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Mattesia oryzaephili (Neogregarinorida: Lipotrophidae), a Pathogen of Stored-Grain Insects: Virulence, Host Range and Comparison with *Mattesia dispora*

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The neogregarine, Mattesia oryzaephili *(Neogregarinorida: Lipotrophidae) has only been reported from the sawtoothed grain beetle,* Oryzaephilus surinamensis*. The pathogen's presence in cadavers of the rusty grain beetle,* Cryptolestes ferrugineus*, in collapsed colonies prompted studies of its potential to control stored-product insects. Respective mortality rates in fourth instar* C. ferrugineus *and* C. pusillus *were 15.3 and 17.7% at 10^2 oocysts/g of diet and 89.4 and 80.5% at 10^5 oocysts/g. The mortality of fourth instar* O. surinamensis *exposed to 10^5 oocysts/g was only 12%. For* C. ferrugineus *larvae, there were no significant differences in mortality and infection between exposure to* Mattesia dispora *and exposure to* M. oryzaephili *($P > 0.05$), but for* C. pusillus *larvae, both responses were significantly higher for* M. oryzaephili *than* M. dispora*. Adult* C. ferrugineus *and* O. surinamensis *were similar in their responses to* M. oryzaephili*, with mortality not exceeding 20%, but differed in their responses to* M. dispora*, with* O. surinamensis *being more susceptible. The median lethal doses for larval Mediterranean flour moths,* Ephestia kuehniella*, were 7.9×10^7* M. oryzaephili *oocysts/g of diet and 2.7×10^3* M. dispora *oocysts/g of diet. In single dose assays of* M. oryzaephili *physiological host range, greater than 75% infection was achieved for* Rhyzopertha dominica *and* Plodia interpunctella*. More than half of oocysts germinated during passage through the guts of susceptible and resistant insects. Second and third instar* Galleria mellonella *were highly susceptible to* M. oryzaephili *infection, but fifth instars were not. Infection percentages in fifth instars exposed to 10^6 oocysts/g were significant only when boric acid or the stilbene, Blankophor® RHK were incorporated into the diet. Host range and general morphology confirm the identity of* Mattesia oryzaephili*.*

Keywords: Mattesia, Cryptolestes, Oryzaephilus surinamensis, Plodia interpunctella, Ephestia kuehniella, *neogregarine, stilbene, boric acid*

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INTRODUCTION

A neogregarine parasite has been associated with rapid declines in colonies of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), in our laboratory. Its development is intracellular, there are two merogonial sequences, and the navicular oocysts are formed in pairs within the gametocyst. These traits place it in the genus *Mattesia*, family Lipotrophidae (Grassé, 1953). Based on the oocyst and nuclear measurements and parallel formation of micronuclear merozoites, I identified it as *Mattesia oryzaephili* Ormières, Loubes, and Kuhl, a pathogen that has been previously reported only from the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Silvanidae) (Ormières *et al*., 1971, 1972). *Mattesia oryzaephili* macronuclear meronts form plasmodia with four to 20 nuclei in individual lobes (Ormières *et al*., 1971). Plasmodial macronuclear meronts are very rare in our isolate, and I have seen none with more than eight nuclei. *Mattesia dispora* Naville is morphologically very similar to *M. oryzaephili* (Naville, 1930), and there are published reports in which their identities may be confused. For example, Finlayson (1950) reported on *M. dispora* pathology for *C.* (*Laemophloeus*) *ferrugineus* and *Cryptolestes pusillus* (Schoenherr) (*Laemophloeus minutus*). The oocyst dimensions of his isolate matched those of *M. oryzaephili*, the description of which his work predated. There is no record of type specimens or type locality for *M. oryzaephili*. Consequently, biochemical or morphological confirmation of my identification is not possible, but host affinities may be useful to distinguish species.

In addition to confirming our taxonomic placement of our isolate, there are other reasons that the host range is of interest. The grain beetles are small insects, and their use for oocyst production is labor-intensive and limits supply. A larger host might produce many times more oocysts with lower resource expense. Furthermore, a broad range of hosts would not only be advantageous in target applicability, but also could help to sustain *M. oryzaephili* populations after field application.

The purpose of the work presented here was to determine the relative susceptibility of insect pests of stored grain with the ultimate goal of identifying targets for microbial control. I was also interested in finding hosts other than the small grain beetles that might be used for production of oocysts. Lastly, I hoped to confirm the identification of the species.

MATERIAL AND METHODS

Mattesia

Mattesia oryzaephili was isolated from *C. ferrugineus* in our laboratory colony, which originated from stored wheat collected in Eastern Kansas several years prior. Inoculum of oocysts was produced in *O. surinamensis* larvae. The *M. dispora* was obtained from *Ephestia kuehniella* (Zeller) (Lepidoptera, Pyralidae) in the colony of Dr. Yolanda Cruz, Oberlin College, and inoculum was produced in *E. kuehniella* larvae.

Insects

All of the test insects were obtained from laboratory colonies. *Cryptolestes ferrugineus*, *C. pusillus* (Schoenherr), *E. kuhniella*, *O. surinamensis*, *Plodia interpunctella* (Hübner) (Lepidoptera, Pyralidae), *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bruchidae), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), *Tribolium castaneum* (Herbst) (Col., Tenebrionidae), and *Trogoderma variable* Ballion (Coleoptera: Dermestidae) have been maintained in colonies at the Grain Marketing and Production Research Center of the Agricultural Research Service (ARS) in Manhattan, KS, for several years and are thought to be of local origin. *Ostrinia nubilalis* (Hübner) (Lepidoptera, Crambidae) was obtained from Kansas State University. *Manduca sexta* (L.) (Lepidoptera, Sphingidae) and *Galleria mellonella* (L.) (Lepidoptera, Pyralidae), were obtained from Carolina Biological Supply, Burlington NC. *Aedes aegypti* (L.) (Diptera, Culicidae) eggs were obtained from ARS,

Gainesville, FL. *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) was obtained from ARS, Stoneville, MS, *Zophobas atratus* Fabricius (Coleoptera: Tenebrionidae) was obtained from Nature's Way, Ross, OH. *Liposcelis bostrichophila* (Badonnel) (Psocoptera: Liposcelidae) was found as a contaminant in a *C. ferrugineus* colony and was reared on a wheat flour, dried milk, and brewer's yeast diet. Beetles were reared on grain or flour diets. *Plodia interpunctella* and *G. mellonella* were maintained on a wheat-based diet with dried milk, brewer's yeast, glycerin, and honey. *Ephestia kuhniella* was maintained on all-purpose wheat flour. Insects were maintained at $30 \pm 1^{\circ}$C and ca. $60 \pm 1\%$ RH except for *C. ferrugineus*, which was maintained at $75 \pm 1\%$ RH over saturated NaCl solution and *L. bostrichophila* which was maintained at $85 \pm 1\%$ RH over saturated KCl solution.

Dose–Response Assays

For dose–response assays with stored-product insects, the oocysts were mixed into all-purpose wheat flour at the appropriate concentrations. Coleoptera were treated in groups of 10 in 30-mL plastic cups with five replicate cups per test. They were exposed to 0.1 g of treated flour for 24 h, then 5 g of crimped hard red winter wheat were added. Adults were treated 5–6 weeks after hatch. The tests were run for 21 days. All living and dead insects were examined microscopically for the presence of oocysts. *Ephestia kuehniella* larvae were assayed at 1 week post-hatch, and the larvae were treated individually in 2-cc wells of assay trays (C-D International, Pitman, NJ) with 0.1 g of oocyst-treated flour. After 3 days, ca. 0.4 g of additional flour was added. There were 48 wells per treatment in each test. Mortality was scored at 21 days, and percent infection was scored at 35 days. All assays were conducted at $26 \pm 1^{\circ}$C and $75 \pm 1\%$ RH and carried out three times on separate days.

Host Range Assays

All insects were treated and incubated individually except for *A. aegypti* and *L. bostrichophila.* All were subject to continuous exposure to *M. oryzaephili* oocysts, except for *H. zea*, *M. sexta*, and *O. nubilalis*. The latter three species were reared to the second instar on preservative-free commercial diets. They were then fed 3 μL of aqueous oocyst suspensions and placed on diets of corn kernels (*H. zea*), green peppers (*M. sexta*), or wheat germ (*O. nubilalis*). Neonate *P. interpunctella* and eggs of *R. dominica* were placed on treated diet that remained undiluted throughout the assays. Other Coleoptera were treated as second instars. *Tenebrio molitor* was incubated in oocyst-treated, undiluted rolled oats. *Zophobas atratus* and *T. castaneum* were exposed to 50 mg of treated wheat flour to which additional diet was added after 2 days. *Aedes aegypti* neonates were exposed overnight to oocysts in 3 mL of water, which was then diluted with 500 mL of water with 2 g of hog chow for completion of development. After emergence, the adult mosquitoes were held in cages with sugar and water provided. *Liposcelis bostrichophila* was treated en masse by exposure in 0.1 g of diet with oocysts and exposed continuously. All were incubated under the above rearing temperature and humidity regimes. Control insects were handled in the same manner as the treatment insects except that no oocysts were added.

Germination Rates

Fourth instar beetles and *G. mellonella* larvae were fed flour-treated with 5×10^7 *M. oryzaephili* oocysts/g and red food coloring. Fourth instar *O. nubilalis* larvae were given 4×10^7 oocysts in water with red food coloring. When the pigment was visible in the gut, the insects were placed on standard diet. Fifteen (Lepidoptera) or 30 (Coleoptera) were treated held in four replicate cups/insect species. Red frass pellets were collected from each rearing cup, and 30 oocysts in each of 10 frass pellets/cup were examined with phase contrast optics for scoring as intact or germinated (empty).

Infectivity Using Adjuncts

Galleria mellonella was chosen as the target host because it is taxonomically close to the Lepidoptera that were found to be susceptible to *M. oryzaephili*, it is large relative to the stored product hosts, and it is subject to infection by many non-specific pathogens, including *M. dispora* (Duhlinska, 1986). Eggs were collected over three 3-day periods to obtain larvae in the second, third and fifth instars. All treatments were mixed into *G. mellonella* diet at doses of 10^6 oocysts/g, 0.1% boric acid, 0.2% of the stilbene fluorescent brightener, Blankophor RKH® (Bayer Corp., Pittsburg, PA). The larvae were exposed continuously to 3 g of treated medium in 50-cc shell vials with screened lids. There were six larvae per vial and five vials per treatment. Incubation was for 14 days at $26 \pm 1^{\circ}$C and $75 \pm 1\%$ RH. Infection percentage was tallied by microscopic examination of squashes of live larvae and recovered cadavers. Missing larvae were tallied as dead, but were not included in infection calculations.

Statistic Analyses

Where appropriate, two-tailed *t*-tests and ANOVA with Student–Newman–Keuls post test were preformed with InStat software (Motulsky & Searle, 1990). The dose response estimates for *E. kuehniella* were generated with the POLO probit analysis program (Russell *et al*., 1977). Beetle dose–response data were too variable for probit analysis. The dose–response data were adjusted with Abbott's formula (Abbott, 1925) for control mortality when it occurred. In all cases, control mortalities were less than 12%.

RESULTS

Dose Responses

Mortality rates of C. *ferrugineus* and *C. pusillus* fourth instar larvae exposed to *M. oryzaephili* were similar over the range of doses with respective lows of 15.3 and 17.7% at 10^2 oocysts/g of diet and highs of 89.4 and 80.5 at 10^5 oocysts/g (Figure 1). Larvae of the three species responded differently to the two *Mattesia* species. Only one dose rate, 10^5 oocysts/g of diet, was used for both *Mattesia* species and all beetles. At that dose, mortality and infection precentage rates of both the fourth instar *C. ferrugineus* and *C. pusillus* were significantly higher with *M. oryzaephili* than with *M. dispora* ($P < 0.01$). In the case of *O. surinamensis*, the mortality of fourth instars did not differ significantly ($t = 1.53$, df = 28, $P = 0.14$), but there was a higher rate of infection with *M. dispora* ($t = 2.20$, df = 28, $P = 0.04$). On the other hand, second instar *O. surinamensis* had significantly greater mortality with *M. dispora* ($P < 0.01$, df = 28, t = 3.93) but higher infection with *M. oryzaephili* ($t = 3.55$, df = 28, $P = 0.04$).

Unlike the larvae, adults of *C. ferrugineus* and *O. surinamensis* had similar mortality and infection responses to *M. oryzaephili* (Figure 2). There were no significant differences in mortality resulting from infection by the two *Mattesia* species at the common doses of 10^5 and 10^6 oocysts/g for *C. ferrugineus* ($t = 1.71$ and $t = 1.56$, df = 28, $P > 0.05$) or *O. surinamensis* ($t = 0.10$ and $t = 0.81$, df = 28, $P > 0.05$). There was a significantly higher percentage infection of *M. oryzaephili* than *M. dispora* in *C. ferrugineus* at 10^5 ($t = 4.27$, df = 28, $P, < 0.01$) and 10^6 ($t = 2.93$, df = 28, $P < 0.01$) oocysts/g of diet. But the higher percent infection of *M. oryzaephili* in *O. surinamensis* was significant only at 10^6 oocysts/g of diet ($t = 2.83$, df = 28, $P < 0.01$).

Mattesia dispora was more infectious and more virulent for *E. kuehniella* larvae than was *M. oryzaephili*. The 21-day median lethal concentrations and 95% confidence intervals were 2.8×10^3 ($0.32–15.7 \times 10^3$) and 7.9×10^7 ($0.31–39.2 \times 10^7$) oocysts/g of diet respectively for a relative potency of 29 000 (Table 1). Infection percentage data were taken at 35 days to allow for the *M. oryzaephili* to develop detectable oocysts, and the median infective concentrations were 3.9×10^3 ($1.2 \times 10^3–1.3 \times 10^4$) and 6.5×10^4 ($1.2 \times 10^4–7.2 \times 10^5$), respectively.

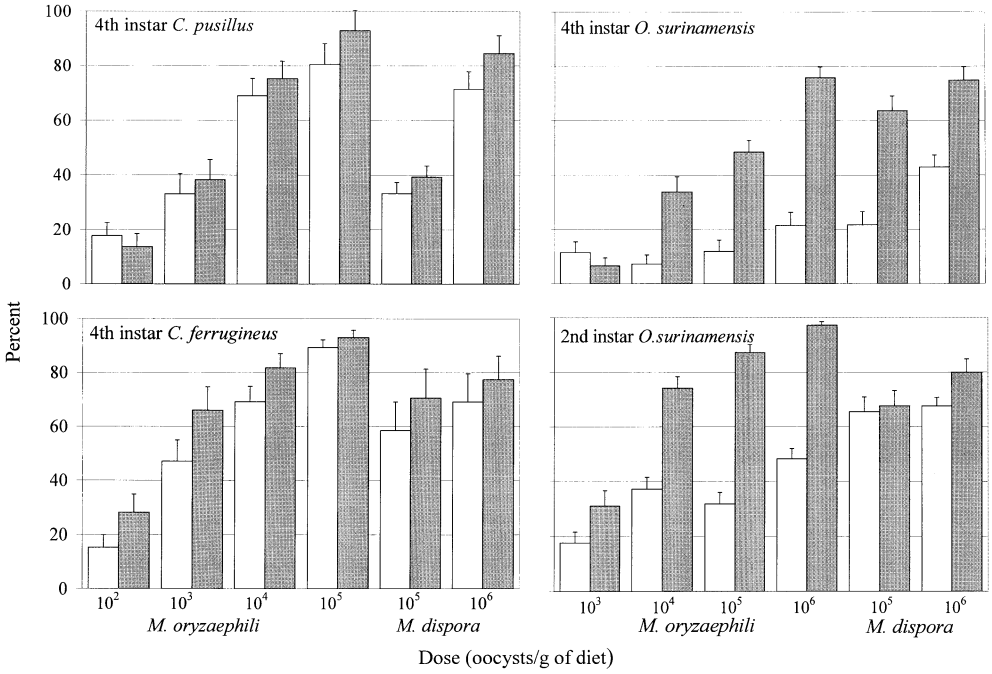

FIGURE 1. Percent±SE mortality (white) and infection (gray) of larval *Cryptolestes ferrugineus*, *Cryptolestes pusillus* and *Oryzaephilus surinamensis* 21 days after treatment with *Mattesia oryzaephili* and *Mattesia dispora*. (Mortality was corrected for control mortality. Infection was evaluated with dead and living insects.)

Host Range

In addition to the grain beetles and *E. kuehniella*, the species in which greater than 10% infection was detected were *P. interpunctella* and *R. dominica* (Table 2). Ten percent of *O. nubilialis* contained oocysts 18 days after exposure to 10^6 oocysts/ml of water, and 6.25% of *T. castaneum* contained oocysts after 14 days of exposure to 10^6 oocysts/g diet. There was no infection of the other test species.

Germination Rates

In the collected frass from *M. oryzaephili*-fed insects, the percent of oocysts that had germinated ranged from 54.8 to 99.5 (Table 3). The mean germination rates for *T. castaneum* and *T. variabile* were significantly higher than for all other tested species ($F = 101.5$, df = 5,206, $P < 0.01$). The percentage rates of infection in the host range assays with these two species were 0–6.3% at the test doses. Only 54.8% germination was recorded in the frass of *O. surinamenisis*, a susceptible host. Accordingly, the gut germination rates did not correspond to the susceptibility of the insects.

Adjunct assay

The percent infection of *M. oryzaephili* in fifth instar *G. mellonella* was significantly greater than zero only with either Blankophor®RHK or boric acid in the diet ($F = 8.48$, df = 5, 24, $P < 0.01$, Table 4). Mortality of fifth instar larvae was significant for only the oocyst–boric acid combination ($F = 6.96$, df = 5, 24, $P < 0.01$). All of the younger larvae, but only 6.6% of the fifth instars that were exposed to *Mattesia oryzaephili* without adjuncts, became infected.

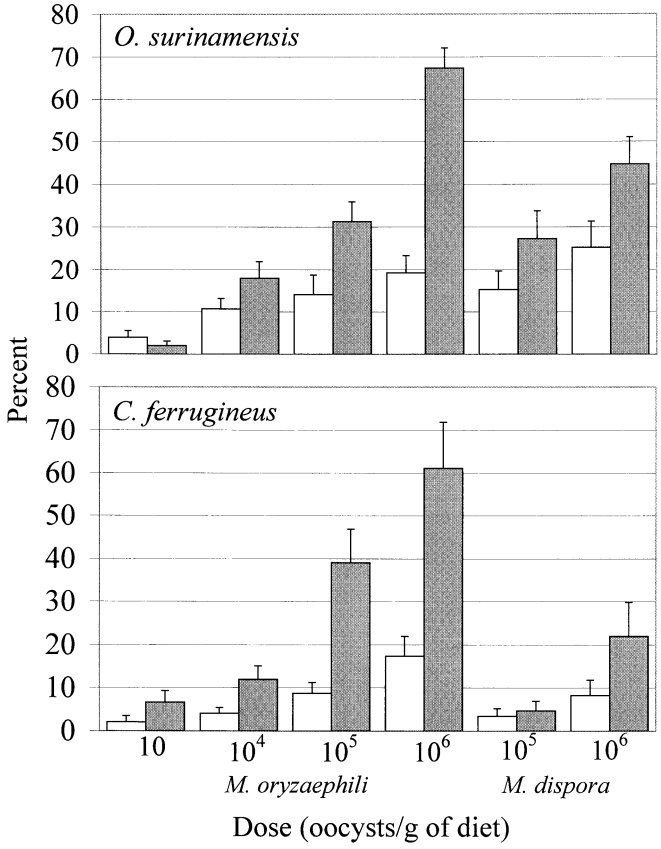

FIGURE 2. Percent ± SE mortality (white) and infection (gray) of adult *Cryptolestes ferrugineus* and *Oryzaephilus surinamensis* 21 days after treatment with *Mattesia oryzaephili* and *Mattesia dispora*. (Infection was evaluated with dead and living insects.)

TABLE 1. Dose response of *Ephestia kuehniella* larvae to *Mattesia oryzaephili* and *Mattesia dispora:* mortality at 21 days, infection at 35 days

		IC_{50} (95% C.I.)	IC_{90} (95% C.I.)	Slope
M. oryzaephili	Mortality	7.9×10^7 ($0.31–39.2 \times 10^7$)	6.5×10^8 (1.0×10^7–)	0.67
	Infection	6.5×10^4 ($1.2–71.9 \times 10^4$)	2.2×10^7 (1.4×10^6–)	0.51
M. dispora	Mortality	2.8×10^3 ($0.32–15.7 \times 10^3$)	1.7×10^5 ($0.026–40.1 \times 10^6$)	0.65
	Infection	3.9×10^3 ($1.2–12.6 \times 10^4$)	1.1×10^5 ($0.28–16.4 \times 10^5$)	0.90

Mortality was higher than in the controls for second instar larvae exposed to either adjunct without oocysts, but only the boric acid-associated mortality of 50% was significant ($F = 15.32$; df = 5, 24; $P < 0.01$). Mortalities of second instars did not differ significantly among *M. oryzaephili* treatments ($F = 15.32$, df = 5, 24, $P > 0.05$). Percent infection of second instars was made unreliable by loss of cadavers to cannibalism, particularly in the boric acid treatments, and is not presented. Among third instar larvae, all those treated with adjuvant-free oocysts became infected and died. Both mortality ($F = 128.9$; df = 5, 24; $P < 0.001$) and

TABLE 2. Infectivity of *Mattesia oryzaephili* for selected insects

Insect	Oocyst dose	Days post exposure	Infected/n
Aedes aegypti	1.7×10^6/ml	21	0/40
Manduca sexta	2.0×10^6/ml	14	0/30
Helicoverpa zea	2.0×10^6/ml	17	0/64
Plodia interpunctella	1.0×10^4/g	21	3/48
	1.0×10^6/g	21	38/48
Ostrinia nubilalis	1.0×10^6/ml	18	4/44
Rhyzopertha dominica	1.0×10^5/g	21	27/36
	1.0×10^6/g	21	28/37
Tenebrio molitor	2.5×10^5/g	22	0/30
Zophobas atratus	1.6×10^7/g	20	0/53
Tribolium castaneum	1.0×10^5/g	14	0/32
	1.0×10^6/g	14	2/32
Trogoderma variable	1.6×10^6/g	28	0/62
Liposcelis bostrichophila	2.0×10^6/g	28	0/40

TABLE 3. Germination rates of *Mattesia oryzaephili* oocysts after passage through the alimentary canals of larval insects; means followed by different letters are significantly different ($P < 0.01$)

Insect	Germination rate (SD)
Tribolium castaneum	99.5 (0.2)a
Trogoderma variabile	97.6 (0.9)a
Galleria mellonella	89.7 (1.3)b
Cryptolestes ferrugineus	80.3 (2.1)c
Ostrinia nubilalis	65.4 (1.4)d
Oryzaephilus surinamensis	54.8 (2.8)e

TABLE 4. Effect of 10^6 *Mattesia oryzaephili* oocysts/g of diet on *Galleria mellonella* of different ages: percent mortality[1] and infection with oocysts in larvae[2] treated with and without potential enhancers[3]

	Instar				
	2nd	3rd		5th	
Treatment	Mortality	Mortality	Infection	Mortality	Infection
Check	3.3c	0.0c	0.0c	0.0b	0.0b
M. oryzaephili	73.3ab	100a	100a	0.0b	6.6b
Boric acid	50.0b	6.7c	0.0c	0.0b	0.0b
M. oryzaephili + boric acid	93.3a	66.7b	86.7b	26.7a	38.0a
Blankophor®RHK	20.0c	3.3c	0.0c	0.0b	0.0b
M. oryzaephili + Blankophor®RHK	90.0a	90.0a	100a	3.3b	43.3a

[1]Missing larvae were scored as dead.
[2]Oocysts in live larvae and recovered cadavers.
[3]Data followed by the same letter within column do not differ significantly ($P < 0.05$).

percent infection ($F = 426.76$; df = 5, 24; $P < 0.01$) among third instar larvae were significantly lower in the oocyst + boric acid treatment.

DISCUSSION

The neogregarine that was found in declining *Cryptolestes ferrugineus* colonies in our laboratory closely fits the description of *M. oryzaephili* (Ormières *et al*., 1971) except for the rarity of the macronuclear plasmodia of the second merogony and their low number of nuclei. Its oocyst measurements are close to those in the *M. oryzaephili* description, the arrangement of micronuclear meronts is unlike any other described species in the genus, and it is infectious for *O. surinamensis*, the only previously recorded host for *M. oryzaephili*. I have observed a great deal of variation in its development between and within host species and do not consider the failure to observe large macronuclear plasmodia to be justification for placement in a new species.

This is the first record of *M. oryzaephili* in the New World. Because our laboratory has received insects from many localities around the world over several decades, we cannot be certain whether our isolate is of domestic or foreign origin. The genus *Mattesia* comprises several species of similar morphology and overlapping physiological host range. This is the case for the two species discussed herein. *Mattesia oryzaephili* has only been reported from the sawtoothed grain beetle, *O. surinamensis* L. (Ormières *et al*., 1971, 1972). Finlayson (1950) reported *M. dispora* from *C. ferrugineus* and *C. pusillus* in England. Based on a comparison of the data presented herein with Finlayson's limited description of its pathology for *C. ferrugineus* and *C. pusillus*, as well as its morphology and the fact that *M. oryzaephili* had not been described at the time of his account, we believe that the species of Finlayson (1950) was *M. oryzaephili*. *Cryptolestes ferrugineus* and *C. pusillus* were the most susceptible of the insects tested, including *O. surinamensis*.

Finlayson also reported that *E. kuehniella* and *P. interpunctella* were susceptible to the pathogen that he reported, although no data were given. The marked difference between *M. dispora* and *M. oryzaephili* in their infectivities and virulence for *E. kuehniella* is further confirmation that the two species are distinct and correctly identified. *Mattesia oryzaephili* develops much more slowly than *M. dispora* in this host as is evidenced by the 1200-fold difference in the 21-day IC_{50} for mortality and the 35-day IC_{50} for infection. One might speculate that the absence of reports of *M. oryzaephili* from Lepidoptera in nature results from inability to detect early infections or incorrect identification.

The literature on host ranges of insect-infecting neogregarines presents a puzzling picture. Purrini (1976, 1977) surveyed the neogregarines among other pathogens of stored-product insects in the Kosovo district of Yugoslavia and reported only *Farinocystis tribolii* Weiser in Coleoptera and *M. dispora* only in Lepidoptera, in particular *E. kuehniella*, which was infected at all the nine localities examined. In addition to Finlayson (1950), there is one previous report of *M. dispora* in beetles. Burkholder and Dicke (1964) referred to a pathogen in *Trogoderma glabrum* (Herbst) as *M. dispora*, but it appears to be the species that was described by Canning (1964) and referred to in later reports as *Mattesia trogodermae*.

The two insects that had the highest infection rates in single dose tests with *M. oryzaephili* are among the most important pests of stored grain, the lesser grain borer, *R. dominica*, and the Indianmeal moth *P. interpunctella*. The lesser grain borer is a primary pest of stored grain. In nature, first instar beetle larvae bore into grain, thereby limiting their exposure to natural enemies that lack search capacity. However, Leliveldt *et al*. (1988) reported that an unidentified *Mattesia* species was present at low prevalence in the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) populations infesting stored corn at many locations in Togo. *Plodia interpunctella* is among the most common and serious pests of stored products (USDA-ARS, 1986). Although *R. dominica* and *P. interpunctella* may not be primary targets for *M. oryzaephili*, some effect on them and amplification of inoculum may result from its application for grain beetles.

The measurement of *M. oryzaephili* oocyst germination during passage through alimentary canals was intended to determine whether this is a host range determinant. The results show that it is not. They do, however, have implications for oocyst dispersal. When oocysts are consumed by scavenging low susceptibility hosts, such as *T. castaneum* and *T. variable*, nearly all of the inoculum is lost to futile germination rather than being dispersed as intact oocysts.

The results indicate that *G. mellonella* larvae can serve as a medium for producing oocysts in larger quantities than are practical with the known beetle hosts. Preliminary trials with *G. mellonella* indicated a marked decrease in susceptibility with age. Accordingly, adjuvants that have been reported to increase the potency of pathogens against other insects were tested in an effort to make use of larger and potentially more productive larvae. Stilbene fluorescent brighteners such as Blankophor®RHK have been shown to enhance baculovirus infection of Lepidoptera (Shapiro & Argauer, 2001). Wang and Granados (2000) demonstrated that the stilbene, calcofluor, enhances virus efficacy by disruption of protein–chitin binding in the peritrophic membrane. Reduction in peritrophic membrane barrier may similarly aid *M. oryzaephili* sporozoite entry into the midgut epithelium. Boric acid's mode of action is not known, but it has recently been shown to synergize the action of the fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin, against the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) (Zurek *et al*., 2002). Synergism between these types of compounds and Protozoa has not previously been reported and will be the subject of further study.

Within the limits of this study and the few reports in the literature, *M. oryzaephili* seems to have an affinity for insects that are associated with stored products. Perhaps this reflects a long association with the habitat. The species that were found to be susceptible in this study include some of the most abundant and damaging pests of stored grain. Laboratory susceptibility to a pathogen (physiological host range) is not a definitive indicator of the true ecological host range, as was demonstrated with microsporidian pathogens of forest Lepidoptera by Solter and Maddox (1998) and Solter *et al*. (2000). Accordingly, inoculative release trials are warranted to assess *M. oryzaephili*'s value as a multiple-target biological grain protectant.

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