Interaction of *Mattesia oryzaephili* (Neogregarinorida: Lipotrophidae) with *Cephalonomia* spp. (Hymenoptera: Bethylidae) and their hosts *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

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Abstract

The neogregarine, *Mattesia oryzaephili*, is pathogenic for several stored-grain pest insects, including the sawtoothed grain beetle, *Oryzaephilus surinamensis*, and the rusty grain beetle, *Cryptolestes ferrugineus*. It also infects their respective bethylid parasitoids, *Cephalonomia tarsalis* and *Cephalonomia waterstoni*. Male wasps do not attack the beetle larvae and do not become infected, but the disease is transmitted per os to nearly all female wasps when they paralyze or feed on infected hosts. The mean survival time of infected female *C. tarsalis* after exposure to heavily infected *O. surinamensis* was 20.0 days, while that of healthy *C. tarsalis* was 38.0 days. For *C. waterstoni* females, the mean survival times were 36.1 days when infected and 45.9 days when uninfected. The long survival time of infected wasps fosters oviposition and inoculum deposition in the hosts' habitat. The wasps sting and bite infected host larvae and sometimes oviposit. Wasp progeny that are deposited on infected host larvae rapidly succumb to *M. oryzaephili*. Two-hour wasp confinement with patentently infected larvae resulted in no transmission from *C. waterstoni* to *C. ferrugineus* and 4% transmission from *C. tarsalis* to *O. surinamensis*. Contamination of wheat by wasps after they had attacked infected beetles resulted in 8.9% infection of *O. surinamensis* that developed from eggs that were placed on the contaminated wheat. It is proposed that *Cephalonomia* spp. can be used as a means to inoculate *M. oryzaephili* into pest grain beetle populations and aid in its dispersal to help suppress pest populations.

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Keywords: *Mattesia oryzaephili*; *Cephalonomia tarsalis*; *Oryzaephilus surinamensis*; *Cryptolestes ferrugineus*; Stored grain; Biological control; Grain beetle; *Cephalonomia waterstoni*

1. Introduction

Because stored grain is a low value commodity for which pest control expenditures are severely limited, suppression by natural enemies is sometimes the only means of protection from pests that is available. Numerous predators, parasitoids and pathogens have been investigated individually (Moore et al., 2000; Schöller and Flinn, 2000), but few data have been reported relative to the interactions between beneficial insects and pathogens in stored products.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), are among the most abundant cosmopolitan pests of stored grain. Both beetles are attacked by parasitoid wasps of the genus *Cephalonomia* (Finlayson, 1950b; Howard et al., 1998) and by the neogregarine, *Mattesia oryzaephili* Ormières (Finlayson, 1950a; Lord, 2003; Ormières et al., 1971). Efforts at biological control of these pests have heretofore been limited to experimentation with individual natural enemies. A validated simulation model (Flinn and Hagstrum, 1995) predicted a substantial impact of...
Cephalonomia waterstoni (Gahan) on a C. ferrugineus population with a well-timed introduction of adequate numbers of wasps. On the other hand, Powell (1938) considered the O. surinamensis parasitoid, Cephalonomia tarsalis (Ashmead), to lack economic importance in the control of its host. M. oryzaephili’s impact on wild populations has not been investigated, but it has caused population crashes in our C. ferrugineus laboratory colonies (Lord, 2003). I have found that M. oryzaephili infects both the wasps. Accordingly, the neogregarine and the wasps might interact in a manner that is complementary or synergistic, providing biological suppression of the pest beetles. As a first step in evaluating their potential for pest suppression, this study was undertaken to determine the wasps’ ability to transmit M. oryzaephili, their susceptibility to it, and how their survival and fecundity would be affected.

2. Materials and methods

2.1. Organisms

Mattesia oryzaephili was isolated from our C. ferrugineus colony (Lord, 2003) and propagated in O. surinamensis larvae. C. ferrugineus, O. surinamensis, C. tarsalis, and C. waterstoni were collected from farm-stored wheat in Kansas and had been maintained in the laboratory for approximately 6–8 years. C. ferrugineus and O. surinamensis were reared on rolled oats with brewer’s yeast. The insects were maintained at 30 ± 1° C and 55 ± 10% RH except for C. ferrugineus, which was maintained at 75 ± 1% RH over saturated NaCl solution. C. ferrugineus was the host in all C. waterstoni experiments and O. surinamensis was the host in all C. tarsalis experiments. Chill anesthesia was used for handling the wasps. Sex was determined by antenna morphology (Rilett, 1949).

2.2. Susceptibility and oviposition

The initial experiments were designed to assess wasp susceptibility to M. oryzaephili via feeding on infected host larvae and the effect of infection on oviposition. To prepare infected hosts, fourth instar beetle larvae were exposed to maximum-challenge dosages of M. oryzaephili (>10⁷ oocysts/g of wheat flour), and still-motile infected larvae were selected for use when infections were detectable by fluorescence under ultraviolet light (Burkholder and Dicke, 1964). Mated, 3- to 5-day-old C. tarsalis or C. waterstoni were sexed and placed in individual 12.5 cm² tissue culture flasks with four infected hosts or four healthy hosts, a thin streak of 50% honey with water, and ca. 5 g of crimped wheat. After 7 days, the wasps were transferred to fresh flasks with four healthy fourth instar beetles to serve as oviposition hosts. After 7 more days of incubation, oviposition, mortality, and infection were scored. Infection was detected microscopically by the presence of oocysts. The experiment was replicated over time with 5 replicates of 10 female wasps-flasks per replicate. Male wasps were used as available in replicates of 6 until n = 60 for C. tarsalis and n = 30 for C. waterstoni had been used. In a separate test, 42 male C. tarsalis were exposed to 10⁷ oocysts/ml mixed with 50% honey water that was streaked on flask sides. In all experiments, incubation was in darkness at 26 ± 1° C and 75 ± 1% RH.

2.3. Survival time

Three to five days after emergence (to obtain adequate numbers and eliminate premature deaths), female C. tarsalis and C. waterstoni were confined individually with two healthy or two patently infected host larvae in 13 × 100 mm glass tubes. Each tube had ca. 5 g of wheat kernels, a narrow streak of 50% honey water, and a cotton ball closure. Two healthy fourth instar host larvae were added to each tube weekly. Survival was checked daily, and infections were confirmed by the presence of oocysts in the cadavers. The data were accumulated from four cohorts of each wasp species and pooled so that C. waterstoni infected n = 61, C. waterstoni uninfected n = 36, C. tarsalis infected n = 44, and C. tarsalis uninfected n = 36.

2.4. Wasp to beetle transmission

To test for transmission from wasps to host larvae, individual wasps of C. tarsalis or C. waterstoni were exposed to 2 live, fluorescent, M. oryzaephili-infected fourth instar host larvae for 2 hours in 13 × 100 mm test tubes with ca. 1 g of wheat. The wasps were then transferred to individual test tubes with two healthy fourth instar hosts, ca. 5 g of whole wheat kernels, and a streak of honey water. After 10 days, the beetle larvae and the wasps were examined for oocysts. Only beetle larvae that were confined with oocyst-positive wasps were included in the analysis. Control beetles were held in tubes without wasps. The experiment with C. tarsalis and O. surinamensis was carried out three times with a total of 183 beetle larvae that were exposed to M. oryzaephili-positive wasps and 208 control larvae. The C. waterstoni and C. ferrugineus experiment was carried out once with a total of 64 beetle larvae exposed to infected wasps and 64 control larvae.

2.5. Fecundity and residual transmission

Two- to five-day-old female C. tarsalis were exposed individually to either two patently infected (fluorescent) fourth instar O. surinamensis for 24 h in 13 × 100 mm glass tubes with 5 g of crimped wheat and a streak of honey water. The wasps were transferred to fresh tubes with wheat and honey but without hosts for 48 h to allow passage of oocysts through the alimentary canal before the tubes were used to test for residual transmission. The C. tarsalis were then transferred to individual 120 ml glass jars with 40 g of wheat, a honey streak and 20 healthy fourth instar host larvae. The jars were covered with 70 mm disks of Whatman No. 1 filter paper. At weekly intervals until death, the wasps were transferred to freshly
prepared jars with 20 additional host larvae. After transfer, the used jars were held for 3 weeks after transfer for counts of wasp progeny after they had emerged as adults. Infections in wasp cadavers were confirmed microscopically. Initially there were 43 *M. oryzaephilii*-exposed *C. tarsalis* and 40 control wasps.

The tubes in which the *C. tarsalis* had been held for 48 hours after exposure to *M. oryzaephilii* were used to test whether the wasps deposited infectious inoculum in their environment. After the wasps were removed, ten *O. surinamensis* eggs were placed in each tube and incubated until the onset of pupation. The beetles were then checked for infection microscopically. Beetles that were exposed to wasps that did not contain oocysts were not included in the analysis.

2.6. Statistical analysis
Statgraphics Plus (Manustics, Rockville MD) was used for Kaplan-Meier survival estimates, ANOVA, and Student’s *t* tests. Square root transformation was used to normalize data. Means were compared with the Tukey–Kramer test.

3. Results

3.1. Susceptibility and oviposition

Exposure of female wasps to patently infected host larvae resulted in a mean rate of detectable infection of 86.5% for *C. waterstoni* and 91.7% for *C. tarsalis* when examined 2 weeks after exposure (Table 1). The infection rates did not differ significantly (*t* = 0.84, *df* = 8, *P* = 0.43). The 14 day incubation was sufficient to detect only the onset of mortality, which did not differ significantly among the treated and untreated wasps of either species (*F* = 1.35, *df* = 3, 16, *P* = 0.29). All *C. waterstoni* (*n* = 14) and *C. tarsalis* (*n* = 17) progeny that were deposited on infected larvae succumbed to *M. oryzaephilii* infection. There was no infection of male wasps, which do not attack the beetles. Exposure of wasps to oocysts in honey water did not result in infections, and the oocysts collapsed and were not infectious.

During the second week after exposure to *M. oryzaephilii*, *C. waterstoni* showed a significant reduction in oviposition relative to controls (*t* = 2.53, *df* = 8, *P* = 0.04) and yielded a significantly lower number of progeny/wasp (*t* = 5.26, *df* = 93, *P* < 0.001; Table 1). In *C. tarsalis* as well, infected wasps showed a significant reduction in the number of progeny produced (*t* = 2.53, *df* = 8, *P* = 0.04), but the percentage of wasps that oviposited did not differ significantly (*t* = 0.52, *df* = 8, *P* = 0.61).

3.2. Survival

Infected *C. tarsalis* females had a mean survival time of 20.2 days (SE = 0.86) after exposure to *M. oryzaephilii*, while mean survival time for uninfected females of the same age class was 38.0 days (SE = 2.49) (Fig. 1). For *C. waterstoni*, the mean survival time for infected females was 36.1 days (SE = 1.25), the mean survival of uninfected females was 45.9 (SE = 2.51) (Fig. 2). The reduction in survival time with infection was significant for both *C. tarsalis* (*t* = 7.30, *df* = 78, *P* < 0.001) and *C. waterstoni* (*t* = 3.89, *df* = 95, *P* < 0.001). Uninfected *C. waterstoni* survived significantly longer than uninfected *C. tarsalis* (*t* = 2.24, *df* = 70, *P* = 0.03). Among the wasps that were exposed to infected hosts, no oocysts were detected in 5% of *C. tarsalis* and 6% of *C. waterstoni*. The exposed but uninfected wasps were not included in the survival analysis.

3.3. Wasp to beetle transmission

When *O. surinamensis* were confined with *C. tarsalis* that had fed on *M. oryzaephilii* beetle larvae, 4.0% (SE 2.0) of a

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**Table 1**

*Cephalonomia tarsalis* and *C. waterstoni* mortality, infection, and oviposition 14 days after feeding on *M. oryzaephilii*-infected host larvae (oviposition from 7 to 14 days post-exposure)

<table>
<thead>
<tr>
<th></th>
<th>% mortality</th>
<th>% infection</th>
<th>Progeny/wasp</th>
<th>% ovipositing</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. tarsalis</em> + <em>M. oryzaephilii</em></td>
<td>4.7 (2.9)a</td>
<td>86.5 (2.3)a</td>
<td>1.7 (0.2)b</td>
<td>88.1 (7.7)a</td>
</tr>
<tr>
<td><em>C. tarsalis</em> control</td>
<td>0.0a</td>
<td>0.0b</td>
<td>2.4 (0.2)a</td>
<td>93.4 (6.6)a</td>
</tr>
<tr>
<td><em>C. waterstoni</em> + <em>M. oryzaephilii</em></td>
<td>6.2 (2.5)a</td>
<td>91.8 (5.8)a</td>
<td>1.6 (0.2)B</td>
<td>72.1 (10.0)B</td>
</tr>
<tr>
<td><em>C. waterstoni</em> control</td>
<td>4.2 (2.6)a</td>
<td>0.0b</td>
<td>3.3 (0.2)A</td>
<td>98.0 (2.0)A</td>
</tr>
</tbody>
</table>

Means within column followed by the same letter and case are not significantly different (α = 0.05).
The week in which death occurred is not included.

Fig. 3. Progeny production of the target hosts. None of the 64 fourth instar total of 183 became infected. The wasps paralyzed 56.7% of the 64 fourth instar C. ferrugineus larvae exposed to M. oryzaephili-fed wasps had microscopically detectible infections, and 37.5% were paralyzed. No control beetles were infected.

3.4. Fecundity and residual transmission to beetle larvae

Calculated over the entire life span, excluding the week in which the individual died, the mean number of progeny/ C. tarsalis wasp/week was significantly greater for control wasps than for M. oryzaephili-exposed wasps ($t = 3.17$, $df = 305$, $P = 0.0017$, Fig. 3). One week after exposure, the progeny production was not significantly different between treatments ($t = 1.11$, $df = 75$, $P = 0.29$). There were significantly fewer progeny per treated wasp in the second week ($t = 2.34$, $df = 60$, $P = 0.02$), third week ($t = 4.30$, $df = 50$, $P < 0.01$), and fifth week ($t = 2.34$, $df = 26$, $P = 0.03$), but not in the fourth week ($t = 1.38$, $df = 33$, $P = 0.18$) nor sixth week ($t = 0.06$, $df = 21$, $P = 0.95$). The mean survival time was 6.8 weeks for untreated wasps and 3.4 weeks for exposed wasps. Accordingly, there was a loss of precision in the data that were taken after the third week with the reduced number of survivors. No exposed wasps survived for more than 6 weeks, and 95% of the cadavers of exposed wasps contained M. oryzaephili oocysts.

When O. surinamensis eggs were placed in the tubes in which wasps had been held for 48 h, 57.6% (SE = 2.9) of those placed in the treatment tubes survived to the fourth instar and 8.9% (SE = 1.9) were infected. Of those placed in control tubes, 56.5% (SE = 2.8) of those placed in control tubes survived to the fourth instar and none was infected.

4. Discussion

Mattesia oryzaephili has a broad host range that crosses orders among stored-product insects (Lord, 2003). This offers the potential for its introduction to suppress several pest species. In contrast to the case of microsporidia (Brooks, 1993), reports of neogregarines infecting both parasitoids and their hosts are rare, perhaps because the parasitoids have been inadequately examined. To my knowledge, Mattesia grandis McLaughlin infection of Bracon mellitor Say, a parasitoid of Anthonomis granis Boheman, (McLaughlin and Adams, 1966) and Mattesia dispensa Naville infection of Habrobracon hebetor (Say), a parasitoid of Ephesia kuehniella Zeller (Leibenguth, 1972), are the only such records. Manning (1965) investigated transmission of an unidentified neogregarine to C. ferrugineus by an undetermined Cephalonomia species. His neogregarine had the characteristics of the Lipotrophidae but not Mattesia, and it did not infect the wasp larvae or adults. Interestingly, I can find no previous records of Protozoa or Microspora infecting Bethylidae. The results presented here demonstrate that two bethylid parasitoids of economically important stored-product pests are susceptible to the neogregarine disease of their hosts. This supports the proposition that the wasps can serve as mechanical vectors and enhance the pathogen’s dissemination.

Several transmission routes are possible to the M. oryzaephili: (1) cadaver transmission, release of the oocysts when the wasps decay after death; (2) fecal transmission, the feces of infected wasps containing oocysts contaminate the beetles food; (3) ovipositor transmission, the neogregarine is transmitted through the oviposition by an infected wasp, either by the external contamination of the ovipositor with the neogregarine or through the injection of oocyst infected glandular fluids or infected eggs. I did not study directly the cadaver transmission, but we believe that this may contribute to the spread of the infection.

This study provides clear evidence for the transmission through fecal matter but weaker implication of ovipositor transmission. When I exposed neonate O. surinamensis to grain in which disease-exposed wasps had been held, the transmission rate was higher when more wasps were confined with last instar hosts. When O. surinamensis were reared from eggs to the fourth instar on substrate in which female C. tarsalis had been confined.

![Fig. 3](image-url)
after exposure to patently infected host larvae, 8.9% of the beetles were infected. The probable source of the *M. oryzaephilii* inoculum in that substrate was the wasp frass. When wasp guts were examined after exposure to infected hosts, most were densely packed with oocysts. I previously demonstrated that the oocysts pass through the guts of many insects with only minimal germination (Lord, 2003). The higher residual than direct transmission could be attributed to the longer exposure period or the greater susceptibility of younger larvae than last instars (Lord, 2003). In either case, it suggests that oocyst deposition in wasp feces is a more important mechanism for transmission from living wasps than direct contact.

Whether transmission takes place through the process of oviposition remains unclear. In this study’s test of direct exposure of last instar beetle larvae to disease-exposed wasps, there was no transmission with *C. ferrugineus* and very low transmission with *O. surinamensis*. Possible routes of transmission are via mandibles and ovipositors that were contaminated while paralyzing donor hosts. *Cephalonomia tarsalis* and *C. waterstoni* females paralyze their hosts by multiple stings while clutching with mandibles (Finlayson, 1950b; Howard et al., 1998). In Naville’s (1930) paper describing *M. dispara* and its biology, he speculated that Ichneumonidae or Braconidae might act as its vectors for *E. kuehniella* by using their ovipositors as injection mechanisms. In his review of the literature on host–parasitoid–pathogen interactions, Brooks (1993) concluded that most studies do not provide conclusive evidence as to whether parasitoids act as mechanical vectors transferring pathogens to food or whether they directly inoculate their hosts via contaminated ovipositors. The data presented here do not support transmission via the ovipositor.

Male *C. tarsalis* and *C. waterstoni* have little or no involvement in *M. oryzaephilii* epizootiology. Even under confinement with infected beetles, no infection of males was achieved. Males of neither species are predatory (Finlayson, 1950b; Powell, 1938). While males will imbibe sugar solutions, *M. oryzaephilii* oocysts that were applied in honey water collapsed from osmotic stress and were not infectious. Consequently, male wasp ingestion of infectious *M. oryzaephilii* would rarely occur under any circumstances, and they cannot be considered hosts or effective dissemination agents for *M. oryzaephilii*.

The mean survival times for healthy wasps in this study (45.9 days and 38.0 days for *C. waterstoni* and *C. tarsalis*, respectively) are similar to previously published values. Finlayson (1950b) reported that female *C. waterstoni* survive 22–44 days on a diet of sucrose, and Powell (1938) stated that the average survival of *C. tarsalis* was approximately 35 days. Infection with *M. oryzaephilii* reduced the mean survival times by 21% for *C. waterstoni* and 37% for *C. tarsalis*. The relatively long survival periods for infected wasps will benefit the biological control efficacy of the wasp–neogregarine combination. The wasps are able to oviposit, albeit at a slightly reduced rate, during this time and thereby propagate and provide pest suppression independently of the protozoan. It has been demonstrated that augmentative release of a beetle parasitoid in stored grain can reduce insect parts in flour (Flinn and Hagstrum, 2001), and a simulation model for *C. waterstoni* indicates that it can suppress *C. ferrugineus* populations (Flinn and Hagstrum, 1995). Another benefit of postexposure survival time is that the wasps are highly mobile and can introduce the disease into healthy beetle populations when exerting independent population suppression.

Pathogens often have deleterious effects on biological control by parasitoids, including premature death of the host or parasitoid, ovipositionally or nutritionally unsuitable hosts, and direct infection of the parasitoid (Brooks, 1993). In a previous study with *O. surinamensis* and *C. tarsalis*, I demonstrated that the fungal pathogen Beauveria bassiana (Balsamo) Vuillemin is as harmful to the wasp as it is to the target pest (Lord, 2001). *M. oryzaephilii* is less virulent to wasps and a better candidate for a combination pest control strategy with them. McLaughlin and Adams (1966) suggested that *B. mellitor* Say might be used as delivery vehicle for *M. grandis* to control the cotton boll weevil, an internal feeder that is difficult to treat directly. They were unable to demonstrate mechanical transmission among beetles as a result of wasp feeding and oviposition activity, but they did not consider frass deposition and cadavers, which appear to be mechanisms for inoculation of *M. oryzaephilii* in the habitats of its hosts.

In summary, the females of two bethylid species are susceptible to *M. oryzaephilii*, a pathogen of their hosts. The prolonged survival and oviposition of infected wasps minimizes the effect of infection on their benefits as independent natural controls. Oocysts in wasp frass and release of oocysts into the beetles’ habitats when infected cadavers are consumed by scavengers or when they disintegrate have potential to make *M. oryzaephilii* a more effective control agent. Its dispersal ability and relatively broad host range make *M. oryzaephilii* a strong candidate for microbial insect control through introduction or conservation. Assessment of its impact on natural pest population will require difficult long-term studies.

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