Evaluation of Remediation Methods for *Nosema* Disease in *Muscidifurax raptor* (Hymenoptera: Pteromalidae)

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Environ. Entomol. 32(5): 1146–1153 (2003)

ABSTRACT Several methods were investigated for managing *Nosema* disease in the parasitoid *Muscidifurax raptor* Girault and Sanders. Treatment of parasitoid eggs or pupae within host puparia with gamma radiation from a cesium-137 source were either lethal to the parasitoids at all dosages tested (eggs) or failed to reduce infection rates (pupae). Exposure of parasitoid eggs within host puparia to heat was effective at several temperatures and exposure times. Optimal results for disease reduction were achieved with a 5-h exposure to 45°C, which resulted in no infection in the resulting adult parasitoids. Continuous rearing at elevated temperatures (30 and 32°C) for three generations resulted in decreased spore loads in infected parasitoids but did not reduce infection rates. Incorporation of the drugs albendazole and rifampicin into rearing media of the parasitoid's host (house fly immatures) resulted in pupae that were of poor quality and did not reduce infection rates in parasitoids that developed in flies reared on drug-treated media. Treatment of adult parasitoids with 3% albendazole and/or rifampicin resulted in decreased rates of transovarial transmission of the disease. Transmission blockage required 3–7 d of exposure to the drug before substantial treatment effects were manifest. Parasitoids that fed for 7 d on rifampicin-treated honey transmitted the disease to 57.7% of their progeny compared with a 99.1% transmission rate among untreated parasitoids. An uninfected colony of *M. raptor* was established by pooling cured parasitoids from heat shock and drug treatment experiments. Parasitoids from the uninfected colony lived longer and produced over twice as many female progeny (201.2) as infected parasitoids (85.2).

KEY WORDS *Nosema*, heat therapy, rifampicin, *Muscidifurax raptor*, *Musca domestica*

MICROSPORIDIA ARE AMONG THE most important groups of protozoan pathogens that infect insects (Brooks 1988). They often cause chronic or debilitating diseases that result in reduced fecundity and longevity, and present serious problems in large-scale insectary production. Because sublethal effects are a characteristic of most microsporidian infections in arthropods (Wilson 1974, Gaugler and Brooks 1975, Goodwin 1984; Becnel and Geden 1994, Geden et al. 1995, Bjornson and Keddie 1999), such infections often go unnoticed in insect cultures. Undetected diseases in organisms used in research can compromise physiological and basic biological studies and lead to incorrect conclusions (Goodwin 1984). Production of healthy arthropods is particularly critical in commercial rearing of biological control agents, because accidental sale and release of diseased parasitoids and predators can reduce the success of the releases. Disease prevention strategies are necessary to monitor and screen for the presence of microsporidia, and once detected, remedial action must be implemented (Becnel and Adreadis 1999).

One of the oldest and simplest disease management strategies was developed independently by Pasteur and Cantoni (Steinhaus 1963) for eliminating pebrine disease caused by *Nosema bombycis* in silkworms, *Bombyx mori.* Because the microsporidium was transmitted transovarially to their progeny, the strategy was to isolate individual females for oviposition and subsequently determine the infection status of the females, pooling the progeny of healthy females to found an uninfected colony. This technique is labor-intensive and requires large numbers of healthy insects to ensure an adequate genetic base for the colony.

Heat therapy has been used successfully to eliminate or reduce microsporidian infection in insects (Raun 1961, Cantwell and Shimanuki 1969, Geden et al. 1995, Kfir and Walters 1997). This method exploits the relatively high tolerance of many hosts to heat compared with the parasites (Becnel and Adreadis 1999). Continuous rearing of infected insects at elevated temperature can also reduce microsporidian prevalence. Heat therapy in combination with the Pasteur and Cantoni methods (Steinhaus 1963) has

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been used to establish healthy colonies (Allen and Brunson 1947, Raun 1961, Hamm et al. 1971).

Drugs have also been tested for activity against microsporidia. Fumagillin, an antibiotic derived from the fungus *Aspergillus fumigatus* was one of the earliest compounds to be tested with some success. It reduced infections caused by *Nosema* pathogens in honey bees (Katznelson and Jamieson 1952, Bailey 1953, Moffet et al. 1969) and the boll weevil, *Anthonomus grandis* (Flint et al. 1972). However, fumagillin was not effective against *Pleistopora* (=Endoreticulatus) *schubergi* found in *Anaitis efformata* (Briese and Milner 1986) or *Nosema muscidifuracis* (Becnel and Geden) in *M. raptor* Girault and Sanders (Geden et al. 1995). The antibiotic rifampicin was found to decrease the levels of microsporidian infection when it was administered per os to *Encarsia* species infected with *Nosema* disease (Sheetz et al. 1997).

Other chemicals that have been used with mixed results against microsporidia in insects include benomyl (Hsiao and Hsiao 1973, Harvey and Gaudet 1977, Brooks et al. 1978). Albendazole, a benzimidazole, caused significant reduction in the number of infected cells in vitro in *Spodoptera frugiperda* cells and also in vivo in *Helicoverpa zea* larvae and adults, but there was resurgence of the infection when the drug was withdrawn (Haque et al. 1993).

Nosema muscidurafacis causes a debilitating disease of the filth fly parasitoid *M. raptor* (Zchori-Fein et al. 1992, Becnel and Geden 1994, Boohene et al. 2003). Previous efforts to manage this pathogen with heat therapy were only marginally successful and resulted in high mortality in the treated parasitoids (Geden et al. 1995). The objective of the current study was to evaluate alternative remediation methods for this disease.

Materials and Methods

Insect Colony Maintenance. The infected *M. raptor* colony was originally collected from dairy farms in New York in 1987 and was the colony from which the type material for *N. muscidifuracis* was originally described (Becnel and Geden 1994). Adult parasitoids were held in a 43 by 43 by 20 cm Plexiglas cage kept inside a 72 by 46 by 55 cm outer box in a rearing chamber maintained at 25°C, 50–80% RH, 16:8 L:D photoperiod. Parasitoids were given house fly pupae as hosts for feeding and oviposition two to three times weekly. The colony was kept in a separate building from uninfected colonies of *M. raptor* and was screened monthly for infection status.

Radiation Treatment. For radiation and heat shock tests, the egg stage of the parasitoid was targeted because this stage contains the fewest *N. muscidifuacis* spores (Zchori-Fein et al. 1992). Eggs within host puparia were obtained by placing 300 house fly pupae (2 d old) with 100 infected *M. raptor* females that were presumed to have mated ($N = 100$) for a 24-h exposure. The pupae were then divided into six batches of 50 pupae in glass vials and subjected to 0 (controls), 20, 40, 60, 80, or 100 Gr of gamma radiation in a cesium-137 radiation source (Precision Machinery, Parsipany, NJ). Twenty Grays was the minimum dosage that was practical with this irradiator. Treated pupae were held at 25°C for fly and parasitoid emergence. Parasitoid progeny (if any) were sexed and scored for *Nosema* infection by making whole mount squashes of the insects and looking for spores under a phase contrast microscope. The experiment was replicated twice. The above methods were also used for the pupal stage of *M. raptor.* In these tests, fly pupae were exposed to parasitoids as described previously and held for 12 d at 25°C to allow parasitoid development to the pupal stage before treatment.

Heat Shock Treatment. Parasitoid eggs within host puparia were obtained by exposing 3,600 house fly pupae to 800 infected, mated female parasitoids for 24 h. Groups of 400 parasitoid-exposed pupae were then subjected to heat in an oven set at either 40 or 45°C with high humidity (70–90% RH) for 1, 3, 5, or 7 h. One group of 400 pupae serving as controls was held at 25°C. The pupae were held after treatment at 25°C (RH, 70%) for parasitoid emergence. Adult parasitoids were counted, sexed, and scored for infection. The experiment was replicated three times.

In a subsequent test, groups of 400 parasitoid-exposed pupae were placed in an oven set at either 47 or 50°C (70–90% RH) for 15, 30, 45, or 60 min. One group of 400 pupae serving as controls was held at 25°C. Adult parasitoids that emerged were counted, sexed, and scored for infection as before. The experiment was replicated three times. Data were analyzed by *G*-tests of independence comparing emergence success and infection status of treated groups with the controls.

Continuous Rearing at Elevated Temperature. House fly pupae (2- to 3-d-old) were weighed out into six groups of 1,000 each and exposed to 250 infected *M. raptor* females (from the infected colony) per group for 24–30 h in 220-ml paper cans for oviposition. Parasitoids were removed from the pupae after this period, and the pupae were mixed together for randomization and sorted into three groups of 2,000 pupae each. The infection level of the ovipositing females, designated as parentals, was sampled for infection by making whole mount squashes and examining under a phase contrast microscope for spores. In addition, 10 females were ground in 3 ml of deionized water in a glass tissue grinder and spore counts were made with a hemocytometer (Cantwell 1970) to determine an initial spore load in the parasitoids. This was repeated three more times (total of four sets of 10 females). The above procedures were repeated for males from the same cohort of insects as the parents used for oviposition.

Paper cans containing parasitized pupae were placed in a rearing box (70 by 47 by 53 cm) over a pan of saturated sodium chloride solution to provide ≈70% RH. The cages were then placed in rearing chambers set to run at 25, 30, or 32°C. Of the 2,000 parasitized pupae set for each treatment group, 500 pupae from each temperature treatment were gel-capped and held for parasitoid emergence, assessment of infection, and spore loads as was done with the parentals.

The rest of the parasitoids that emerged were provided with host pupae at a constant host:parasitoid ratio of 20:1 to provide progeny for the next generation (G-2). When the next generation emerged, the above procedures were again carried out up to the third generation (G-3). Results were analyzed with the PROC GLM Procedure of SAS (SAS Institute 1992) by evaluating the infection level and spore load as a function of the rearing temperature. Means were separated by the method of Tukey.

Drug Treatment: Incorporation of Drug into Host. Albendazole and rifampicin were mixed with house fly larval rearing medium in an effort to incorporate the drugs in the tissue of the developing fly. Fly larval diet (a mixture of 50 parts wheat bran, 30 parts alfalfa meal, and 20 parts corn meal; Hogsette 1992) was mixed with water at a ratio of 5:4 by volume and weighed out into 25-g batches in 30-ml plastic cups. Drugs were weighed and mixed with the diet to obtain rates of 0.1, 1.0, or 10% (AI) by weight. House fly second instars ($N = 50$/cup) were removed from untreated media and placed in the treated cups. Cups were covered with screen-topped lids, placed in a 25°C chamber (70% RH), and checked daily for moisture loss (remoistening as needed) and pupation. Pupae were counted, weighed, and exposed to infected *M. raptor* females at a host:parasitoid ratio of 5:1 for oviposition. Parasitoids were removed after 24–36 h, and the pupae were held until emergence. Emerged parasitoids were counted, sexed, and assessed for infection as before. Drug trials were replicated on three occasions using different batches of flies and parasitoids.

Drug Treatment: per os Administration to Adult Parasitoids. Drug-treated honey solutions were prepared by mixing 100 mg of each drug with 2 g of honey, 200 μl of glycerol, and 0.5 ml of water to give a final drug solution of ≈3% (Sheetz et al. 1997). Preliminary bioassays showed that 3% was near the upper limit of tolerance of these drugs by the parasitoids. Female *M. raptor* ($N = 400$) that had been starved for 12–24 h were placed in a 1-liter plastic container together with 200 males. A pipette was used to dispense 0.5 ml of the drug solution onto a 5 by 7 cm microscope slide covered with tissue paper (Stirling Co., Bound Brook, NJ). The slide was then placed inside the plastic container with the parasitoids for feeding. The sides of the containers were also streaked with the drug solution and the slide was replenished daily with fresh drug solution. After feeding for 24 h on the drug solution the parasitoids were given host pupae for oviposition for 24 h at 1, 3, 5, and 7 d after the initial exposure to the drug. Pupae were removed at the end of the 24-h exposure and held at 25°C for emergence. In this way, parasitoids were confined with treated honey without hosts on days 2, 4, and 6. Progeny from each successive oviposition interval were counted and scored for infection status as described previously. Mortality was assessed at 1, 3, 5, and 7 d postdrug exposure. The experiment was replicated three to four times for each drug. In another set of experiments a combination of both drugs in 1:1 ratio of 100 mg each was made, and the procedures described above were carried out.

Mortality, emergence success, progeny production, and infection rate were analyzed and evaluated with drug treatment as the dependent variable in the GLM procedure of SAS (SAS Institute 1992) and means were separated using the method of Tukey.

Fitness of Cured and Infected Colonies. Uninfected colonies were established from females that emerged in the heat shock and drug treatments. A total of 150 female parasitoids from the heat shock treatments were placed individually in 30-cm cups and given 60 house fly pupae each for 3 d of oviposition. Females were assessed for infection after oviposition, and progeny of uninfected females pooled to establish a clean line. Similar methods were used to obtain drug-cured parasitoids by isolating female progeny, resulting from the final oviposition (7 d after drug administration). Heat- and drug-cured colonies were pooled into a single uninfected colony when no resurgence of infection was observed for two generations.

Infected and uninfected parasitoids, 1–2 d old, were counted into groups of five females and two males (12 groups per infection status), placed in 30-ml cups, and given 100 house fly pupae per group. Parasitoids were checked and pupae replaced daily with fresh pupae (host:parasitoid ratio of 20:1) for 7 d. Pupae were held at 25°C for house fly and parasitoid emergence. Differences in parasitoid survival, host attacks (no. killed pupae), and progeny production as a function of infection status were evaluated by the GLM procedures of SAS (SAS Institute 1992).

Results

Radiation Treatment. The egg stage of *M. raptor* was very sensitive to radiation from a Cs-137 source, and all doses tested were lethal (data not presented). There was no emergence of adult parasitoids even from the lowest radiation dose of 20 Grays, whereas emergence from controls was ≈60%. In contrast, the pupal stage was relatively tolerant of the radiation treatments. All treatments of the pupal stage had successful emergence to the adult stage and varied from 87 to 114% of the controls. None of the tested treatment doses had a therapeutic effect, however, and infection was 100% in all treatment groups.

Heat Shock. Relative survival of *M. raptor* subjected to heat shock at 40°C decreased with time and was ≈52% compared with controls after a 7-h exposure (Table 1). At 45°C, survival was ≈6% of the controls after a 5-h exposure. Treatment for 7 h was lethal at 45°C, and survival after 5 h was only ≈5% compared with controls. Substantial curing (90–100%) was obtained at 45°C for 3 and 5 h, although survival was very low at 5 h (Table 1). Reductions in infection rates were significant at all exposures to 47 and 50°C (Table 1). Over 85% reduction in infection was observed when parasitoids were exposed for 60 and 75 min at 47°C, and survival was 58–71% compared with controls. There was 100% cure (0% infection) at 50°C when pupae were exposed for 45 and 60 min, with relative survival of 18 and 8%, respectively (Table 1).

Table 1. Relative survival and *Nosema*-infection status of adult *M. raptor* after subjecting parasitoid eggs (within host puparia) to heat at 40, 45, 47, or 50°C

Temperature (°C)	Time	Mean (SE) emergence	Relative survival[a]	χ^2	*N*	Percent infected	χ^2
40	1 h	199.5 (6.4)	97.0	0.6ns[b]	75	96.0	2.5ns[b]
40	3 h	172.0 (17.0)	83.6	6.0*	117	93.3	8.5**
40	5 h	157.0 (50.9)	76.3	11.8**	120	93.3	7.0**
40	7 h	106.0 (12.0)	51.5	53.1**	90	96.7	1.9ns
45	1 h	166.5 (13.4)	80.7	7.9**	68	88.2	12.2**
45	3 h	101.5 (9.2)	49.3	58.9**	108	10.2	239.7**
45	5 h	11.5 (6.4)	5.6	362.1**	23	0.0	117.2**
45	7 h	0.0	0.0	490.1**	—	—	—
47	15 min	184.0 (45.3)	89.5	0.2ns	118	83.9	11.5**
47	30 min	170.0 (46.7)	82.6	1.6ns	119	73.9	25.6**
47	45 min	135.0 (33.9)	65.6	13.5**	229	35.8	127.8**
47	60 min	120.0 (26.2)	58.3	23.3**	198	14.6	214.1**
47	75 min	130.0 (47.4)	63.2	16.4**	158	12.7	208.4**
50	15 min	123.0 (49.0)	59.8	34.9**	166	84.3	54.5**
50	30 min	106.0 (48.0)	51.5	53.1**	129	65.1	7.9**
50	45 min	38.5 (16.3)	18.7	209.3**	40	0.0	43.7**
50	60 min	17.5 (3.5)	3.4	317.6**	30	0.0	32.6**
50	75 min	0.0	0.0	490.0**	—	—	—
Controls		205.7 (2.8)	100.0	—	130	99.2	—

Newly parasitized fly pupae were exposed to heat treatments in batches of 400 pupae per temperature and exposure time and then held at 27°C for parasitoid emergence. Controls were held at 27°C throughout the test. The experiment was replicated three times.

[a] Emergence success as a percent of control emergence.

[b] ns, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$ (*G*-tests of independence comparing emergence success and infection status of treated groups with controls.

No parasitoids emerged from pupae exposed to 50°C for 75 min.

Rearing at Elevated Temperature. All of the parasitoids reared at 25 and 30°C were infected after one generation (Table 2). There were small but statistically significant reductions of infection levels in the first generation when parasitoids were reared at 32°C; infection rates were 93.0 and 95.8 for females and males, respectively (Table 2). In the second and third generations, there were no significant effects of rearing temperature on infection rates.

Spore loads were higher for females overall compared with the males, and decreased between generations one and three when parasitoids were reared at the two higher temperatures (Table 2). Parasitoids reared at 32°C had about one-third as many spores as the 25°C controls after a single generation. Rearing at 32°C for three generations resulted in a 7- to 8-fold reduction in spore loads compared with the controls. Spore loads of males showed similar patterns, with third generation males reared at 25°C having spore loads about 9-fold higher than parasitoids reared at 32°C.

Drug Treatment: Incorporation of Drug into Host. Both albendazole and rifampicin had strong deleterious effects on house fly immatures. There were pronounced differences in the pupal size and appearance even at the middle dose 1%. Larvae that were fed on diets containing albendazole had reduced pupation success and pupal weights at all doses tested (Table 3). Parasitoids that emerged from all concentrations of the albendazole-treated pupae were still infected (data not shown). Larvae that were reared on rifampicin-treated diet produced pupae that were small, hollow, and light in color indicative of less tanning. No

Table 2. Infection status and spore loads of *Nosema*-infected *M. raptor* reared at three constant temperatures for three generations

Temperature (°C)	Sex	Percent Infected (mean [se]) in generation			No. *Nosema* spores/parasitoid × 10^6 in generation		
		1	2	3	1	2	3
25	Females	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	13.8 (0.5)a	15.1 (0.5)a	13.1 (0.5)a
30		100.0 (0.0)a	100.0 (0.0)a	96.4 (2.3)a	7.3 (0.3)b	7.6 (0.6)b	3.5 (0.4)b
32		93.0 (2.1)a	98.0 (1.7)a	96.3 (2.2)a	4.8 (0.7)c	3.7 (0.2)c	1.9 (0.3)b
ANOVA *F*[a]		7.78*	1.43 ns	0.86 ns	77.73**	162.45**	222.18**
25	Males	100.0 (0.0)a	100.0 (0.0)a	98.4 (1.5)a	10.3 (0.5)a	9.2 (0.4)a	8.9 (0.2)a
30		100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	5.6 (0.3)b	4.6 (0.6)b	2.7 (0.4)b
32		95.8 (1.1)b	90.0 (0.0)b	97.5 (2.0)a	3.3 (0.1)c	2.5 (0.1)c	1.0 (0.1)c
ANOVA *F*[a]		34.04**	>300**	0.54 ns	112.44**	79.44**	232.72**

Infection status was determined by examining 30 individuals of each sex per generation. Spore concentrations were determined by hemacytometer counts of three groups of 10 pooled parasitoids of each sex per generation. Means followed by the same letter within a column for each sex are not statistically different at $P = 0.05$ using Tukey's means separation method.

[a] $P > 0.05$, ns; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 3. Effect of albendazole-treated rearing medium on house fly pupation success and pupal weights

Drug concentration	*N*	Mean (SE) no. of pupae	Mean weight (SE) of pupae (mg)
Control	183	45.8 (1.0)a	16.2 (0.5)a
0.1	136	34.0 (3.1)b	10.6 (0.9)b
1	146	36.5 (2.6)b	10.3 (1.0)b
10	148	37.0 (1.0)a,b	9.6 (0.9)b
ANOVA F^a		5.75*	13.17**

[a] $P > 0.05$, ns; * $P \leq 0.05$; ** $P \leq 0.01$; df = 3, 12.

Means followed by the same letter within a column are not statistically different at $P = 0.05$ using Tukey's means separation method.

Four groups of 50 larvae fed on a diet containing various concentrations of albendazole until pupation.

parasitoids emerged from pupae in the rifampicin-treated groups.

Drug Treatment: per os Administration to Adult Parasitoids. Albendazole and rifampicin had no significant effect on parasitoid mortality for the first 5 d of treatment (Table 4). Mortality in the rifampicin-treated group was higher (72.5%) than for the controls (54.4%) and the albendazole treated parasitoids (53.8%) on day 7. Drug treatment of adult parasitoids also had little effect on progeny production. Albendazole had no significant effect on progeny production in any exposure interval. Rifampicin-treated parasitoids produced fewer progeny than the other treatments in all of the exposure periods, but the effect was only significant on day 1. All of the drug treatments resulted in significant reductions of infection of progeny by day 3, although infection levels were still >93% (Table 4). By day 7, progeny from rifampicin-treated females had the lowest infection rate (57.7%), followed by albendazole (78.6%) and the rifampicin-albendazole mixture (81.7%). Untreated parasitoids produced progeny with an infection level of >99% on day 7.

Fitness of Cured and Infected Colonies. There were no differences in the survival of female parasitoids for the first 5 d of observation (Table 5). Uninfected females had higher survival rates on days 6 and 7, however. By day 7, only 16% of the infected females were alive compared with 60% of the females from the uninfected colony. No difference in survival was observed among infected and uninfected males. Uninfected parasitoids produced a total of 201.2 female and 70.2 male progeny, compared with 85.2 and 61.4 female and male progeny from infected parasitoids, respectively (Table 5). Progeny of uninfected parasitoids were 74.1% females compared with 58.2% females among progeny of infected parasitoids.

Rates of host attacks (mortality caused by parasitism and feeding) were higher for the cured (uninfected) parasitoids and ranged from 1.1 to 12 times higher than the infected parasitoids (Table 6). Overall, uninfected parasitoids killed 397.5 pupae compared with 279.5 pupae for the infected parasitoids.

Discussion

Nosema muscidifuracis in *M. raptor* is maintained by a transmission cycle that involves both vertical and horizontal transmission. The pathogen is acquired horizontally when uninfected immatures cannibalize infected immatures in superparasitized puparia and also when uninfected adults feed on infected parasitoid immatures within host puparia. Vertical transmission occurs transovarially from mother to offspring through infected eggs (Geden et al. 1995). Microsporidian development can be influenced by environmental factors such as temperature and host nutritional sources and levels (Becnel and Undeen 1993).

Our strategy in attempting to manage the disease was to kill the vegetative stages or inactivate the spores

Table 4. Effect of per os treatment with 3% albendazole and rifampicin on mortality and progeny production by *Nosema*-infected *M. raptor*

Treatment[a]	Mortality, progeny production, and infection status of progeny on day since beginning drug treatment			
	1	3	5	7
	Mortality (mean [SE]) of adult parasitoids			
Control	6.9 (1.7)a	23.3 (1.8)a	36.6 (2.2)a	54.4 (1.1)b
Albendazole	5.5 (2.3)a	19.0 (5.2)a	31.2 (6.3)a	53.8 (5.9)b
Mixture	8.6 (0.4)a	25.3 (2.0)a	40.1 (2.1)a	61.7 (1.4)a,b
Rifampicin	12.7 (3.0)a	38.9 (11.2)a	54.8 (10.2)a	72.5 (1.4)a
ANOVA F^b	2.19 ns	1.86 ns	2.69 ns	5.51*
	No. adult progeny produced			
Control	1026.3 (82.2)a	671.3 (32.8)a	518.0 (81.7)a	73.0 (2.6)a
Albendazole	923.7 (46.5)a,b	776.7 (83.3)a	365.0 (106.5)a	98.0 (49.2)a
Mixture	937.3 (107.7)a,b	652.3 (43.7)a	315.4 (93.2)a	179.0 (55.0)a
Rifampicin	557.3 (128.4)b	423.8 (119.1)a	150.0 (50.6)a	59.3 (34.3)a
ANOVA F^b	4.66*	0.76 ns	3.14 ns	1.85 ns
	Infection status of progeny			
Control	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	99.1 (1.0)a
Albendazole	98.6 (0.8)a	97.1 (0.7)b	91.5 (3.4)b	78.6 (8.2)b,c
Rifampicin	98.5 (0.8)a	97.3 (1.0)b	73.5 (6.7)c	57.7 (4.4)c
Mixture	98.9 (1.1)a	93.4 (1.9)b	86.5 (3.9)b,c	81.7 (1.6)b
ANOVA F^b	1.1 ns	10.24**	15.55**	22.96**

[a] 3% of drug solution.

[b] $P > 0.05$, ns; * $P \leq 0.05$; $P \leq 0.01$. df = 3.

Means with the same letter within a column are not significantly different at $P = 0.05$ using Tukey's means separation method.

Table 5. Survival and progeny production of infected and uninfected *M. raptor* cured of *Nosema muscidifuracis*

Day	No. alive on day (mean [SE])			No. adult progeny produced on day (mean [SC]).		
	Uninfected colony	Infected colony	F^a	Uninfected colony	Infected colony	F^a
			Females			
1	5.0 (0.0)	4.9 (0.1)	1.0 ns	23.2 (1.7)	11.0 (1.6)	26.06**
2	4.9 (0.1)	4.9 (0.1)	0.0 ns	52.7 (2.9)	25.6 (2.3)	53.45**
3	4.7 (0.1)	4.8 (0.1)	0.19 ns	44.4 (2.9)	26.0 (2.7)	21.45**
4	4.6 (0.1)	4.7 (0.1)	0.16 ns	31.9 (3.5)	13.3 (2.6)	18.37**
5	4.6 (0.2)	4.3 (0.3)	0.85 ns	24.7 (3.4)	6.9 (1.6)	21.96**
6	4.2 (0.3)	2.8 (0.4)	6.64*	13.3 (1.5)	2.4 (0.9)	39.57**
7	3.0 (0.5)	0.8 (0.3)	20.61**	11.0 (4.4)	0.0 (0.0)	6.36*
Total female progeny produced				201.2 (2.8)	85.2 (2.2)	45.22**
			Males			
1	2.0 (0.0)	1.9 (0.1)	1.0 ns	18.2 (1.6)	13.5 (1.4)	4.66**
2	2.0 (0.0)	1.8 (0.1)	2.2 ns	15.9 (1.5)	17.7 (1.5)	0.62 ns
3	1.8 (0.1)	1.8 (0.1)	0.23 ns	12.3 (1.4)	17.0 (2.2)	3.14 ns
4	1.8 (0.1)	1.8 (0.1)	0.23 ns	7.3 (1.5)	6.9 (2.7)	0.04 ns
5	1.8 (0.1)	1.4 (0.2)	3.48 ns	7.5 (1.50)	4.9 (1.9)	1.12 ns
6	1.8 (0.2)	1.3 (0.3)	3.77 ns	5.8 (0.8)	1.4 (0.7)	18.34**
7	1.0 (0.2)	0.5 (0.3)	1.36 ns	3.2 (1.1)	0.0 (0.0)	8.64*
Total male progeny produced				70.2 (1.6)	61.4 (1.3)	7.88**

[a] $P > 0.05$, ns; * $P \leq 0.05$; ** $P \leq 0.01$.
df = 1, 22 for days 1–6: 1, 10 for day 7.
Twelve groups of five females and two males observed for 7 d after emergence at 25°C, host:parasitoid ratio of 20 pupae/female/day.

in the egg stage so that the resulting progeny would be free of the disease. The spore load in the egg stage and other immature stages are usually low in comparison to the adult (Zchori-Fein et al. 1992). Several reports (Baribeau and Burkhardt 1974, Wilson 1974, Teetor and Kramer 1977, Kelly and Anthony 1979) have shown that sunlight or UV radiation can inactivate microsporidian spores, and there is at least one report (Undeen et al. 1984) in which spore viability measured by both infection rate and intensity of infection in *N. algerae* was lost when purified spores were subjected to gamma radiation. In our experiments gamma radiation treatments were lethal to the parasitoid eggs and were ineffective in causing any curing effect in the pupal stage at the doses tested.

Muscidifurax raptor seems to be a good candidate for heat therapy. Some of the parasitoid eggs were able to survive heat shock conditions as extreme as 50°C for 60. Exposure of *M. raptor* at 50°C for 45 min was very effective in managing *Nosema* disease, resulting in 100% cure. This treatment gave a relative survival of 18%, which is an acceptable level of mortality for most applications. Our results indicate that this method could be adopted to salvage colonies that are 100% infected. Other temperatures and exposure times were also effective in reducing the infection level with less parasitoid mortality. For example, an exposure time of 3 h at 45°C resulted in ≈90% cure with a relative survival of almost 50%. If the intent is to minimize parasitoid mortality, this treatment could be combined with the Pasteur method to establish a healthy colony. In other parasitoid species, high sensitivity to heat shock treatments by the parasitoids may not allow use of this approach (Ferriera de Almeida et al. 2002).

Table 6. Attack rates (house fly pupae killed) of infected and uninfected *M. raptor* cured of *Nosema muscidifuracis*

Day	No. hosts killed on day[a]		F^b	df
	Uninfected	Infected		
1	61.2 (2.9)	43.9 (2.9)	16.92**	1, 16
2	79.4 (1.5)	72.2 (4.9)	1.18 ns	1, 14
3	85.0 (3.2)	69.6 (4.5)	5.09*	1, 16
4	60.1 (5.6)	49.8 (5.5)	1.45 ns	1, 16
5	47.4 (4.1)	30.8 (4.2)	8.54*	1, 16
6	35.6 (3.6)	10.9 (2.7)	30.64**	1, 20
7	28.8 (9.6)	2.3 (1.2)	7.56*	1, 10
Total	397.5 (10.2)	279.5 (7.7)	35.15**	1, 16

[a] Includes successfully parasitized hosts, aborted parasitism, and pupae killed by host-feeding.
[b] ns, $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$.
Twelve groups of 5 females and 2 males observed for 7 days after emergence at 25°C, host:parasitoid ratio of 20 pupae/female/day.

The heat shock results obtained here represent an improvement of those obtained by Geden et al. (1995), where infected *M. raptor* eggs within host puparia were submerged in water baths to transfer heat to target tissues rapidly. Although exposure of infected *M. raptor* eggs in a 47°C water bath for 45–60 min greatly suppressed *N. muscidifuracis*, the treatment resulted in poor parasitoid survival. Submerging the parasitoid in water may have limited exposure times tolerated by the immature parasitoid because of asphyxiation and may have resulted in the lower survival.

Elevated temperature can inhibit or reduce the growth of microsporidia in insects (Wilson and Sohi 1977). We observed slight reductions in the infection level when infected parasitoids were reared at continuous elevated temperature of 30 and 32°C for up to three generations (Table 2). These reductions were not significant for females but were significant for males at 32°C for the first and second generation. Rearing at even higher temperatures is not practical for this species, as 32°C is near the upper limit of

tolerance for *M. raptor* under constant temperatures (Geden 1997). Although rearing at elevated temperatures had a negligible impact on infection rates, it did have substantial effects on spore loads in infected parasitoids. It is possible that rearing for additional generations may have had more therapeutic value. Wilson (1979) also observed a reduction in the spore production of *N. fumiferanae* in the spruce budworm when colonies were reared continuously at 30°C. Nevertheless, there was resurgence of the infection when the hosts were returned to lower temperatures. Because spores are generally resistant to heat, they may have survived and been able to resume the infection when they were moved to lower temperatures. Wilson and Sohi (1977) were able to completely eliminate *N. disstriae* from *Malacosoma disstria* when cultures were reared at 35°C for 28 d.

There has been renewed interest in microsporidia research in recent years because of their emerging recognition as important enteric pathogens in immunocompromised patients such as those with HIV infection or patients treated with immunosuppressive drugs (Costa and Weiss 2000, Weiss 2001). Consequently many compounds are currently being tested for antimicrosporidial activity. Albendazole is a benzimidazole that has been used extensively and has shown some promise in curbing microsporidian infections. It is a microtubule inhibitor and is effective against *Encephalitozoon* species that infect humans and other mammals (Didier et al. 2000). In our tests with albendazole in which we attempted to incorporate the drug in the diet of the house fly host, this drug was ineffective in reducing the infection and was somewhat detrimental to the fly pupae, resulting in lower pupal yields and weights. The antibiotic rifampicin was also ineffective when used in this manner and was detrimental to the larvae. Although many of the larvae were able to pupate, the resulting pupae were small, hollow, and nonviable. The antibiotic nature of rifampicin might have killed many of the microorganisms required by fly larvae for nutrition, thereby denying the larvae an essential source of nutrients (Schmidtmann and Martin 1992).

Per os administration of either albendazole, rifampicin, or a mixture of both drugs gave some cure, ranging from ≈20% for albendazole and the mixture to ≈40% with rifampicin during the fourth exposure to host pupae for oviposition. The first and second exposure periods showed a slight reduction in the infection level, but this was not significant. Rifampicin was the most effective of the treatments. Rifampicin was also used successfully in eliminating a *Nosema* pathogen from an *Encarsia* parasitoid without any evidence of resurgence (Sheetz et al. 1997). There is often a resurgence of infection once the hosts are taken off the drugs (Lynch and Lewis 1971, Briese and Milner 1986).

A concern in subjecting *M. raptor* to heat and drug therapy was parasitoid fitness after elimination of the disease. Our results showed there were no apparent fitness trade-offs as a result of these treatments. Cured parasitoids lived somewhat longer and produced almost twice as many females per day than infected females. Progeny production of infected females was much lower than uninfected females before differential mortality was observed (days 1–5), demonstrating that differences in apparent progeny production by infected colonies are not due mainly to differences in female survivorship.

Male survival was similar among infected and uninfected parasitoids, suggesting that infection is more severe in the females than in the males, perhaps because of lower spore loads in males (Table 2). The role of the infected males in this disease cycle is limited because there is no evidence of paternal or venereal transmission of the pathogen (Geden et al. 1995). However, spores have been observed in the meconium of immatures in both male and females and also in the feces of adult males (C.K.B., unpublished observations). These environmental spores may be involved in horizontal transmission and infection of new hosts.

In summary, our results indicate that *Nosema* diseases in *M. raptor* can be eliminated from colonies of *M. raptor* using simple heat treatment or drug therapy methods followed by isolation and combination of clean family lines and that such therapy results in enhanced fitness of the parasitoids. These methods can be used easily by commercial insectaries or researchers with an interest in parasitoid biology.

Acknowledgments

The authors thank R. Clark, P. Seaman, A. Malcom, and H. McKeithen for assisting with the experiments and insect rearing.

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Received for publication 5 March 2003; accepted 15 May 2003.