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Seasonal Activity of *Entomophthora muscae* (Zygomycetes: Entomophthorales) in *Musca domestica* L. (Diptera: Muscidae) with Reference to Temperature and Relative Humidity

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The fungus, *Entomophthora muscae* (Cohn) Fresenius, commonly infects house flies, *Musca domestica* L., on feedlots and dairies in southeastern Nebraska. During two fly breeding seasons, disease prevalence increased from <1% in May, June, and July to 25 and 77% in August and September, respectively. Effects of relative humidity (RH) and temperature were examined with regard to infectivity and conidial yield of two isolates of *E. muscae*. House flies were exposed to conidial discharge at 23, 43, 75, and 98% RH. Infection rates of a Nebraska isolate (NE) were 83–94% regardless of relative humidity. Under constant temperature (25°C) the NE isolate produced conidia for 15–21 h, whereas a California isolate (CA) produced conidia for 18–24 h. Temperatures of 10, 20, 30, and 38°C were examined for effects on conidial yield and duration. More conidia were produced at 10 and 20°C for both isolates. The duration of the conidial discharge was temperature dependent. Shortest conidial discharge occurred at 38°C for the NE and CA isolates (9 and 6 h, respectively). Cadaver weight was correlated to spore yield at 20°C for both isolates and at 30°C for the CA isolate. These results suggest that *E. muscae* strains are suited to unique environmental conditions and may influence natural or induced epizootics. © 1993 Academic Press, Inc.

KEY WORDS: House fly; epizootiology; entomopathogenic fungus.

INTRODUCTION

Entomophthora muscae (Cohn) Fresenius epizootics have been observed in adult Diptera since the 19th century (Cohn, 1855; Yeager, 1939; Mullens *et al.*, 1987). Several agricultural pests associated with livestock may become infected, including house flies (*Musca domestica* L.), lesser house flies (*Fannia canicularis* L.), and *Ophyra aenescens* Wiedemann (Mullens *et al.*, 1987). Fly control is a growing concern for poultry and livestock producers, particularly in areas of expanding urbanization (Thomas and Skoda, 1993). Cultural and chemical controls have been the primary management tools for these filth flies. However, the importance of natural enemies, including *E. muscae*, is being reevaluated (Rutz and Patterson, 1990). Recent studies demonstrated that *E. muscae* has an impact on naturally occurring fly populations in poultry houses (Mullens *et al.*, 1987), but little is known of *E. muscae* epizootics on midwestern feedlots and dairies.

Mechanisms that contribute to the spread of *E. muscae* are of particular importance to epizootiology. Typically epizootics occur in the fall of the year. In some cases, epizootics coincided with cool, moist conditions (Cohn, 1855; Yeager, 1939); in others, epizootics occurred with relatively warm, dry conditions (Baird, 1957). These differences may indicate that *E. muscae* isolates function differently under certain environmental conditions. Recent epizootiological studies have not determined any direct relationships between wet conditions and *E. muscae* epizootics (Wilding and Lauckner, 1974; Mullens *et al.*, 1987; Carruthers *et al.*, 1988), although laboratory studies have demonstrated the importance of cuticular moisture and ambient relative humidity to the infection and transmission of this fungus (Kramer, 1980a,b; Mullens and Rodriguez, 1985).

Temperature has a demonstrative effect on *E. muscae* conidial discharge, germination, and incubation period (Eilenberg, 1987; Carruthers and Haynes, 1986; Mullens, 1990). Temperature effect is a probable factor in field studies as well. Mullens *et al.* (1987) described an epizootic in house fly populations in southern California as a low level infection in the spring, decreasing or nonexistent in midsummer, then increasing with the arrival of cooler fall weather. They observed decreases in the number of infected flies in hot weather with daily averages of 25–38°C. Subsequent experiments indicated

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conidia production was reduced when temperatures were above 27°C.

Comparative studies of differences in basic mechanisms influencing disease transmission, i.e., conidial yield and infectivity, under various abiotic conditions may help elucidate factors affecting epizootics under varied field conditions within a complicated taxon. *Entomophthora muscae* was characterized as a species complex of several varieties with distinct morphology and a varied host range (Keller, 1984, 1987). These fungi were collectively classified as one of four forms of the species (Keller, 1984). Form A with 4–7 nuclei per conidium was revised as *Entomophthora schizophorae* Keller and Wilding, a pathogen of *Pollenia, Delia* and *Psila* species, but not house flies (Keller, 1987). In contrast, form C with 12–16 nuclei per conidium is pathogenic to house flies. Experiments presented herein were performed using two taxa of *E. muscae* (form A or *E. schizophorae*, and form C), both of which were highly pathogenic to house flies in our study and, in this text, are considered *E. muscae*.

Our study involved both an observational field study and a laboratory study where certain abiotic factors could be manipulated. First, we investigated the prevalence of *E. muscae* in house flies associated with livestock facilities in Nebraska. To further understand the importance of host availability and macroclimatic factors, host densities, temperature, and precipitation were monitored. Second, we examined the effects of relative humidity and temperature under controlled laboratory conditions on the infection rate, conidial yield, and sporulation dynamics of a California isolate and a Nebraska isolate of *E. muscae*.

MATERIALS AND METHODS

Field Collections

Adult house flies were collected with a sweepnet from feedlots and dairies in southeastern Nebraska. Because none of the facilities were alike, fly collections were made from several similar fly aggregation sites (i.e., from feed bunks and spilled feed around the bunks, and from the open front of several calf shelters which also included areas of spilled feed and manure-soiled straw bedding). Sampling periods were limited to 15 min/facility. Collection sites at each facility were sampled weekly and pooled for each farm. Because moribund flies are sedentary and cannot fly (Mullens *et al.*, 1987), collecting active flies minimized cross-transmission within the holding cages. To reduce stress, collected flies were given a water-soaked paper towel for moisture and transported to the laboratory in an air-conditioned vehicle.

Once in the laboratory, the flies were provided either a 10% sugar–water solution or sugar and water separately. Cages were positioned on solid shelves with cloth barriers between the cages to minimize cross transmission of the fungus from adjacent cages. The flies were held at room temperature (22 ± 2°C) and observed daily for 7 days for patent infection (i.e., cadavers exhibiting fungal sporulation) (Mullens *et al.*, 1987). If *E. muscae*-induced mortalities were observed during the first 2–3 days after collection, the cadavers were removed as soon as possible and discarded. The minimal incubation period for *E. muscae* at 20°C was 5 days; therefore, all collected flies were frozen at 7 days to further reduce the likelihood of secondarily infecting field-collected flies. We assumed that flies infected secondarily would not complete the sporulation process before 7 days. Freeze-killed flies were counted and the number of patent infections was determined.

Adult house flies were collected from two livestock facilities in 1987 and 1988. In 1987 weekly collections were made from April 23 to September 28 (24 weeks). Both facilities were located in Seward County, Nebraska, on a 250-cow dairy (R1) and a 150-holstein heifer feedlot (R2). Again in 1988, house fly populations were sampled for 17 weeks, from May 26 to October 29. Temperature and precipitation data were compiled from the Climatological Data, Nebraska, USDC/NOAA station located at Seward.

Isolation of Cultures

A culture of *E. muscae* was established in a colony of laboratory-reared house flies for use in subsequent experimentation. This isolate, designated the Nebraska (NE) isolate, was cultured from house flies collected near Seward, Nebraska. The NE isolate is a member of the *E. muscae* complex (Form A) with primary conidia containing 4–8 large nuclei (4.15 μm) per spore (Keller, 1984). The NE isolate is similar to a New York isolate described by Kramer and Steinkraus (1981). *In vivo* cultures of the NE isolate have been maintained by direct fly-to-fly transmission for 5 years.

The California (CA) isolate of *E. muscae* was originally collected from infected house flies at a dairy facility near Riverside, California (Mullens, 1985). The CA culture also was characterized as a member of the *E. muscae* complex, with 12–16 small nuclei (3.5 μm) per primary conidium. The CA culture has been maintained at the University of California, Riverside since 1982.

Laboratory Studies

A series of laboratory experiments was conducted to obtain a better understanding of the effects of relative humidity (RH) and temperature on *E. muscae*. Experiments were performed to compare infectivity of both isolates under various RHs. Specific RHs were obtained by using the following saturated salt solutions: sodium phosphate (Na_2HPO_4), 97% RH; sodium chloride

(NaCl), 75% RH; potassium carbonate (K_2CO_3), 43% RH; and potassium acetate (CH_3CO_2K), 23% RH (Rockland, 1960). Solutions were placed in separate desiccators and the desiccators held in an incubator at 25°C with an 18:6 (L:D) photoperiod.

Infection rates of the NE isolate were examined by placing four, 400-ml screened (window screen) plastic cups containing 25 newly eclosed house flies in each of the four desiccators. One container of flies was protected from pathogen exposure by placing a clean petri dish over the screen. The other three containers were exposed to conidia by placing three fresh cadavers on the screened lid of each container in the four desiccators. Desiccators were sealed and placed in an incubator for 18 h (25°C). Then each group of flies was transferred to a separate cage with food and water. Flies were held at room temperature (22 ± 2°C) and examined daily for patent infection. The experiment was replicated two times, and each experiment was terminated after 7 days to prevent secondary infection.

Similarly, we examined the effects of RH on the conidial yield under constant temperature (25°C). Following a method similar to that described by Mullens and Rodriguez (1985), three fresh cadavers with legs and wings removed were fixed by the head to a microscope slide with a drop of clear fingernail polish. Each slide supporting the cadavers was suspended 25 mm above a clean microscope slide. Primary conidia were allowed to shower onto the clean slide directly below the fly cadavers. Slides were exchanged at 3-h intervals until the conidial discharge was complete (about 24 h). Conidia were determined by visual count of all conidia located within 100 fields of vision. Counts were made with the aid of a phase contrast microscope at 200× power and a guide scored longitudinally at 5-mm intervals to prevent overlapping counts.

To determine the effect of temperature on conidial yield, cadavers were weighed and randomly assigned to treatments (temperature), with six cadavers per treatment and each fly served as a replicate. Cadavers were fixed to microscope slides as previously described and placed in desiccators at 75% RH. Desiccators were placed in separate incubators at 10, 20, 30, and 38°C. Clean slides were placed directly below each cadaver at 3-h intervals until sporulation ceased. Numbers of conidia were determined by counting the conidia as previously described.

Statistical Methods

Statistical comparisons of total fly number, and number of *E. muscae*-infected flies, between farms and years were analyzed with ANOVA ($P \leq 0.05$). Simple and multiple regression techniques (Statview) were used to determine whether the dependent variable, proportion of *E. muscae*-infected house flies, was affected by independent variables: temperature, precipitation, and host density. Weights of fly cadavers were used to determine whether larger flies produced more conidia than smaller flies at different temperatures. Regression analysis was performed to determine the relationship of fly weight to conidial yield, as independent and dependent variables, respectively. Separate analyses of covariance (ANCOVA, $P \leq 0.05$), with fly weight as a covariate, were performed on the total conidial yield data at various temperatures and RHs (Minitab, 1988). Spore yield comparisons were made between treatments within isolates. Because experiments were not run concurrently, no direct statistical comparisons between the NE and CA isolates were possible.

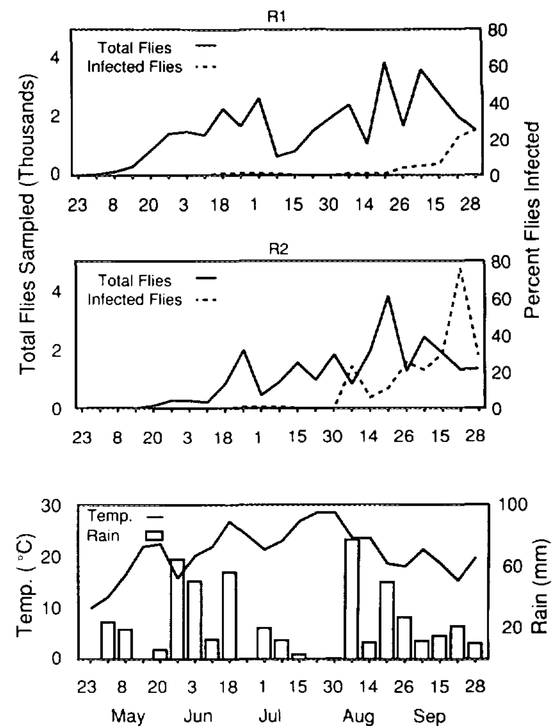

FIG. 1. Seasonal abundance and *Entomophthora muscae* infection rates for house flies at two confined cattle facilities and climatological data in 1987.

RESULTS AND DISCUSSION

Field Studies

Based on sweepnet sampling, peak fly numbers in 1987 occurred between mid-August and mid-September at R1 and R2 (Fig. 1). The weekly mean (±SE) number of house flies collected was 1577 ± 221 and 1009 ± 206 for R1 and R2, respectively ($F = 2.75$, $df = 45$). Fly production was variable throughout the 1988 season, and peak fly populations occurred during the same period at both facilities (Fig. 2). Weekly mean numbers of flies

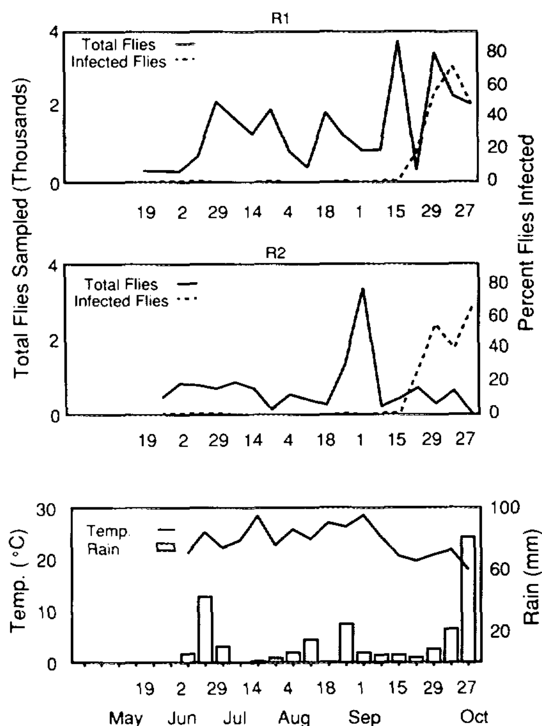

FIG. 2. Seasonal abundance and *Entomophthora muscae* infection rates for house flies at two confined cattle facilities and climatological data in 1988.

collected in 1988 were 1265 ± 233 and 727 ± 182 on R1 and R2, respectively (F = 3.16, df = 33). F statistics for these means (between sites and within years) were not significant (ANOVA $P \leqslant 0.05$). Accordingly, site-specific means between years were not significantly different (F = 0.99 and F = 1.41) for R1 and R2, respectively (df = 39, ANOVA $P \leqslant 0.05$). Despite this fact, flies were less abundant in 1988 than in 1987, with sweepnet collections yielding an average of 312 fewer house flies/week at R1 and 283 fewer flies/week at R2 in 1988 than in 1987. We suspected that the reduction in overall fly production was attributable to dry conditions and therefore, less fly breeding habitat was available (West, 1951). Summaries of the climatological data (NOAA 1987–1988) indicated the precipitation for Seward County was lower in 1988 (7.2 mm less rainfall/week) than in 1987, while the 1988 average temperature was higher (2.7°C) (Figs. 1 and 2).

Entomophthora muscae infections were first observed in the house fly collections in mid-June 1987. The prevalence of disease was <1% during much of the summer but increased to 25 and 76% by late September at R1 and R2, respectively (Fig. 1). The seasonal pattern of *E. muscae* activity was similar in 1988 (Fig. 2). Again <1% of the house flies collected in May and June was infected with *E. muscae*. Few infected flies were collected during July and early August and on some dates no infected flies were found. The prevalence of infected flies increased in late August and continued to a high of 77% at R1 by mid-October, and a high of 71% at R2 1 week later. As the proportion of infected house flies increased, an apparent decrease in the total number of flies was observed for both years (Figs. 1 and 2). It was difficult to discern whether this effect was due to *E. muscae* or whether it was part of the natural seasonal population flux. *Entomophthora muscae* did not respond immediately to the increase in available hosts observed in June but demonstrated a lag period and disease prevalence remained below 1% throughout most of the summer. The number of infections appeared to increase in response to host density and favorable temperatures about 6 weeks after abundant house fly populations were reached. Although *E. muscae* prevalence increased, there was no apparent impact on the host population, until perhaps late September, when there was a slight reduction in the numbers of hosts. Mullens *et al.* (1987) also noted that peak house fly numbers were attained about 3 months before *E. muscae* infections were found under favorable field conditions. A similar lagging density-dependent disease response was observed in onion flies and may be common to *E. muscae* epizootics (Carruthers *et al.*, 1985).

Differences between site and year were elucidated with analysis of variance (ANOVA, $P \leqslant 0.05$). Mean percentage infection between R1 and R2 (3.0 ± 1.4 and 9.8 ± 3.7, respectively, F = 4.93, df = 17) were significantly different in 1987 but were not in 1988 (12.4 ± 6.0 and 12.6 ± 5.7, respectively, F = 2.2, df = 17) (Figs. 1 and 2). Although inoculum was low in May, June, and July 1988, disease prevalence increased rapidly in September, exceeding those observed in 1987 at R1, but not R2. Based on these observations, the dynamics of *E. muscae* appeared to be associated with certain macroclimatic conditions, e.g., decreasing temperatures and, although less apparent, increasing precipitation (Figs. 1 and 2).

Irrespective of these trends, few significant correlations between fly densities and infection rates were observed from season-long data (May through September) except when *E. muscae* infection was weakly correlated with fly density at R1 in 1988 (R^2 = 0.337, slope 0.363 ± 0.132, $P \leqslant 0.05$). Significant negative correlations were observed between temperature and *E. muscae* infections collected from August through September on R1 (R^2 = 0.367, slope −26.02 ± 12.92, $P \leqslant 0.05$) and R2 (R^2 = 0.717, slope −64.63 ± 15.36, $P \leqslant 0.05$) in 1987. Examining the combined effects of temperature, precipitation, and host density on *E. muscae* infection strengthened the R^2 values on all facilities studied, but not significantly (Table 1).

Our results tend to support observations that correlations of *E. muscae* epizootics to environmental conditions are often weak or inconclusive (Carruthers and

TABLE 1

Multiple Regression to Determine the Relationships of *Entomophthora muscae* Infections to the Combined Effects of Temperature, Precipitation, and Host Density

Year/location	Coefficient	SE	P
1987			
R1 (R^2 = 0.508)			
Intercept	879.12	330.34	0.25
Temperature	−29.24	13.06	
Precipitation	−2.54	2.23	
Host density	−0.28	0.06	
R2 (R^2 = 0.743)			
Intercept	1860.86	421.67	0.10
Temperature	−66.37	17.48	
Precipitation	−1.95	2.85	
Host density	−0.02	0.08	
1988			
R1 (R^2 = 0.415)			
Intercept	586.16	2378.78	0.25
Temperature	−33.43	90.82	
Precipitation	−7.25	11.80	
Host density	−0.33	0.26	
R2 (R^2 = 0.319)			
Intercept	647.50	379.33	0.25
Temperature	−24.24	16.81	
Precipitation	−1.78	1.88	
Host density	−0.02	0.05	

Soper, 1987). However, certain similar trends were apparent. Increased *E. muscae* prevalence was associated with relatively cool and/or wet weather conditions, appropriate host densities, and sufficient inoculum. Similar observations were made by Kramer (1980a), Mullens *et al.* (1987), and Carruthers and Soper (1987).

Epizootics in house fly populations in California were described as low levels of infection in the spring, decreasing or nonexistent during midsummer, and then increasing with the onset of cooler fall conditions (Mullens *et al.*, 1987). Although significant relationships between certain abiotic factors and two springtime and summer–autumn pests, *F. canicularis* and *O. aenescens* were found, Mullens *et al.* (1987) could not attribute *E. muscae* infections in house flies, a mid- to late summer pest, to temperature and precipitation. These authors found that when weekly mean temperatures were above 25°C, infection rates declined. In our study weekly mean temperatures exceeded 25°C for 4 weeks in 1987, and 6 weeks in 1988. Because high temperatures are unfavorable to the development of fungal infections (Carruthers *et al.*, 1992; Watson *et al.*, 1993) extreme conditions in the field probably affect *E. muscae* epizootics as well.

Epizootics are generally density-dependent and, therefore, a population density threshold must be attained before epizootics can proceed (Brown, 1987). Graphically, epizootics are depicted as a sharp decline in host numbers shortly after the epizootic threshold is reached. In Nebraska the seasonal increase of *E. muscae* infection in the fly population occurred from August through September and was disrupted by a killing frost which decimated the host population and possibly that of *E. muscae*. Characteristic of our data was the absence of a springtime peak common to other *E. muscae* studies (Mullens *et al.*, 1987; Carruthers *et al.*, 1985; Carruthers, 1981). The absence of *E. muscae* in the spring reflects an unknown means of overwintering. No resting conidia were observed, and house fly numbers most likely were below the epizootic threshold level (Brown, 1987). We suspect that if favorable environmental conditions were sustained, a more complete seasonal cycle may have been observed in house flies and *E. muscae*.

Laboratory Studies

Relative humidity effects. House flies were infected with the NE *E. muscae* under all RHs examined, and no untreated insects developed the disease (Table 2). Under the conditions of this experiment, infection rates ranged between 83 and 94%, regardless of RH. Mullens and Rodriguez (1985) established that the CA isolate sporulated well at 20, 50, and 80% RH. Subsequently, Mullens (1986) observed infection rates near 100% from RHs of 30–60%.

We observed a diurnal periodicity in *E. muscae*-infected flies collected from the field which was maintained in the *in vivo* laboratory culture. Infected flies routinely died in the late afternoon. Fly cadavers started to discharge conidia about 3 h after death and continued to produce conidia through the night and early morning hours of the next day. The NE isolate exhibited a similar periodicity as that described by Mullens and Rodriguez (1985) for the CA isolate. Furthermore, diurnal periodicity was observed under natural conditions when infected flies routinely died in the late afternoon (Mullens, 1985). In our RH experiments, primary conidia production began soon after 9 pm for all treatments, approximately 3 h post mortem. The duration of conidial discharge for the NE isolate was 15–21 h (Table 2). Essentially all cadavers ceased primary conidia production in 15 h at 23, 43, and 75% RH for the NE isolate. Five cadavers produced conidia for 18 h, and one cadaver produced primary conidia after 21 h at 97% RH. Mullens and Rodriguez (1985) studied sporulation dynamics from fly cadavers held at 20, 50, and 80% RH. Humidities of 50% and 80% extended the duration of the conidial discharge beyond those observed at 20% RH, but cadavers held at 20% RH still produced a large number of conidia.

The duration of the conidial discharge for the CA isolate was 21–24 h (Table 2). Responses were similar for all four treatments. Generally, sporulation of the cadavers was well synchronized, indicating initial infec-

TABLE 2

Mean Primary Conidia Production of Two Isolates of *Entomophthora muscae* (Nebraska and California) during a 24-h Period at Four Relative Humidities (% RH)

	Mean (SE) number of conidia ($\times 10^3$) at 3-h intervals post mortem[a]									
% RH	0	3	6	9	12	15	18	21	24	Mean[b]
					Nebraska					
23	0	2.1	4.6	1.7	0.3	0.3	0	0	0	8.8
		(2.0)	(1.9)	(1.7)	(0.8)	(0.7)				(3.7)
43	0	2.1	4.3	1.1	0.1	0.001	0	0	0	7.8
		(0.4)	(1.6)	(1.3)	(0.1)					(2.6)
75	0	2.8	4.8	3.0	0.5	0.002	0	0	0	11.3
		(1.3)	(0.8)	(2.2)	(0.8)					(2.6)
97	0	2.3	4.2	3.1	0.6	0.3	0.04	0.002	0	10.5
		(1.1)	(0.8)	(1.1)	(0.9)	(0.6)				(2.2)
					California					
23	0	0.2	0.9	1.0	0.7	0.3	0.3	0.01	0.003	3.5
		(0.3)	(0.4)	(0.6)	(0.5)	(0.3)	(0.3)			(1.9)
43	0	0.4	0.8	0.8	0.6	0.4	0.05	0.06	0.01	3.1
		(0.5)	(0.5)	(0.5)	(0.5)	(0.4)				(1.6)
75	0	0.5	0.8	0.5	0.4	0.01	0.04	0	0	2.4
		(0.6)	(0.8)	(0.3)	(0.4)					(1.3)
97	0	0.4	0.8	0.6	0.5	0.2	0.06	0.02	0.005	2.6
		(0.3)	(0.5)	(0.6)	(0.5)	(0.2)				(1.9)

[a] Values are means for six cadavers.
[b] Mean/fly.

tions were consistent. However, one cadaver held at 97% RH ceased sporulation within 18 h while the remaining five cadavers continued to produced conidia for 24 h.

Secondary conidia production also was determined in this experiment. The relative abundance of these conidia for both isolates indicated secondary conidia production peaked 12–15 h postmortem, 6–9 h after the peak primary conidia production (Fig. 3). Mullens and Rodriguez (1985) suggested that secondary conidial discharge coincides with early morning fly aggregation periods and, therefore, secondary conidial discharges were considered important to disease transmission. Experiments conducted by Mullens (1986) indicated that secondary conidia are highly infective and primary conidia were less infective and probably served as a dispersal mechanism for the secondary conidia. Subsequently, the infectivity of primary conidia of a Denmark strain of *E. muscae* (similar morphologically to the NE strain) was significantly greater than the primary conidia from the CA strain (Bellini *et al.*, 1992). Furthermore, the secondary conidia of the CA *E. muscae* were more infective than primary conidia from either strain.

The NE isolate produced the greatest mean number of primary conidia (11,260 and 10,521) at 75 and 97% RH, respectively (Table 3). Fewer conidia were produced at 23 and 43% RH (8791 and 7872, respectively). The greatest number of conidia for the CA isolate was produced at 23% RH (3515) and 43% RH (3037) and fewer conidia were produced at 75% (2250) and 97% RH (2594). Although cadaver weight was suspected to influence the number of conidia produced (Mullens and Rodriguez, 1985), adjusting for cadaver weight did not impact the analysis in this study (ANCOVA, $P \leq 0.05$) for either isolate ($F = 0.05$, $df = 23$; $F = 0.01$, $df = 23$, CA and NE, respectively).

Temperature effects. Temperature had a greater effect on sporulation of the NE isolate than did RH (Table 3). The duration of the conidial discharge was temperature dependent (Fig. 4a). Peak sporulation occurred 3 h after the cadavers were fixed to the glass slide for all treatments. Conidial discharge was as short as 9 h at 38°C. The lowest mean spore count occurred at 38°C (313 ± 280/cadaver). Cadavers held at 30°C produced a mean of 1116 ± 230 conidia in a 12-h period. All but one cadaver ceased sporulation 9 h after the experiment began. At 20°C cadavers discharged conidia for 24 h with a mean of 9931 ± 2503 conidia/cadaver. Primary conidia production reached a peak 12 h after the cadavers were fixed to the slide. At 10°C a mean of 2520 ± 405 conidia was produced. The sporulation curve was bimodal at 10°C, because sporulation had all but ceased within the first 6 h of the experiment and then increased to a second smaller peak 33 h later. This was probably a slowed

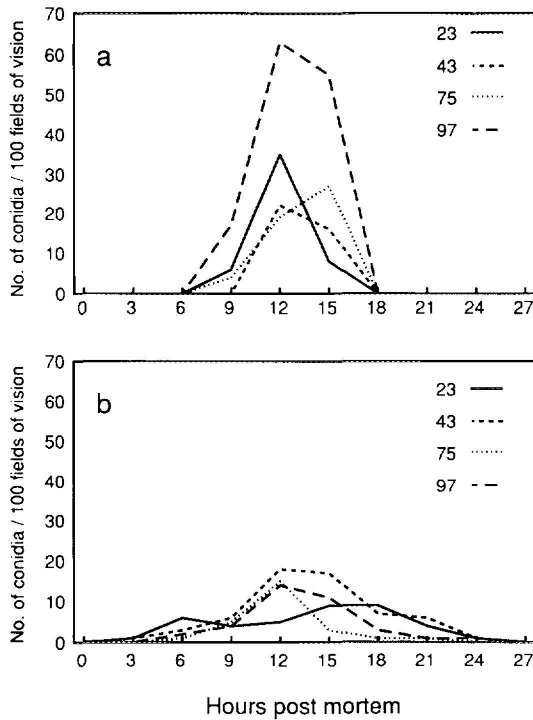

FIG. 3. Effect of relative humidity on the dynamics of secondary sporulation of two isolates of *Entomophthora muscae*. a, Nebraska; b, California.

developmental response after cadaver transfer to the low temperature. The duration of the conidial shower at 10°C was 41 h, almost twice as long as at 20°C. One cadaver produced 2115 conidia, ceased sporulation after 6 h, and did not resume sporulation.

Temperature effects on sporulation of the CA isolate were similar to those of the NE isolate (Table 3 and Fig. 4b). The shortest sporulation period occurred at 38°C for 6 h and the longest at 10°C for 48 h. Cadavers held at 38°C produced only 201 conidia with most sporulation occurring 6 h after the experiments began.

At 30°C cadavers produced a mean of 1430 ± 366 conidia over a 15-h period. Peak conidial production occurred 9 h post mortem. At 20°C the CA isolate produced 2818 ± 893 conidia over a 24-h sporulation period. Peak sporulation occurred 9 h into the conidial shower.

Cadavers held at 10°C produced conidial discharge for 48 h and mean conidial yield was 3559 ± 1326 conidia. This was more than the number of conidia produced by the NE isolate at the same temperature. Peak sporulation occurred 12 h post mortem. The dynamics of the conidial discharge did not produce the bimodal curve observed with the NE isolate. Conidial production for both isolates was greater at cooler temperatures than at warm temperatures. For the NE *E. muscae*, 79,284 conidia were counted, and 19.1 and 70.1% were produced at temperatures of 10°C and 20°C, respectively (Table 3). Fewer conidia were produced at 30 and 38°C (8.4 and 2.4%, respectively). Of the 60,783 conidia counted for the CA isolate, 10°C produced the most (57.7%), followed by 20°C (27.8%). Fewer conidia were produced at 30 (14.1%) and 38°C (0.3%). These results suggest that the CA isolate may be better adapted to cooler temperatures, unlike the NE isolate, which produced most conidia at 20°C.

As discussed previously, cadaver weight did not affect conidial yield in this study (Table 3), but regression analysis suggests an interplay between cadaver weight and temperature on the pathogenicity of *E. muscae*. Cadaver

TABLE 3

Relative Humidity and Temperature (°C) Effects on Conidial Yield from Two Isolates (Nebraska and California) of *Entomophthora muscae*

	Nebraska		California	
Relative humidity	Mean (SE) cadaver wt.	Mean (SE) number of *E. muscae* conidia	Mean (SE) cadaver wt.	Mean (SE) number of *E. muscae* conidia
97%	31.2 (4.8)a	10521 (903)a	23.2 (5.3)a	2593 (990)a
75%	31.0 (4.9)a	11259 (1056)a	23.0 (5.3)a	2249 (537)a
43%	34.3 (5.4)a	7872 (1072)b	25.3 (4.9)a	3036 (621)a
23%	32.5 (6.0)a	8790 (1534)ab	23.3 (3.7)a	3515 (781)a
Temperature				
38	25.5 (4.7)a	313 (280)a	23.2 (4.7)a	34 (20)a
30	26.2 (2.4)a	1116 (230)b	20.5 (4.1)a	1430 (366)b
20	26.8 (3.1)a	9931 (2503)c	21.5 (4.6)a	2818 (893)b
10	24.8 (3.4)a	2520 (405)d	20.2 (2.7)a	3559 (1326)b

Note. Means within treatment columns (relative humidity or temperature) followed by the same letter are not significantly different. ANCOVA $P \leq 0.05$.

weight and conidial yield were correlated at 30°C (R^2 = 0.840) and, with one outlier removed, at 20°C (R^2 = 0.842) for the CA isolate (Table 4). Cadaver weight and conidial yield were correlated for the NE isolate at 20°C (R^2 = 0.878). Cadaver weight and conidial yield were not correlated at 10°C for either isolate. Mullens and Rodriguez (1985) observed that cadaver weight and conidial yield were correlated under temperature conditions between 20 and 26°C and variable RH for the CA isolate. These results indicate that although RH was constant (75%), larger cadavers produced more infective conidia only within "favorable" temperature ranges.

Conclusions

Although *E. muscae* is a common disease of house flies in Nebraska its impact on the house fly population is limited. Despite the increasing number of potential hosts during the warm summer months, *E. muscae* prevalence declined to near nonexistent levels. Increased prevalence of disease only occurred with the onset of cooler fall conditions and the impact on the house fly populations was not fully demonstrated because the epizootic cycles were disrupted by frost.

Abiotic conditions in the field were not strongly correlated to increased prevalence of disease. Laboratory studies examining the effects of temperature and relative humidity indicated that temperature was perhaps the most important factor influencing conidial yield. Thus, temperature probably influences the transmission of disease under natural conditions.

Comparisons of two isolates of *E. muscae* indicate that they differed in this response to temperature and RH. The CA isolate appeared to function better at low RH and low temperature by producing relatively more conidia, especially the highly infective secondary conidia compared with the NE isolate. In light of these results, macroclimatic conditions probably play an important role in the transmission, infection, and maintenance of *E. muscae* in the house fly population. Efforts directed toward monitoring these factors and host behavior may provide a more complete view of *E. muscae* epizootics in house fly populations.

This study contrasts the biology of two members of the Entomophthorales taxa once thought to be a species complex (*E. muscae*). However, the taxonomy of the NE isolate remains in question. Based on the revision of the *E. muscae* complex by Keller (1987), the NE isolate resembles *E. schizophorae* in all characteristics except the host range. If the description of *E. schizophorae* is applicable to the NE isolate, this study demonstrates an expanded host range for *E. schizophorae* on the North American continent.

TABLE 4

Regression Analysis of Conidial Yield and Cadaver Weight at Four Temperatures

Temperature (°C)	Isolate			
	Nebraska		California	
	R^2	Slope ± SE	R^2	Slope ± SE
10	0.259	147048.1 (954.7)	0.055	258176.9 (3505.7)
20	0.878*	1877612.1 (2391.2)	0.842*	409581.5 (977.2)
30	0.332	135046.2 (514.1)	0.840*	198929.8 (401.6)
38	0.263	−30721.5 (268.7)	0.114	−3517.6 (51.2)

* $P \leq 0.05$.

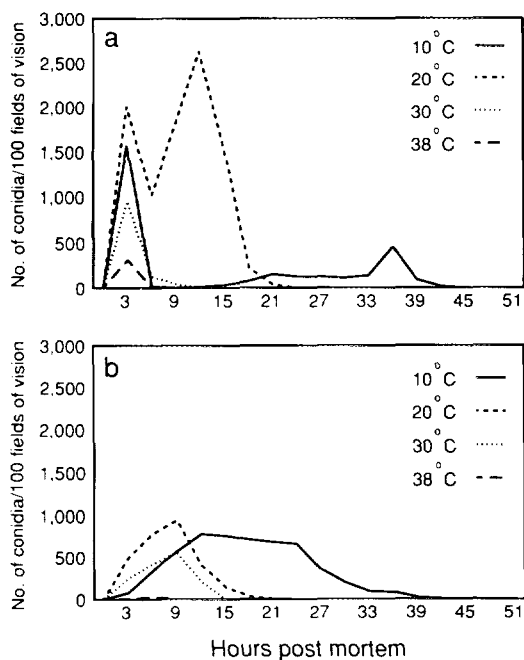

FIG. 4. Effect of temperature on the dynamics of primary sporulation of two isolates of *Entomophthora muscae*. a, Nebraska; b, California.

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