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Behavioral Fever Response of *Musca domestica* (Diptera: Muscidae) to Infection by *Entomophthora muscae* (Zygomycetes: Entomophthorales)

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House flies, *Musca domestica* L., infected with *Entomophthora muscae* (Cohn) Fresenius were exposed to 40°C to determine the effects of high temperature on diseased house flies. In no-choice experiments, high temperatures early in the incubation period of two isolates of *E. muscae* increased the survival time of diseased house flies. House flies surviving *E. muscae* infections induced by a Nebraska (NE) isolate were 87, 78, and 37% if exposed to high temperature for 8 hr during Days 1, 2, and 3 of the incubation period, respectively. House fly survival rates declined to 17 and 13% if exposed to high temperature on Days 4 and 5 of the usual 5-day incubation period. House flies surviving infections induced by a California (CA) isolate were 93, 97, and 92% if exposed to 40°C temperatures for 8 hr during Days 1, 2, and 3 of the incubation period. Survival rates declined to 75, 38, and 17% if high temperature exposures occurred on Days 4, 5, and 6 of the typical 7-day incubation period. Short-term exposures (4–6 hr) of *E. muscae* (NE) infected house flies (24 hr old) to 40°C increased fly survival 90%. Similar results were not observed at 35°C. In free-choice experiments, house flies infected with the NE isolate of *E. muscae* for 24–48 hr exhibited a behavioral fever and were able to use heat therapy to eliminate pathogenic effects of *E. muscae*. The response was not consistent throughout the *E. muscae* incubation period. Behavioral fevers were not exhibited by infected house flies on Days 1, 4, and 5 of the incubation period. On Day 5 of the incubation period, dying flies moved to the cool regions of the gradient.

KEY WORDS: House fly; mycosis; behavioral fever; heat therapy.

INTRODUCTION

Behavioral fevers occur when ectothermic or poikilothermic organisms, including arthropods, modify their behavior to regulate body temperature (Louis *et al.*, 1986). Behavioral fevers have been observed in microsporidian-infected *Melanoplus sanguinipes* (Boorstein and Ewald, 1987), rickettsia-infected crickets (Louis *et al.*, 1986), heat-killed *Aeromonas hydrophila*-injected crayfish (Casterlin and Reynolds, 1977), *Escherichia coli* endotoxin-injected cockroaches (Bronstein and Conner 1984), and prostaglandin E_1-injected lobsters and shrimp (Casterlin and Reynolds, 1979). Little information exists on the effects of temperature on the pathogenesis of fungal pathogens of arthropods or the exhibition of behavioral fevers by insects infected with pathogenic fungi. Carruthers *et al.* (in press) reported that clear-winged grasshoppers were able to elevate body temperature through basking behavior and almost eliminate *Entomophaga grylli* infections.

Entomophthora muscae (Cohn) Fresenius attacks its insect host through the exoskeleton. The pathogenesis of *E. muscae* within the host was described by Brobyn and Wilding (1983). Spores germinated within 24 hr and penetrated the fly exoskeleton. The germ tubes grew into the insect hemocoel, fungal cytoplasm streamed into the germ tubes, and hyphal bodies budded off. The sphere-like hyphal bodies replicated within the fly hemolymph in 24 to 48 hr. These hyphal bodies consumed hemolymph and some internal organs of the host from 48 to 72 hr following infection. The abdomen of the infected host developed a cream-white coloration after 72 to 96 hr. Hyphal bodies consumed the wing muscles from 96–144 hr, and the fly died. The fungus continued development, and the conidiophores grew through the host exoskeleton at the intersegmental membranes. Conidiophores develop a single spore at the apex which was forcibly ejected from the conidiophore when mature.

Several studies in muscoid fly hosts other than *Musca domestica* L. have indicated that *E. muscae* is greatly influenced by temperature. Carruthers and Haynes (1985) demonstrated that *in vivo* incubation in

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Delia antigua Meigen was prolonged at low temperatures (minimum of 5°C) and that the fungus apparently did not survive at a constant 32°C. Eilenberg (1987) showed that both *in vivo* incubation and the duration of conidial discharge were much longer at low temperatures. Cumulative conidial production by *E. muscae* in *D. antigua* was greatest at 16°C and was reduced markedly at temperatures <10°C or >27°C (Carruthers 1981). Mullens *et al.* (1987) showed that infection levels for *E. muscae* in *M. domestica* in the field were low during periods of hot weather (>25°C), and Mullens (1990) stated that infected *M. domestica* held at 34°C in the laboratory produced no conidia.

Olesen (1984) was the first to mention that *E. muscae*-infected *M. domestica* could exhibit a behavioral fever when exposed to a thermal gradient. Available data (Mullens, 1990; Watson, 1991) suggest that strains in the *E. muscae* group are severely limited by temperatures >32–35°C. The present study was designed to determine if short-term exposures of *E. muscae*-infected *M. domestica* to high temperatures would affect fungal pathogenesis and to determine whether infected flies would exhibit a behavioral fever response when allowed to thermoregulate in a heat gradient.

MATERIALS AND METHODS

The primary *E. muscae* culture used in this study was established from house flies collected from a dairy near Seward, Nebraska, in 1987. The culture was maintained continuously by direct fly-to-fly transmissions for 3 years (Mullens, 1986). This isolate was characterized by R. A. Humber, USDA, ARS, Insect Pathology Research Unit, Boyce Thompson Institute, Ithaca, New York, as a member of the *E. muscae* complex similar to the New York isolate originally isolated from *Pollenia rudis* (F.) (Kramer and Steinkraus, 1981). The Nebraska (NE) isolate produces large spores (averaging 11.96 by 15.87 μm), with nuclei larger than 4.5 μm in diameter and 4–8 nuclei per spore. One set of experiments was conducted using a second strain of *E. muscae* isolated from *M. domestica* in southern California (Mullens, 1986). The California (CA) isolate has 12–20 nuclei (about 3 μm in diameter) per spore. Typical incubation periods for the NE and CA isolates are 5 days (20°C) and 7 days (21°C), respectively (Watson, 1991; Mullens, 1986).

Temperature Effects on Pathogenesis

To determine the effects of temperature (40°C) on mortality rates of *E. muscae* (NE isolate) infected house flies, 320 laboratory-reared house flies (24 hr old) were exposed to conidial showers produced from 25 actively sporulating fly cadavers. Exposures were made in 550-ml plastic containers with screened lids for 18 hr at room temperature (22.5 ± 2°C). Twenty exposed flies were randomly selected and added to each of 16 cups (550-ml plastic containers with screened lids) provisioned with a sugar and dry milk mixture and a water-soaked cotton ball. Six additional containers each held 20 healthy flies (controls). Four containers, three holding diseased flies and one holding unexposed (healthy) flies, were placed in a 40°C incubator for a single 8-hr exposure on either Day 1, 2, 3, 4, or 5 following the initial infection period. Thus, separate groups were exposed to 40°C each day of the *E. muscae* incubation period. Flies were maintained at room temperature (22.5 ± 2°C) at all other times during the experiment. Three cups of diseased flies and 1 cup of healthy flies were held at room temperature throughout the experimental period. The experiment was replicated two times. Fly mortalities were monitored daily for 8 days (NE isolate), and cause of death was determined as either *E. muscae*-induced or from unknown causes. Patent infections were characterized as fly mortality resulting in sporulating fungi, a distended abdomen and extended wings, legs, and proboscis. Because *E. muscae* sporulation may be reduced or aborted at high temperature, dead flies exhibiting the typical postmortem signs were considered positive in that experiment. Similar experiments were conducted using the CA isolate of *E. muscae* in *M. domestica* at the University of California, Riverside. To account for a longer incubation period, heat exposures were conducted daily for 6 days and fly mortalities were monitored daily for 10 days following the initial infection. All further experimentation described herein was conducted using only the NE isolate of *E. muscae*.

To determine the effect of short-term exposures to high temperature on the survival of *E. muscae*-infected adult house flies in the early stages of infection, 180 laboratory-reared house flies (<24 hr old) were exposed to conidial showers for 18 hr. Only flies infected 24–48 hr were used in these experiments. Twenty exposed flies were added to each of nine plastic cups (550-ml) with screened lids and provisioned with food and water. Nine additional containers held 20 healthy flies each. Sixteen containers (eight each with diseased or healthy flies) were placed in a 40°C incubator, and one diseased cup and one healthy cup were held at room temperature throughout the experiment. One cup from each group, diseased and healthy, was removed from the incubator at 1-hr intervals for 8 hr and then held at room temperature until patent infections developed. Fly mortalities were monitored daily. This experiment was replicated three times at 40°C. Additionally, the experiment was repeated three times at 35°C.

Behavioral Fever Response

To measure the behavioral response of *E. muscae*-infected house flies to temperature, a heat gradient was established. A paper towel was cut and taped to the bottom of an aluminum baking pan (5.0 by 24.5 by 34.0

cm). A Plexiglas[3] cover (0.3 by 28.2 by 38.3 cm) was placed over the aluminum pan to hold the house flies in the pan. A flexible electrical heat tape was placed outside and across the bottom of the aluminum pan and the top of the Plexiglas cover at one end. Small holes (2 mm) were drilled in the Plexiglas cover at 7.5-cm intervals so a probe could be inserted to measure the surface temperature within each gradient zone created by the heat tape. Temperatures established in the gradient were in five zones: 42, 35, 31, 28, and 26 ± 2°C.

Newly eclosed house flies were exposed to conidial showers for 18 hr. Experiments were started 24 hr after exposure to allow sufficient time for spore germination and penetration of the cuticle. Flies were anesthetized with cold and released in the center of the heat gradient. Twenty diseased flies were placed in one heat gradient pan and 20 healthy flies were placed in a second, identical heat gradient pan. An additional 20 diseased flies were held at room temperature. After the flies were released in the heat gradient pan, the location of the flies was recorded at 2-hr intervals for 8 hr. After the heat-exposure period flies were removed from the pans and placed in 550-ml screened plastic cups provisioned with food and water. Flies were held at room temperature until all infected flies died (about 8 days). The experiment was replicated three times.

Further experiments were conducted to determine if *E. muscae*-infected or uninfected house flies exhibited similar behavioral responses to high temperature each day of the 5-day incubation period typical of the NE isolate. Two hundred flies were exposed to conidial showers as previously described and an additional 100 were held as unexposed controls. For 5 days, 20 flies from the healthy group and 20 from the *E. muscae*-exposed group were placed in separate heat gradient pans for 8 hr at 24-hr intervals. The location of diseased and healthy flies was monitored 2, 4, 6, and 8 hr after being placed in the heat gradient. An additional 20 flies from the *E. muscae*-exposed group were held continuously at room temperature to monitor the development of disease.

Statistical Analysis

Weighted means were calculated from the incubation period data as follows: add the number of patent infections per day, times the day each death occurred, and divide the sum by the total number of patent infections occurring at the completion of the experiment. Effects of high temperature on the duration of the incubation period were tested using χ^2 tests of differences. For the analysis, Days 2, 3, and 4 for the NE isolate and Days 1, 2, and 3 for the CA isolate were pooled and compared to the last day of the incubation period (Days 5 and 6, respectively) and to the control groups. Additionally, χ^2 analysis was used to test differences between the incubation periods of the isolates. The distribution of infected and uninfected flies in the heat gradient experiments was tested using χ^2 tests of differences. Alpha levels of 0.05 were used throughout the study.

[3] Mention of a propriety product in this paper does not imply its approval by the USDA to the exclusion of other products that may be suitable.

RESULTS

Effects of Temperature on Infection

Fly mortality and the number of flies dying with patent infections induced by the NE isolate increased with exposure to 40°C for 8 hr each consecutive day of the incubation period (Table 1). No flies died with patent infections when they were exposed to high temperatures on the first day of infection. Increasingly more of the flies exhibited patent infections if exposed to high temperatures on the second, third, fourth, and fifth day of infection. Of the infected flies held at room temperature, 90 (75%) died with patent infections. As expected, none of the healthy fly mortalities (total <5%) were attributed to *E. muscae*.

Similar results were observed in experiments investigating the effects of high temperature on the CA isolate of *E. muscae*. Few flies died with patent infections after exposure to 40°C for 8 hr each day of the 6-day incubation period (Table 1). Four flies developed patent infections, albeit they were exposed to high temperature on the first day of infection. Although daily means of patent infections were low, total mortalities increased each consecutive day of the incubation period. Sixty-eight percent of the *E. muscae* (CA) infected flies held at room temperature throughout the experiment died with patent infections. Maximum mortality in any healthy group averaged 8%.

Duration of the incubation period of the CA isolate (8.1 days) was significantly longer than the 4.1-day period observed with the NE isolate (Table 1). However, effects of exposure to 40°C on the incubation period did not significantly extend the incubation period for either isolate of *E. muscae* under these experimental conditions (Table 1).

The effect of shorter exposures (0–8 hr) to high temperatures indicated that increased exposures to 40°C increased the survival rate of infected house flies (Fig. 1). In three replicates a mean of 88% of the flies died with patent infections in flies held continuously at room temperature. After 1 hr of exposure to 40°C, 63% of the diseased flies died with patent infections, and fly survival generally increased with increased exposure time to 40°C. Less than 10% of the flies developed patent infections after 5 hr and no patent infections occurred in flies exposed to 40°C for 8 hr.

The time required to kill 50% of the flies at risk was

TABLE 1

Effect of 8-Hr Exposure to 40°C on the Mortality of House Flies (60 Flies/Day) Infected with One of Two Isolates of *E. muscae* through a 1- to 5-Day (Nebraska) or a 1- to 6-Day (California) Incubation Period

	Nebraska isolate				California isolate			
	Deaths				Deaths			
Exposure day	Average mortality	Average positive	Percentage survival	Incubation[a] period	Average mortality	Average positive	Percentage survival	Incubation[a] period
1	8	0	87	—	4	4	93	9.1b
2	13	7	78	7.1a	3	2	97	9.3b
3	38	17	37	6.9a	5	2	92	9.8b
4	50	20	17	5.1a	15	1	75	9.0b
5	52	38	13	4.2a	38	0	38	—
6	—	—	—		50	8	17	8.6b
Positive control[b]	54	45	10	4.8a	19[c]	14	5	8.1b
Negative control	1	0	95	—	2	0	92	—

Note. Mortality was monitored 8 d (NE) or 10 d (CA) following the initial infection period.

[a] Numbers within and between columns with identical letters are not significantly different, χ^2 ($P \leq 0.05$).

[b] Flies were held at 22.5 ± 2°C throughout the experiment.

[c] n = 20. Incubation Period = average number of days for patent infections to develop, calculated as: (No. patent (day) + No. patent (day))/(Total no. of patent infections).

prolonged by exposure to 40°C. Infected house flies held at room temperature had a mean incubation of 5 days, whereas those exposed to 40°C for 1–2 hr had a mean incubation time of 6.5 days.

In three replicates conducted at 35°C for 0–8 hr, an average mortality rate of 91% occurred in infected flies held at room temperature. After 1 and 2 hr exposure to 35°C a mean of 52 and 45% of the flies died with patent infections, respectively; thereafter no effect was apparent (Fig. 1).

Behavioral Responses

Flies were not distributed uniformly within the gradient (Fig. 2). Diseased flies exhibited a change in behavior that was not apparent in the healthy flies. Diseased flies were generally lethargic and tended to remain in certain temperature zones within the gradient for long periods. Healthy flies generally were active

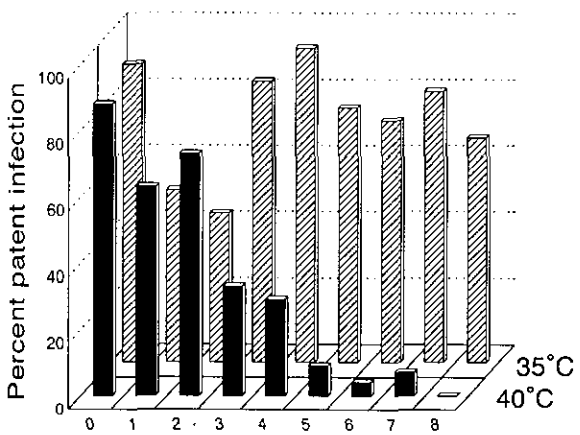

FIG. 1. Percentage patent infections occurring in *E. muscae* (NE isolate) infected house flies after short-term exposures to high temperature.

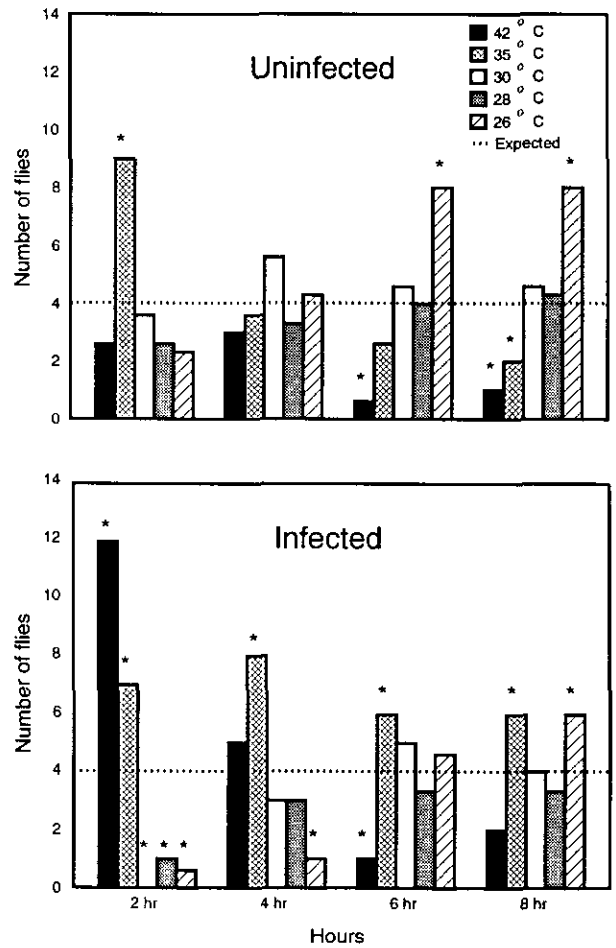

FIG. 2. Distribution of uninfected and infected house flies in a temperature gradient. χ^2 expected value of four flies/temperature zone was used to represent a hypothetical uniform distribution. * = $P \leq 0.05$.

throughout the gradient. Most healthy flies (57%) were located in the 35°C zone during the first 2 hr after being released in the gradient. Four hours after release in the temperature gradient healthy flies were not aggregated in specific temperature zones, but 6 and 8 hr after release, 40% of the flies were located in the 26°C zone. Zones of preference for infected flies within the heat gradient changed with time. Two hours after being placed in the heat gradient, 92% of the diseased flies were located at temperatures ≥35°C, and 58% were located at 42°C. Four hours after being released in the heat gradient 63% of the diseased flies were located at ≥35°C. After 4 hr, diseased flies generally avoided temperatures above 35°C. Survival rates for the diseased flies held at room temperatures were 2%, whereas those for the diseased flies released in the temperature gradient were 93%. Three dead flies were observed in the healthy, heat-treated group, none of which was attributed to *E. muscae*.

House fly behavior in the heat gradient differed with each consecutive day of incubation. Flies infected for less than 24 hr preferred 35°C significantly more often than those in the healthy group (Fig. 3). Flies infected with *E. muscae* for 2 days (24–48 hr incubation) exhibited a behavioral fever. Within 2 hr of release in the heat gradient, significantly more infected flies than uninfected flies were observed at 42°C. After 4 hr most diseased flies had moved to 35°C. Significant numbers of flies exhibited a behavioral fever for 4 hr on the third day of infection when compared to the uninfected group. Infected flies responded negatively to 42°C on Days 4 and 5 of the incubation period, and significantly more infected flies were found in the cool regions of the gradient when compared to the uninfected flies. When flies were left in the gradient after the observation period, 34 (57%) of the patent cadavers were found in the 26°C region and 14 (23%) were located at 28°C. No cadavers with patent infections were recovered above 30°C.

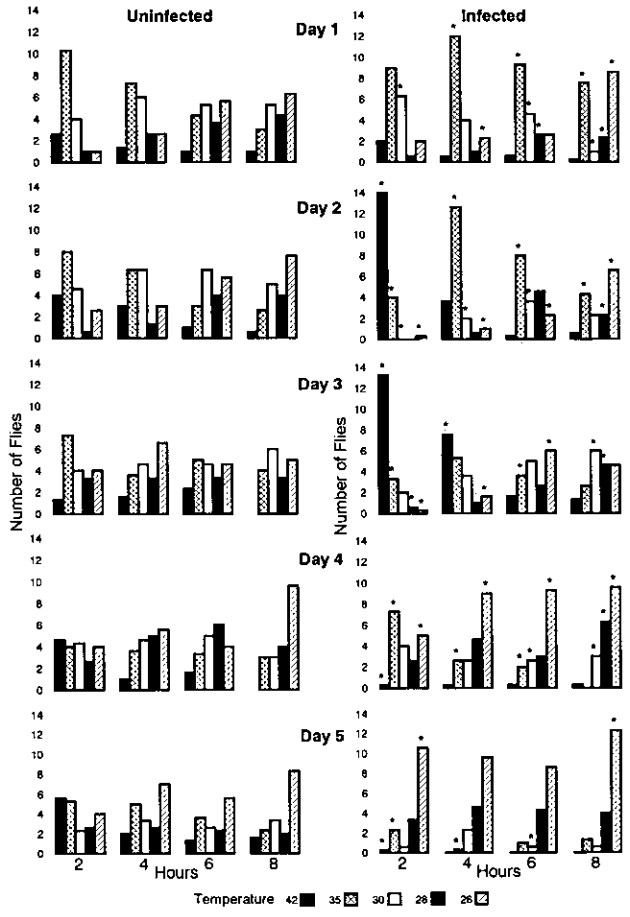

FIG. 3. Distribution of infected and uninfected house flies in a heat gradient on 5 consecutive days of the incubation period. χ^2 values were calculated from infected (observed) and uninfected (expected) observations. * = $P \leq 0.05$.

Numbers of flies dying with patent infections varied with each day of the incubation period (Table 2). Patent infections were above 90% for all infected flies held at room temperature throughout the experiment. Fifty-one percent of the flies infected for 1 day and released in the heat gradient for 8 hr developed patent infections. Flies infected for 2 days exhibited a behavioral fever and had the lowest numbers of patent infections (3%). Although flies infected for 3 days also exhibited a behavioral fever, 35% of these flies died with patent infections. No behavioral fever was observed in flies infected for 4–5 days and 80% of these flies died with patent infections. The controls produced minimal numbers of patent infections (2% in one replicate).

DISCUSSION

Examination of the effects of temperature was prompted because of a characteristic reduction in the numbers of infected flies found in the field during the summer months (Mullens *et al.*, 1987; Watson and Petersen, unreported data). Recent studies have shown *E. muscae* conidial discharge to be temperature-dependent (Eilenberg, 1987). High temperature also adversely effects the sporulation, germination, and infectivity of *E. muscae* (Mullens, 1990; Carruthers and

TABLE 2

Number and Percentage of Flies Dying with Patent Infections Infected with *E. muscae* (NE Isolate) then Released in a Heat Gradient (26–42°C) for 8 Hr or Held at Room Temperature (22.5°C) for 5 Consecutive Days of the Incubation Period

	Heat gradient		Room temperature
Day	Uninfected	Infected	Infected
1	0	31 (51%)	59 (98%)
2	1 (2%)[a]	2 (3)	59 (98)
3	0	21 (35)	60 (100)
4	1 (2%)[a]	48 (80)	55 (93)
5	1 (2%)[a]	48 (80)	59 (98)

Note. $n = 60$; 20 flies/treatment and the experiment was replicated three times.

[a] Positive *E. muscae* infections occurring in one replicate.

Haynes, 1986). Our experiments were conducted to determine factors other than the direct effects of temperature on the sporulation, germination, and infection processes. Olesen (1984) observed that temperature may disrupt the normal pathogenesis of *E. muscae* in house flies. Our results indicate that exposure to high temperatures during Day 1 to Day 3 of the incubation period can eliminate *E. muscae* infection. Eight-hour exposure to 40°C on Days 4 and 5 in the incubation period does not rid the fly of the infection. Many flies of the latter group exhibited the characteristic cream-white abdomen of infected flies, but once exposed to 40°C, these flies often died without completing the sporulation process or assuming the typical postmortem posture. Our results indicate flies at Days 1 and 4 of incubation did not exhibit behavior fevers, and flies actually exhibit a negative response to high temperature on Day 5 of the incubation period. When critically ill flies were left in the gradient to die, most fly cadavers were found at 26°C, the lowest temperature available within the gradient. Interestingly, cooler temperatures are beneficial to the sporulation of these fungi (Carruthers, 1981).

High temperatures probably contribute to the observed decrease in the number of infected house flies in field studies (Mullens *et al.*, 1987). Temperatures examined in this study are not uncommon in Nebraska during the summer months and may exceed 40°C on buildings or structures in direct sunlight. The first experiment of this study demonstrated that house flies can overcome the *E. muscae* infection with exposure to high temperature during the first 1–2 days of the incubation period. Furthermore, flies exposed to high temperatures for 5–8 hr have a good chance of survival, and brief exposures may extend the incubation period. However, low initial infection levels can affect the duration of the incubation period (Mullens, 1985). Because the number of infective spores was not measured in this study, combined with an 18-hr conidial exposure time, the significant effects of temperature on the duration of the incubation period were not evident. Yet, temperature effects on fungal incubation periods has been documented. Carruthers *et al.* (in press) reported that high temperatures (35°C) retarded the development of *E. grylli*-infected grasshoppers. Grasshoppers and house flies use basking behavior as a means of increasing the body temperature before flight or other activities (Carruthers *et al.*, in press; Mullens *et al.*, 1987). In the case of house flies this may have therapeutic value. House flies prevented from basking on pighouses had higher infection rates than those allowed to bask (Olesen, 1986).

Behavioral fevers in insects have not been well studied. Kluger and Rothenburg (1980) indicated that fevers may be a mechanism for the control of disease, but little documentation exists on behavioral fevers in naturally infected arthropods or fever therapy. Therapeutic use of heat was observed by Louis *et al.* (1986) when crickets were able to use heat therapy to control rickettsial infections. Our study documents behavioral fever in house flies induced by a fungal infection and indicates that house flies use basking behavior to rid themselves of disease.

The physiological processes inducing behavioral fever are in need of investigation. Physiologically, prostaglandins have been implicated in the induction of fever, and they recently have been observed in arthropods (Wakayama *et al.*, 1985; Stanley-Samuelson and Loher, 1983). Current research indicates that eicosanoids and prostaglandins may be a component of the insect immune system (Stanley-Samuelson *et al.*, 1991). Several arthropods respond to prostaglandin injections by exhibiting behavioral fevers (Casterlin and Reynolds, 1979; Cabanac and Le Guelte, 1980). The duration and intensity of the fever was dosage-dependent (Casterlin and Reynolds, 1978), and after exhibiting the fever, test animals returned to normal temperature zones (Cabanac and Le Guelte, 1980). In our study house flies exhibited a behavioral fever for 4–6 hr and then moved to cooler temperature zones. Comparing the fever response to the no-choice incubator studies, the duration of the fever coincides with increased survival rates observed at 5–8 hr at 40°C. We suspect that the fungal infection may elicit a physiological response in the house fly that is a function of humoral immunity.

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