in Michigan from 11 September to 6 October,

Table 2. Prevalence of Japanese beetle larval pathogens at secondary sites

Site	Turf type	Larvae per 0.1m^2		*n*	% infected JB larvae		Gregarinidae
		JB	Other		2000 Date	*Paenibacillus popilliae*	
1	BB	1.4	0.0	55	10 May	0	47
2	BB	1.3	0.0	36	19 May	0	3
3	BB	8.0	0.0	32	24 Oct	0	3
4	BB	4.5	0.0	14	24 Oct	0	40
5	BB	2.7	0.0	7	24 Oct	0	25
6	BB	7.5	0.0	30	24 Oct	0	75
7	BB	7.8	0.0	31	24 Oct	6	52
8	GC	15.0	0.0	30	26 Sep	0	0
9	GC	3.0	0.0	20	4 Oct	0	0
10	GC	5.3	0.0	21	12 Oct	0	84
11	GC	3.7	0.0	15	12 Oct	0	70
12	GC	8.8	0.0	35	12 Oct	0	66
13	GC	4.9	0.0	18	12 Oct	0	66
14	GC	14.4	0.0	59	13 Oct	10	78
15	GC	18.0	0.0	30	30 Oct	3	0
16	LM	2.1	0.0	38	15 May	0	89
17	LM	1.6	3.3	5	11 Sep	0	60
18	LM	6.3	9.4	30	11 Sep	0	90
19	LM	4.0	0.4	15	11 Sep	0	0
20	LM	0.1	0.6	2	6 Oct	0	0
21	LM	3.2	1.4	42	6 Oct	0	0
22	LM	0.1	0.7	18	26 Oct	0	64
23	LM	0.2	9.2	10	28 Oct	0	0
24	LM	0.5	1.1	8	1 Oct	0	0

BB = grass in commercial blueberry field; GC = golf course; LM = non-irrigated low-maintenance turf. JB = Japanese beetle; Other = European chafer, Asiatic garden beetle, or *Phyllophaga* spp. *n*, Japanese beetle larvae evaluated for pathogens.

a total of 33.1% were infected with *Stictospora* sp., compared with 55–96% of all Japanese beetle larvae collected in Connecticut from 15 to 30 September (Hanula and Andreadis 1988). Furthermore, within Michigan, *Stictospora* sp. is more abundant in older (>20 yr old) infestations compared with more recent (<10 yr old) infestations (Table 3). The oldest continuous infestations of Japanese beetle in Michigan are located in the two southernmost layers of counties (Wellso and Fischer 1971; Fig. 2). Infestations in other counties (Fig. 1: sample sites A-F, 9, 15, 19, 20, 21, 23, and 24) are much more recent. The mean rate of *Stictospora* sp. infection in the two southernmost layers of counties are 38.8 and 53.6% from our primary and secondary research sites, respectively, compared with12.3 and 0.0% for all other counties (Table 3). Although we did not know for how many years Japanese beetle had been found at each of our sample sites, we were able to obtain a data set from the USDA/APHIS Cooperative Pest Survey (CAPS) that reports the first year that Japanese beetle is "known to be established" in each county (USDA 2001). We used these reports of first established Japanese beetle populations in each county to assign an age of infestation to each of our sample sites. This put considerable noise into the estimated age of each infestation because some of the first established populations were the targets of eradication programs, and there is also a lot of variation in when different locations within a county first became infested. Even so, when sample sites are sorted into those with infestations >20 yr old or <10 yr old, according to USDA/APHIS data, differences in *Stictospora* sp. infection levels are clear. In counties where the first Japanese beetle infestation is >20 yr old, *Stictospora* sp. infected 43.2% of Japanese beetle larvae, compared with 15.4% in counties where the first infestation is <10 yr old.

Larval densities at our research sites were not different among new and old infestations (Table 3). However, our experimental design is not well-suited for comparing larval density in new and old infestations, particularly in the secondary sites, because some of the new infestations were in a very early stage of establishment before populations had a chance to

Table 3. Incidence of *Stictospora* sp. in Japanese beetle larvae from the most southern counties where Japanese beetle was first found, compared with the same in counties that have become infested more recently

Type of research site	Location in Michigan	No. of sites	Larvae/0.1 m^2	% larvae infected with *Stictospora* sp.
Primary sites	In 13 southern-most counties[a]	5	7.6 ± 4.3	38.8 ± 12.8
	All other sites	6	11.0 ± 6.2	12.3 ± 18.0
Secondary sites	In 13 southern-most counties	17	5.6 ± 4.3	53.6 ± 29.9
	All other sites	7	4.1 ± 6.3	0 ± 0

Primary sites were extensively sampled in 1999 and 2000 (Table 1; Fig. 1) while secondary sites were sampled once in 2000 (Table 2).
[a]Sample sites in bottom two layers of counties in Michigan (Fig. 1).

build, and because secondary sites included both irrigated and nonirrigated locations.

Whether or not *Stictospora* sp. or *Ovavesicula* are important pathogens of Japanese beetle is not clear. Hanula and Andreadis (1988) suggest that the Japanese beetle *Stictospora* sp. is avirulent because of it's ubiquity in Connecticut. Generally, gregarines have been considered as weak pathogens having little affect on their hosts (Maddox 1987; Tanada and Kaya 1993; Undeen and Vavra 1997). However, few gregarines have been studied carefully enough to determine the affect of infection on their hosts. More testing is needed to determine what affect *Stictospora* sp. has on Japanese beetle larvae.

Ovavesicula popilliae infection can reduce Japanese beetle fecundity by 50%, and delay oviposition (Hanula 1990). In Connecticut the epizootiology of *O. popilliae* was investigated at four sites over a 3-yr period (Hanula 1990). At two sites, the density of Japanese beetle larvae decreased from 8.0/0.1 m^2 to 1.0–3.0/m^2 as the incidence of *O. popilliae* increased from 40 to 80%. The role of *O. popilliae* as a pathogen of Japanese beetle deserves more attention.

Nematodes. Entomopathogenic nematodes were observed at three of 11 primary sites in spring 1999, for an overall average infection rate of 1.4% (Table 1). Nematodes at site "D" were *Steinernema* sp.; nematodes at sites E and I were *Heterorhabditis* sp. No infected grubs were detected on other sample dates or at the secondary sites. Nematode incidence data underestimates total mortality, as only recently infected, nondegraded cadavers can be observed. However, it appears that in a variety of habitats and sample dates, nematodes inflict minimal mortality on Japanese beetle in Michigan. Our data are consistent with previous reports indicating that while entomopathogenic nematodes may be widespread in turf soils (Campbell et al. 1995, 1998; Klein and Moyseenko 1997) and may cause occasional epizootics (Poinar et al. 1987; Kaya and Gaugler 1993), they are not consistently significant mortality factors. An unidentified mermithid parasite was collected from a single grub collected at site H in September 1999. Mermithidae are generally uncommon pathogens in Japanese beetle, though infection rates from 5 to 60% have been observed for *Psammomermis* sp. in New England (Klein et al. 1976).

Fungi. Metarhizium anisopliae and a fungus believed to make larvae susceptible to *M. anisopliae* infection, *Entoderma colletosporium*, were not observed in the field during this study Hanula and Andreadis 1991). Infected grubs did occur in Japanese beetle samples collected and held in the laboratory for 1–2 wk; however even under these conditions the infection rate was <0.1%. Metarhizium anisopliae prevalence in natural Japanese beetle populations is generally "very low" (Fleming 1968). Hanula and Andreadis (1988) detected this pathogen in 1.2% of Japanese beetle larvae in Connecticut.

The parasites and pathogens evaluated in this study cause minor mortality of Japanese beetle in Michigan. Although present, neither *P. popilliae* or species of entomopathogenic nematodes were epizootic at any of the observed sites. The absence of the parasitoids *T. vernalis* and *I. aldrichi* suggest that these species have not yet colonized the state, or the climate in Michigan is not conducive to their survival. The observation of *Ovavesicula popilliae* in just one geographic locality, suggests that this pathogen is in the early stages of colonization. *Stictospora* sp. is abundant in areas where Japanese beetle has been well established for more than 20 yr, but is absent or scarce in new infestations. At this time it is not known how *Stictospora* sp. affect populations of Japanese beetle. The absence of *T. vernalis* and *I. aldrichi* from all sites, and the absence of *O. popilliae* from 33 of 35 sites suggests that the introduction of these natural enemies may be beneficial, especially when considering their prevalence in Connecticut.

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References Cited

Andreadis, T. G., and J. L. Hanula. 1987. Ultrastructural study and description of *Ovavesicula popilliae* N.G., N. Sp. (Microsporida: Pleistophoridae) from the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). J. Protozool. 34: 15–21.

Campbell, J. F., E. Lewis, F. Yoder, and R. Gaugler. 1995. Entomopathogenic nematode (Heterorhabditidae and Steinernematidae) seasonal population dynamics and impact on insect populations in turfgrass. Biol. Control 5: 598–606.

Campbell, J. F., G. Orza, F. Yoder, E. Lewis, and R. Gaugler. 1998. spatial and temporal distribution of endemic and released entomopoathogenic nematode populations in turfgrass. Entomol. Exp. Appl. 86: 1–11.

Cherry, R. H., and M. G. Klein. 1997. Mortality induced by *Bacillus popilliae* in *Cyclocephala parallela* (Coleoptera: Scarabaeidae) held under simulated field temperatures. Fla. Entomol. 80: 261–265.

Crutchfield, B. A., and D. A. Potter. 1995a. Tolerance of cool-season turfgrasses to feeding by Japanese beetle and southern masked chafer (Coleoptera: Scarabaeidae) grubs. J. Econ. Entomol. 88: 1380–1387.

Crutchfield, B. A., and D. A. Potter. 1995b. Damage relationships of Japanese beetle and southern masked chafer (Coleoptera: Scarabaeidae) grubs in cool-season turfgrasses. J. Econ. Entomol. 88: 1049–1056.

Fleming, W. E. 1968. Biological control of the Japanese beetle. U.S. Dep. Agric. Tech. Bull. 1383.

Fleming, W. E. 1972. Biology of the Japanese beetle. U.S. Dep. Agric. Tech. Bull. 1449.

Gardner, T. R. 1938. Influence of feeding habits of *Tiphia vernalis* on the parasitization of the Japanese beetle. J. Econ. Entomol. 31: 204–207.

Hadley, C. H., and I. M. Hawley. 1934. General information about the Japanese beetle in the United States. USDA Circ. 332.

Hanula, J. L. 1990. Epizootiological investigations of the microsporidium *Ovavesicula popilliae* and bacterium *Ba-*

cillus popilliae in field populations of the Japanese beetle (Coleoptera: Scarabaeidae). Environ. Entomol. 19: 1552–1557.

Hanula, J. L., and T. G. Andreadis. 1988. Parasitic microorganisms of Japanese beetle (Coleoptera: Scarabaeidae) and associated scarab larvae in Connecticut soils. Environ Entomol. 17: 709–714.

Hanula, J. L., and T. G. Andreadis. 1991. Entoderma colletsporium gen. and sp. nov., a unique cuticular fungus infecting Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). J. Invertebr. Pathol. 58: 327–334.

Humber, R. A. 1997. Fungi: identification, pp. 153–185. *In* L. A. Lacey (ed.), Manual of techniques in insect pathology. Academic, San Diego, CA.

Hutton, P. O., and P. P. Burbutis. 1974. Milky disease and Japanese beetle in Delaware. J. Econ. Entomol. 67: 247–248.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38: 181–206.

King, J. L. 1950. The spring tiphia, an imported enemy of the Japanese beetle. U.S. Bur. Entomol. And Plant Q. E-799.

King, J. L., and J. K. Holloway. 1930. *Tiphia popilliavora*, a parasite of the Japanese beetle. USDA Circ. 145.

Klein, M. G., and T. A. Jackson. 1992. Bacterial diseases of scarabs *In* T.R. Glare and T.A. Jackson (eds.), Use of pathogens in scarab pest management. Intercept, Hanover.

Klein, M. G., and J. J. Moyseenko. 1997. Isolation and infectivity of entomopathogenic nematodes from Ohio. J. Nematol. 29: 590.

Klein, M. G., Nickle, W. R., Benedict, P. R., and D. M. Dunbar. 1976. Psammomermissp. (Nematoda:Mermithidae): a new nematode parasite of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). Helminthol. Soc. Wash. 43: 235–236.

King, J. L. 1931. The present status of the established parasites of *Popillia japonica* Newman. J. Econ. Entomol. 24: 453–462.

Lucarotti, C. J., and R. E. Clopton. 1998. Incidence of *Leidyana canadensis* (Apicomplexa: Eugregarinida) in *Lambdina fiscellaria* larvae (Lepidoptera: Geometridae). Can. Entomol. 130: 583–594.

Maddox, J. V. 1987. Protozoan diseases, pp. 417–452. *In* J. R. Fuxa and Y. Tanada (eds.), Epizootiology of insect diseases. Wiley, New York.

Pettersson, B., K. E. Rippere, A. A. Yousten and F. G. Priest. 1999. Transfer of *Bacillus lentimorbus* and *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov. Int. J. Syst. Bacteriol. 49: 531–540.

Poinar, G. O., Jr., T. Jackson, and M. Klein. 1987. Heterorhabditis megidis sp. n. (Heterorhabditidae: Rhabditida), parasitic in the Japanese beetle (Coleoptera: Scarabaeidae), in Ohio. Proc. Helminthol. Soc. Wash. 54: 53–59.

Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis, and control. Ann Arbor Press, Chelsea, MI.

Regniere, J., and W. M. Brooks. 1978. Entomogenous microorganisms associated with the Japanese beetle, *Popillia japonica*, in eastern North Carolina. J. Invertebr. Pathology 32: 226–228.

Smitley, D. R., T. W. Davis, and N. L. Rothwell. 1998. Spatial distribution of *Ataenius spretulus*, *Aphodius granarius* (Coleoptera: Scarabaeidae), and predaceous insects across golf course fairways and roughs. Environ. Entomol 27: 1336–1349.

Tanada, Y., and H. K. Kaya. 1993. Insect Pathology. Academic, San Diego.

Thurston, G. S., H. K. Kaya, and R. Gaugler. 1994. Characterizing the enhanced susceptibility of milky disease-infected scarabaeid grubs to entomopathogenic nematodes. Biol. Control 4: 67–73.

Undeen, A. H., and J. Vavra. 1997. Res. methods for entomopathogenic protozoa. *In* L. Lacey (ed.), Manual of techniques in insect pathology. Academic, San Diego, CA.

USDA. 2000. National Agricultural Pest Information System: Japanese beetle (http://www.ceris.purdue.edu/napis/pests/jb/index.html).

USDA. 2001. First detection and first known establishment of Japanese beetle in Michigan counties. USDA-APHIS National Agricultural Pest Information System (NAPIS) and the Cooperative Agricultural Pest Survey (CAPS) (http //napis.ceris.purdue.edu/htbin/sr, p. 4.com).

Vittum, P. J., M. G. Villani, and H. Tashiro. 1999. Turfgrass insects of the United States and Canada. Cornell University Press, Ithaca, NY.

Wellso, S. G., and R. L. Fischer. 1971. Insects taken at Japanese beetle traps baited with anethole-eugenol in southern Michigan in 1968. Mich. Entomol. 4: 105–108.

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