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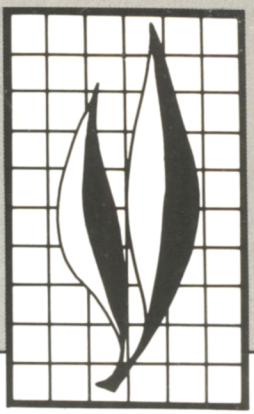

An Epizootiological Study of *Entomophthora muscae* in Muscoid Fly Populations on Southern California Poultry Facilities, with Emphasis on *Musca domestica*

Bradley A. Mullens, John L. Rodriguez, and Jeffery A. Meyer

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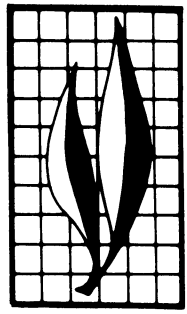

ABSTRACT

Infection by the pathogenic fungus *Entomophthora muscae* was monitored in muscoid fly populations on four caged-layer poultry facilities in southern California. Adult flies were captured every 1 to 2 weeks with sweep nets and held for 7 days in the laboratory to assess incidence of infection over a 2-year period. Patent *Musca domestica* and *Ophyra aenescens* infections were found throughout the year. Average prevalence of infection in *M. domestica* was highest (45 percent) in fall and lowest (< 1 percent) in the hot summer months. Infection in *O. aenescens* at times approached 100 percent, with three peaks of *E. muscae* activity (March, June, November) coincident with peak population densities. Infections in *Fannia canicularis* and *F. femoralis* were evident primarily in late spring. Peak average infections in *F. canicularis* (45 percent) lagged behind peak adult population density by 3 weeks in 1983. Infections in *Fannia* spp. were rare in 1984 due to low rainfall and subsequent low adult densities. Infection prevalence was higher in male than female hosts, but was probably influenced by the shorter incubation period in males and the short holding period. Flies were not infected with *E. muscae* as larvae or while emerging from manure. Cool fall weather stimulated morning *M. domestica* aggregation on the south walls of the primary poultry house study site, where cadavers killed by *E. muscae* were most common. Flies likely were infected primarily by morning exposure to secondary conidia expelled from these structure surfaces. Possible management techniques to optimize *E. muscae* activity are discussed.

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An Epizootiological Study of *Entomophthora muscae* in Muscoid Fly Populations on Southern California Poultry Facilities, with Emphasis on *Musca domestica*[1]

INTRODUCTION AND LITERATURE REVIEW

TRADITIONALLY, MANAGEMENT PROGRAMS for filth-breeding muscoid flies have relied on chemical and cultural control measures. During the last 20 years, however, both scientists and producers have begun to appreciate the role that natural enemies play in regulating fly populations. Research emphasis has centered on predators and parasites of immature fly stages in the manure environment (Axtell 1981; Axtell and Rutz 1986). In California, a considerable amount of work on these natural enemies has been done (Peck and Anderson 1969; Legner and Olton 1971; Legner et al. 1975), and it has led to the use of integrated management schemes in some areas of the state (Legner and Dietrick 1974). In spite of the excellent efforts of numerous individuals to define the role of natural enemies in regulating fly populations, natural biotic factors affecting adult fly stages have received little attention.

A widely recognized natural enemy of adult muscoid flies is the fungus *Entomophthora muscae* (Cohn) Fresenius. This pathogen has been found infecting several species of Diptera in the Muscidae, Calliphoridae, Anthomyiidae, Sarcophagidae, Drosophilidae, and Syrphidae, and often has been reported from North America, Europe, and India (MacLeod, Müeller-Kögler, and Wilding 1976). Historically, a commonly reported host has been *Musca domestica* L., which reflects the close association of this fly with human dwellings. In spite of its ready visibility, the disease has not been critically examined in the field for any species of manure-breeding fly, including *M. domestica.* To date, the only intensive studies of *E. muscae* epidemiology have been in agricultural pest populations, notably the wheat bulb fly, *Leptohylemyia coarctata* Fall. (Wilding and Lauckner 1974) and the onion maggot, *Delia antiqua* (Meigen) (Carruthers, Haynes, and MacLeod 1985).

The literature on *E. muscae* is voluminous and often old, and it is not our intention to review it in its entirety. The following, however, is a review of published literature that, in our opinion, is appropriate background to the biology of the pathogen and/or of relevance to a study of its epizootiology in this system. Some other references may be found in Müller-Kögler (1965), Greenberg (1971), or MacLeod, Müller-Kögler, and Wilding (1976). *Entomophthora muscae* was first described by Cohn (1855), and Brefeld (1871) first studied in detail the process of host invasion and propagation for *E. muscae.* This process also was documented by Brobyn and Wilding (1983) and much of the following account is from that work.

[1]Accepted for publication January 27, 1987.

Conidia adhere to the insect cuticle with the aid of wall material and some cytoplasm from the ruptured conidiophore (Eilenberg, Bresciani, and Latge 1986). They then germinate, and cuticle penetration is accomplished by the germ tube, probably with the aid of enzymes. Since long germ tubes have been observed only from secondary conidia (Kramer 1980), this could be the infective form, though Brobyn and Wilding (1983) were not able to determine whether the germ tubes originated from primary or secondary conidia. Penetration may occur almost anywhere on the body of the host, though the abdomen most frequently is entered. The contents of the conidia pass through the germ tube, sometimes forming a "thin-walled, bladderlike hypha" at the entry site within the abdominal cuticle. Within 28 hours of inoculation, hyphal bodies can be found throughout the hemocell, and they proliferate in the abdomen, especially the fat body. Eventually, most internal tissues are destroyed. Brobyn and Wilding (1983) state that oocytes and tracheae survive undamaged, but the senior author has observed apparent resorption of yolk from mature oocytes in the later stages of infection. Hyphal bodies fill the hemocell shortly before host death, and the fungus begins to penetrate the intersegmental membranes within a few hours of host death. Brobyn and Wilding (1983) did not observe any specialized attachment structures, but Belazy (1984) has reported rhizoid formation ("holdfasts") by *E. muscae* from the labellum of several families and species of muscoid Diptera. Some stages in the *E. muscae* life cycle are shown in figure 1.

Epizootics have been observed on numerous occasions, usually in fall, for *M. domestica* in temperate regions of Europe and North America (Cohn 1855; Lebert 1856; Brefeld 1871; Thaxter 1888; Graham-Smith 1916, 1918). Brefeld (1871) thought that the pathogen could overwinter in fly populations in warmer regions and move northward in spring with dispersing flies or could persist in hibernating fly populations due to the prolonged disease incubation at cool temperatures. Lakon (1919) did not agree with Brefeld's assessment regarding overwintering of *E. muscae,* believing that the resting spore stage and transmission of the pathogen among multiple host species allowed it to persist year-round in temperate regions, such as Germany. The pathogen can persist in flies year-round in warm regions, such as southern Italy and Brazil (Brefeld 1871, 1908); Ystrom (1980) reported *E. muscae* was present year-round in *M. domestica* in Denmark. Some references to reports of *E. muscae* in the U.S. can be found in Greenberg (1971).

In general, house fly cadavers have been found outdoors infrequently, even during epizootic periods (Thaxter 1888; Hewitt 1914; Graham-Smith 1916; Gussow 1917). These authors most commonly found cadavers indoors, especially at windows. Resting spores also have not been found consistently in house flies. According to Thaxter (1888), they were first described by Lebert (1856). Goldstein (1923) stated that resting spores were formed late in fungal development and under dry conditions; Winter (1881) and Gustaffson (1965) found them in moist conditions. Brefeld (1871) and Thaxter (1888) never found resting spores.

Some authors have reported epizootics of *E. muscae* during wet periods (Petch 1934; Yeager 1939), but the pathogen also is able to function under drier conditions. Baird (1957) observed an epizootic in a colonized tachinid at 50 percent relative humidity (RH), and Kramer (1980) showed that conidia could be produced and infect flies over a wide range of humidities. Mullens and Rodriguez (1985) showed that house fly cadavers produced many primary and secondary conidia at 20, 50, and 80 percent

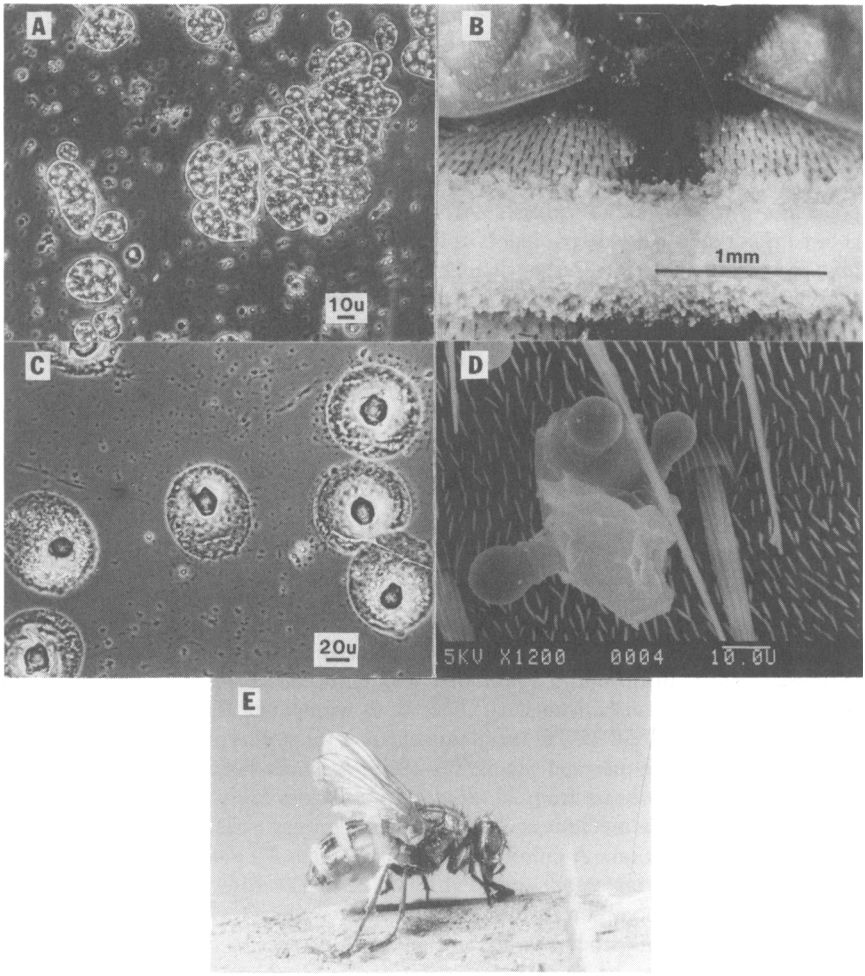

Fig. 1. Selected stages in the *E. muscae* life cycle. (*a*) Hyphal bodies (note variable size and shape) in hemocell of infected *M. domestica* during the last 24 to 48 hours of the incubation period. (*b*) Intersegmental membrane of cadaver, showing lush conidiophore growth. Each conidiophore bears a single primary conidium. (*c*) Appearance of primary conidia discharged onto glass slide. Note the "halo" of wall material and cytoplasm around each conidium from the ruptured conidiophore. Conidium in *center* best shows typical shape for this species complex. (*d*) Discharged primary conidia which have formed secondary conidia on cuticle of *M. domestica* cadaver. (*e*) Cadaver of *M. domestica* killed 18 hours before by *E. muscae.* Note outstretched legs, wings, and proboscis, fungal growth on intersegmental and ventral regions of abdomen, and conidia adhering to wings, hind tibiae, and substrate. Photo by Max Badgley, University of California, Riverside. Other photos by senior author.

RH. Conidial production began 4 to 5 hours after host death, reached peak levels 10 to 12 hours postmortem, and largely was completed in 20 hours. On a glass substrate, secondary conidia were discharged 3 to 9 hours after the primary conidia (Mullens and Rodriguez 1985). Temperature probably is important in the seasonal occurrences of *E. muscae.* Carruthers (1981) showed that conidial discharge was reduced dramatically at temperatures less than 10°C or over 25°C for *E. muscae* from *D. antiqua* in Michigan. Though little is known about the dynamics of the disease in wild *Musca* populations, Wilding and Lauckner (1974) and Carruthers, Haynes and MacLeod (1985) showed that the primary determinants of infection in *L. coarctata* and *D. antiqua* were host and inoculum density, rather than climatic factors. Host age structure also was a significant factor for *E. muscae* in *L. coarctata* populations (Wilding and Lauckner 1974); infections in older female hosts primarily resulted in resting spore formation. It is known from laboratory studies that young *M. domestica* are more susceptible to *E. muscae,* have a shorter incubation period, and are more likely to produce conidia than are older hosts (Mullens 1985).

The taxonomic state of *E. muscae* is currently unresolved. MacLeod, Müller-Kögler, and Wilding (1976) presented data from the literature showing considerable variability in dimensions of conidia and resting spores. A given strain can exhibit variability in its ability to infect different host species (Kramer and Steinkraus 1981; Mullens, unpublished). Keller (1984) also provided preliminary data suggesting that four forms of *E. muscae* may exist, based on conidial dimensions and number and dimensions of nuclei. Presently, all forms are still referred to as *E. muscae* (R. A. Humber, personal communication). The pathogen has been colonized in vitro for laboratory studies (Schweizer 1948; Srinivasan, Narasimhan, and Thirumalachar 1964), but, like many Entomophthorales, can be somewhat difficult to manipulate (Latge 1982). It is not particularly difficult, however, to maintain at least some strains of the pathogen in vivo in the laboratory (Kramer and Steinkraus 1981; Mullens 1986). The southern California strain of *E. muscae* from *M. domestica* also does fairly well (vegetatively) in Grace's tissue culture medium and also will grow on egg yolk agar (R. A. Humber, personal communication). A culture of the fungus from *M. domestica* is being maintained in vivo in our laboratory and in vitro at the USDA Insect Pathology Research Laboratory, Boyce Thompson Institute, Ithaca, New York.

The purpose of this study was to determine which species of muscoid flies were infected by *E. muscae* on caged layer operations in southern California and to document seasonal variability in infection prevalence. This information was related to biotic and abiotic factors to attempt to explain the epizootiology of the disease in fly populations at these sites.

MATERIALS AND METHODS

Study Sites

Four commercial egg operations south of Riverside in Riverside County, California, were selected for study. Monitoring at the Lohr Ranch began in November 1982. Three other nearby sites were added over the following 8 months. These were Kramer

1 (K1) and Kramer 2 (K2) in January 1983 and Loma Linda University (LL) in June 1983 (fig. 2). Sites were monitored continuously until January 1985. Producer management practices were monitored, but no attempt was made to alter them in any way. Manure cleanouts, removal of old hens and introduction of new ones, and any use of pesticides, especially insecticides, were noted.

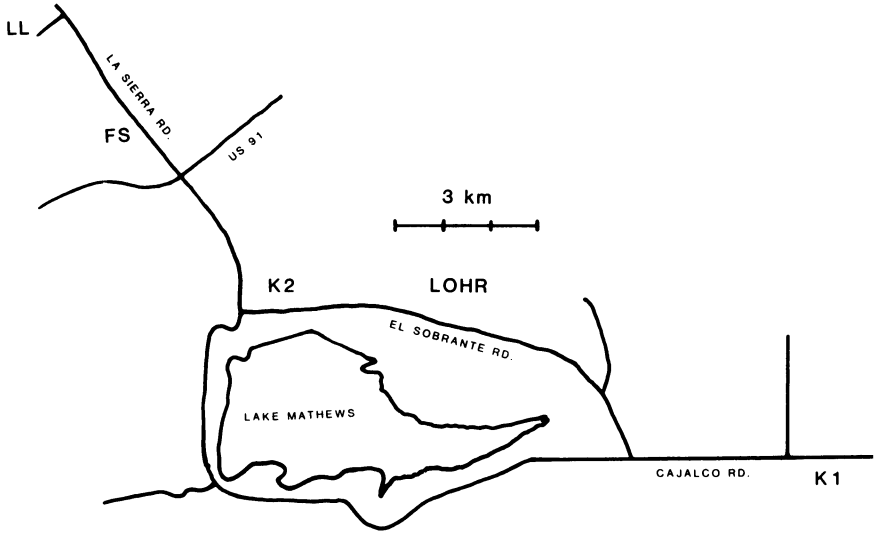

Fig. 2. Locations of the four caged layer poultry ranches in *E. muscae* epizootiology study. LL = Loma Linda University, FS = Fire Station (outside weather data), K2 = Kramer Ranch 2, Lohr = Lohr Ranch, and K1 = Kramer Ranch 1.

The Lohr site was a ranch of 42,000 hens confined in wire cages suspended from the ceiling framework. Manure was allowed to accumulate on the ground beneath the birds and was removed at approximately 6-month intervals. When manure cleanout occurred, a layer of dry manure, 20 cm deep, was left. This served to help absorb moisture from the fresh droppings and was a refugium for parasites and predators useful in natural biological control of pest flies. The six chicken houses were wood frame and tin roof structures with sides of wooden lath and chicken wire. The open construction allowed good air circulation to assist manure drying, also critical for keeping fly levels down and maintaining suitable conditions for beneficial arthropods. The houses were equipped with sprinklers at the roof peak, set to operate during periods of high temperature. These usually occurred for several hours each afternoon during

summer. This general house design was similar for all four study sites. The immediate area around the houses was largely devoid of vegetation, as was generally the case at the other sites.

The K1 site was a larger operation, with 116,000 hens held in five houses. Hens were contained in cages as at Lohr, but were supported on a wooden framework, and manure accumulated on boards beneath the hens. Boards were scraped daily, and manure was hauled to another location (see K2). There was thus little fly breeding at this site, but the ranch did serve as an aggregation area for flies, especially *Fannia canicularis* (L).

The K2 ranch consisted of 10 interconnected chicken houses (74,000 hens total). Manure was allowed to accumulate on the concrete beneath the hens and was cleaned out at weekly intervals. Occasionally manure accumulated for 2 or 3 weeks, however. A field adjacent to this facility was used for thin-bed drying of manure from this and several other egg facilities. Some fly breeding occurred at K2 and in the adjacent drying field. Cooling was by roof sprinklers and misters on the eaves.

The LL site was a ranch of 40,000 birds held in nine houses. Manure accumulated beneath the hens and was removed twice a year. Like Lohr, this ranch supported large breeding fly populations.

Recording hygrothermographs were placed at the end of the manure rows (0.5 m height) inside one house at Lohr and K1 in July 1983. They were operated continuously through January 1985. Other weather data (maximum and minimum daily temperatures and rainfall) were obtained from a fire station (FS) 2.5 km southeast of the LL site (fig. 2). Climate in this area of southern California is moderate from late October to late May, and most of the annual average 28 cm of rainfall occurs then. Freezing temperatures are uncommon, and daytime high temperatures usually are between 15° and 25°C in winter. The remainder of the year (June to October) is warmer, and rainfall is rare. From July to September, temperatures can exceed 40°C, while low temperatures usually are around 20°C. Easterly desert winds occur sporadically during fall and may reduce relative humidities (RH) below 10 percent. Typically, humidities are in the range of 40 percent RH during the day, but often exceed 95 percent RH for 4 to 8 hours at night.

Monitoring Fly Populations

Sticky fly tapes (Aeroxon) were used at all four sites to monitor fly populations. They were hung from overhead rafters around the perimeter of one house at each site. Both ends were attached, allowing the tapes to drape downward in a shallow arc. Tapes were hung inside at Lohr and LL and outside but under the eaves at K1 and K2. Three tapes were hung on each side (at each end and in the middle) of each house. Because the K2 aggregate of houses was very large, all six tapes were hung along a single side (south exposure) at this site. Tapes were changed weekly and brought back to the laboratory. After a week in the field, tapes had accumulated sufficient flies and dust that they could be loosely placed together, by site, in a paper sack, with minimal loss or transfer of captured flies from the tapes. The numbers of *Musca domestica* L., *Fannia canicularis* (L.), *Ophyra aenescens* (Wiedemann), *Muscina stabulans* (Fallen), *F. femoralis* (Stein), and *Stomoxys calcitrans* (L.) were recorded after visual identification and counting. A 3X set of lenses, worn on the head, was useful for observing wing veination, body shape, color, and markings in some specimens on the tapes.

Monitoring Infections

Flies were collected with sweep nets at various locations at each site. Collections were made every 2 weeks throughout most of the year and weekly during critical *E. muscae* transmission periods in spring and fall. Collections were made between 10 a.m. and 2 p.m., and flies were collected from all sites on the same day. Sweeps were made several cm above fly resting or aggregation sites, such that flies had to take flight quickly to be caught. The areas in which flies were collected were fairly constant within a site through time. At Lohr and LL, flies were collected from the manure surface, containers holding dead chickens and broken eggs, and from the inside and outside walls of the houses. At K1 and K2, most flies were collected from the outside walls, and less commonly from manure surfaces or adjacent vegetation.

Flies captured at each site were placed in a 3.8-L cardboard carton with a screened lid. A 6-cm hole was cut on the side near the bottom of each carton and was plugged with a large rubber stopper; this served as an opening to add flies. Each carton held a container of dry milk and sugar, as well as a water cup with a lid and drinking wick. Cartons at first were autoclaved before each use, but it subsequently was shown that cartons free of flies and cadavers for a full week were unable to serve as a source of infection. This was due to the short life of conidia and the limited time of secondary conidial discharge (Mullens and Rodriguez 1985).

Preliminary studies showed that flies collected in this way rarely died earlier than day 3 postcapture, with most dying between day 4 and day 7. Flies were held at 22 ± 2°C and natural photoperiod for 7 days postcapture. The shortest incubation period observed at this temperature in over 3 years of experimentation has been 5 days with very young hosts and high exposure levels. The 7-day holding period thus minimized the risk of misinterpretation due to retransmission within the holding carton. After 7 days the flies were placed in a freezer (−10°C) for a few hours, then sorted by species and sex into three groups. Flies that were still soft and flexible were considered to have been alive when placed in the freezer. Flies that were dried out or stiff were considered dead of unknown causes. Flies that had died with obvious external signs of *E. muscae* (outstretched wings, legs and proboscis, swollen abdomens with fungal growth visible in intersegmental regions) comprised the third category (fig. 1*e*). To reduce variability due to different observers, the senior author conducted all of the sorting. Primary conidia were periodically collected from fly cadavers and examined for shape and size, which are distinctive for the *E. muscae* group (MacLeod, Müller-Kögler, and Wilding 1976). Representative cadavers were sent to R. A. Humber, USDA-ARS Insect Pathology Research Unit, Boyce Thompson Institute, Ithaca, New York; he confirmed the pathogen identity.

On two dates in fall 1984, flies were captured from the Lohr and K2 sites as usual and were anaesthetized with CO_2 in the laboratory and placed on ice. A total of 100 flies (mostly *M. domestica*) was randomly selected from each group, and these were held individually in clean 22-ml plastic cups with nylon organdy lids. Each cup contained a small amount of dry milk and sugar and received a large drop of water on the mesh each morning from which each fly could drink. The remainder of the flies were returned immediately to the original 3.8-L carton and held as usual under exactly the same environmental conditions as the individually held flies. Individually held flies were checked daily, and mortality recorded as above. After 7 days, the flies

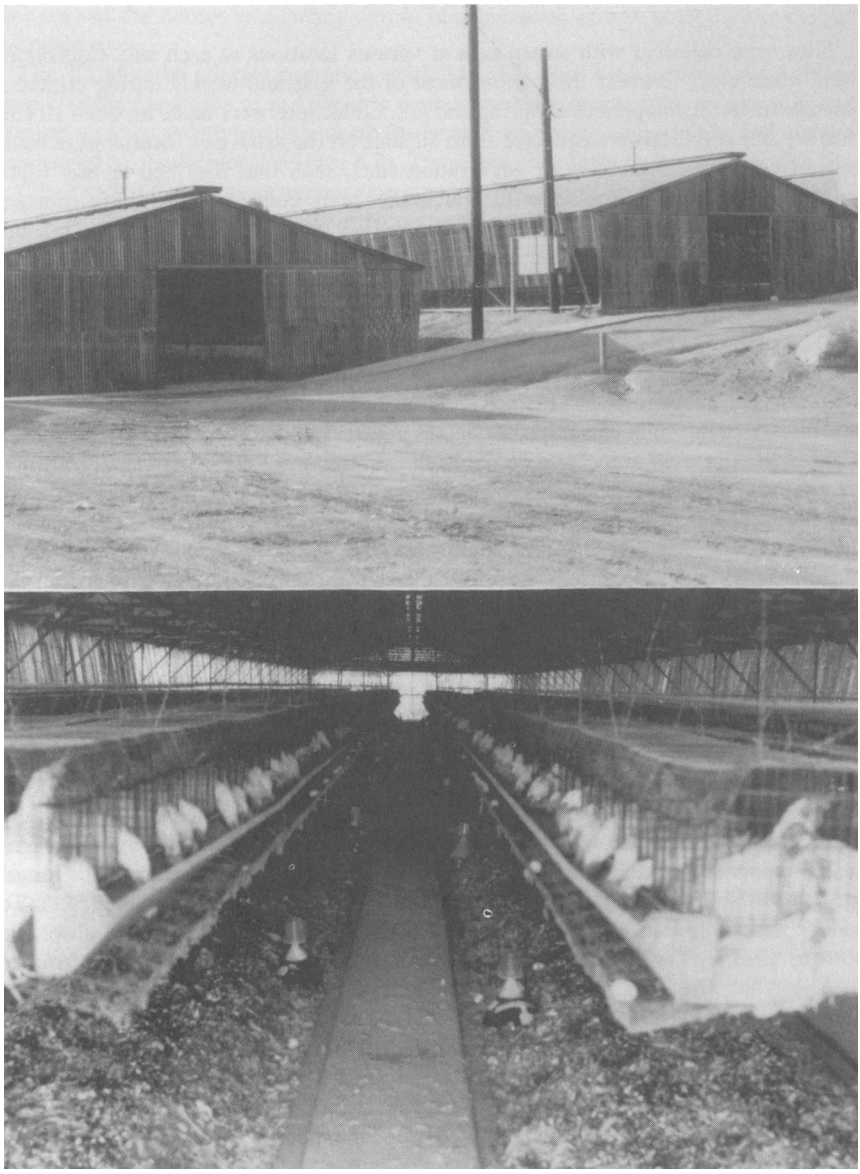

Fig. 3. Lohr study site showing open house construction, suspended cages, and emergence traps at edge of manure accumulations.

held as a group were killed and sorted as usual, to compare their mortality with that of the individually held flies over the same period of time. The latter group also was held an additional 5 days (12 days total) to allow any remaining patent infections to be detected.

Attempts to Infect Larvae

Larval *M. domestica* were exposed to high levels of *E. muscae* conidia in 9-cm plastic petri dishes. Two larval ages were used: third-instar larvae within 1 to 2 days of pupation (mostly ceased feeding) and third-instar larvae within 2 to 3 days of pupation (still feeding). Ten larvae were placed in each dish.

Older larvae (n = 110) were placed into 11 dishes. Larvae in three of these dishes were allowed to pupate in the bare dish (controls). Larvae in the remaining dishes were placed together with 70 *M. domestica* cadavers killed by *E. muscae.* Six of the eight treatment dishes contained only larvae and cadavers, while two also received a wet paper towel to ensure saturated conditions. After exposure to *E. muscae* conidia, pupae from half of the treatment dishes were surface sterilized with a 0.5 percent solution of sodium hypochlorite for 1 minute. All pupae were removed from the petri dishes and held separately (by treatment) for adult fly emergence in 237-ml cardboard cartons with mesh lids. Flies were given free access to a water wick and dried milk and sugar, and were held at 22 ± 2°C for 14 days after emergence. Adults were observed daily for signs of infection.

The young larval group (n = 60) was held in six dishes. A thin layer (2 mm) of rearing medium was scattered on the bottom of each dish. Half the dishes were held as controls; the other half received 70 *M. domestica* cadavers killed by *E. muscae.* Larvae in all dishes were covered with moist paper towels to ensure saturated conditions. Pupae were separated as before but were not surface sterilized. Adults were held and observed as described above.

Emergence Trap Collections

As some species of Diptera may become infected with *E. muscae* when emerging from soil (Carruthers, Haynes, and MacLeod 1985), we decided to examine this possibility for *M. domestica* and *Fannia* spp. emerging from chicken manure in the field. For 3 consecutive weeks in April and October 1983, 40 emergence traps were placed arbitrarily at 3-m intervals on manure in natural fly pupation sites along the center and peripheral walkways at Lohr (fig. 3). Each trap covered a circular surface area of 661 cm^2. Care was taken not to disturb the manure surface directly beneath the traps, which were left in place for 4 days. A piece of fresh potato was placed in the collecting head of each trap as a source of food and water for emerging flies. After 4 days, flies were taken to the laboratory. Flies from each trap were held separately at 22 ± 2°C in clean, 237-ml cardboard containers with nylon mesh tops, and were supplied with a water wick and dried milk and sugar. The *M. domestica* were held for 12 days and the *Fannia* spp. for 19 days. They were observed daily during this time and were sorted as mentioned earlier after freezing on day 12 or 19.

Cadaver Distribution and Fly Activity

Studies were conducted at Lohr and K2 during fall 1984, when *M. domestica* infection rates were high. Visual searches of the chicken houses and nearby vegetation were made several times at Lohr to identify cadaver attachment sites. More systematic and intense observations were made at K2 on the mornings of November 1, 14, 16, and 20 in 1984.

The south wall of the K2 site was divided from the west end into 15 linear sections of 1 m each, with 2 m between sections. Within each 1-m linear section, three regions were examined for fly cadavers killed by *E. muscae.* Ground vegetation within 1.5 m of the base of the wall was carefully examined. The wall itself was divided into two regions—lower ($\leq$ 2 m) and upper (2-3.5 m). The lower region included chicken wire near the ground and the lower section of reinforced plastic curtain, as well as part of the upper section of curtain. The upper region included the upper curtain section and chicken wire up to the roof. Observations also were made of the lower surface of the eaves. On November 14 similar observations were made in 10 linear sections of 1 m each. On this date, however, the inside south wall was searched in the same linear 1-m sections, and the inside rafters were examined in these 1-m sections up to the first crossbeam. The outside north wall also was examined. On November 16 cursory observations were made on the outside south and north walls, and counts were made in ten sections of the outside east and west walls as described earlier. Cadavers were counted and categorized as fresh (cadavers still moist, swollen, and flexible) or old (cadavers dried out but still attached) and recorded. Searches of vegetation also were made around the house in adjacent trees and ground vegetation on November 16 and 20 in conjunction with fly activity studies.

Diel patterns of fly activity were studied on November 16 and 20 at K2, with emphasis on fly aggregation on the south wall. The first day was cloudy; November 20 was a more typical, sunny day. Ten sections, 0.3 $\times$ 0.3 m, were outlined on the curtain at a 2.0 m-height. From 7 a.m. to 4 p.m., the number of *M. domestica* resting in each square was counted at 30-minute intervals. After the 10 counts were taken for each time interval, temperature and humidity were taken with a battery powered psychrometer at a 1-m height and 1 m away from the south wall. Observations were made on fly activity on the manure surface beneath the hens and on relative fly aggregation on the ceiling inside the house.

Statistical Analysis

This study was primarily descriptive in nature, but many comparisons were made with chi-square analyses to determine whether observed infection levels differed by site, host sex, species, etc. Correlation analyses were used to compare fluctuations in infection levels between host species through time. Stepwise multiple regression analysis was utilized to attempt to determine the relationship of infection levels at Lohr to various independent variables. These included average weekly maximum, minimum, and average temperatures; average weekly minimum relative humidity, and relative adult fly density according to the weekly sticky tape counts. Separate analyses were conducted for the three host species, *M. domestica, F. canicularis,* and *O. aenescens.* Stepwise multiple regression was conducted with the SAS statistical analysis package (SAS 1982) using the option Maxr (maximum r^2 improvement).

RESULTS

Assessing Infection Rates

The validity of the holding technique for assessing *E. muscae* infection in *M. domestica* was demonstrated in experiments done during the fall 1984 epizootic period. Consistent with previous observations in the group holding cartons, flies held individually usually died from 4 to 7 days postcapture (fig. 4). A few died as early as day 2 or as late as day 11. For the individually held flies killed by *E. muscae,* 97 percent of the males and 88 percent of the females (94 percent total) died by day 7, the normal holding period. Comparative data for the flies held as a group and those held individually are presented in table 1. Pooled estimates for the two sites were not significantly different ($P > .05$) for flies held individually and those held as a group for the 7-day interval, though a relatively higher proportion of flies seemed to die from *E. muscae* in the individually held flies captured at K2. The 12-day totals were slightly higher due to inclusion of the low number of flies dying of the infection between day 7 and day 12.

Dissections of these flies dying without external signs of mycosis showed infection trends very similar (within 1 to 2 percent) to those for flies in which only patent infections were included (table 1). Thus, it appeared that infected flies did not die without signs in numbers high enough to alter estimates of infection based on live flies and patent infections alone.

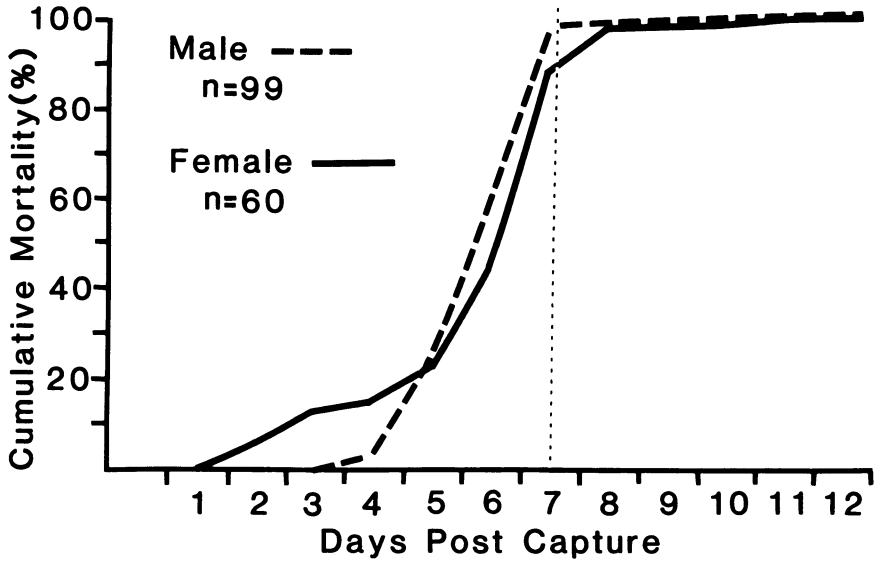

Fig. 4. Cumulative percentage of *E. muscae* cases in *M. domestica* according to time postcapture.

TABLE 1. MORTALITY IN FIELD-COLLECTED *M. DOMESTICA* DUE TO *E. MUSCAE* ACCORDING TO DURATION AND METHOD OF HOLDING FLIES POSTCAPTURE (NOV. 21 AND DEC. 4, 1984)

Site	Holding method*	% Patent *E. muscae*†				% All *E. muscae*‡			
		n	♂	♀	Total	n	♂	♀	Total
Lohr	7-day group	538	22	27	24	562	22	28	25
	7-day individual	138	22	27	25	172	25	27	26
	12-day individual	138	22	31	27	172	26	31	28
K2	7-day group	692	64	53	62	769	65	56	62
	7-day individual	162	74	69	72	198	71	72	71
	12-day individual	162	76	77	77	198	73	78	75
Both	7-day group	1230	49	38	45	1331	50	40	46
	7-day individual	300	54	44	50	370	52	47	50
	12-day individual	300	56	51	54	370	54	52	53
χ^2	Individual vs. group (7-day)		2.55 ns§				1.71 ns		

*Held together in 3.8-L carton (group) or separately in 22-ml cups (individual).

†% Patent *E. muscae* = Patent cases/(live + patent cases) × 100.

‡All *E. muscae* = Patent cases + dead with internal fungus/(live + patent cases + all other dead) × 100.

§ns = not significant ($p > 0.05$).

Group-held flies did die regularly without signs of *E. muscae* infection, and fly deaths due to unknown causes are included for *M. domestica* in figure 5. In general, there was marked variability from week to week and among sites. Insecticides used were dimethoate (Cygon) spray (used irregularly at Lohr as a spot-treatment larvicide and as a residual application) and methomyl fly bait (Golden Malrin). At the LL site pyrethrins were used (every 1 to 3 days) for adult fly knockdown and tetrachlorvinphos + dichlorvos (Ravap) was used as a larvicide and residual spray. Methomyl bait was used in bait stations outside the houses at K1. The K2 ranch occasionally used bait stations, but generally used only a very few sprays of naled (Dibrom). In many cases, fly deaths from unknown causes were associated with insecticide use, as in the 1983 naled spray at K2 or the use of fresh methomyl fly bait at K1 in 1984 (fig. 5). Deaths in the unknown causes category averaged 5 percent of total flies collected, and rarely exceeded 10 percent overall, though occasionally up to 15 to 20 percent of the *M. domestica* died of unknown causes. These deaths tended to be more prevalent in late summer and early fall, when field temperatures were at a maximum and fly populations (and insecticide use for *M. domestica*) were high. Deaths in this category for the other fly species also were irregular and varied from week to week and among sites. Dead flies rarely were dissected, but some did contain fungal fragments and likely were killed by *E. muscae.* It was not feasible, however, to dissect all flies on a regular basis. The markedly irregular percentage of deaths from unknown causes tended to be low and was related much more to other stress factors (such as insecticides, weather) than to seasonal activity of *E. muscae.* Data in table 1 support this view. Because of this, data on seasonal infection rates were calculated from the live and patently infected flies only.

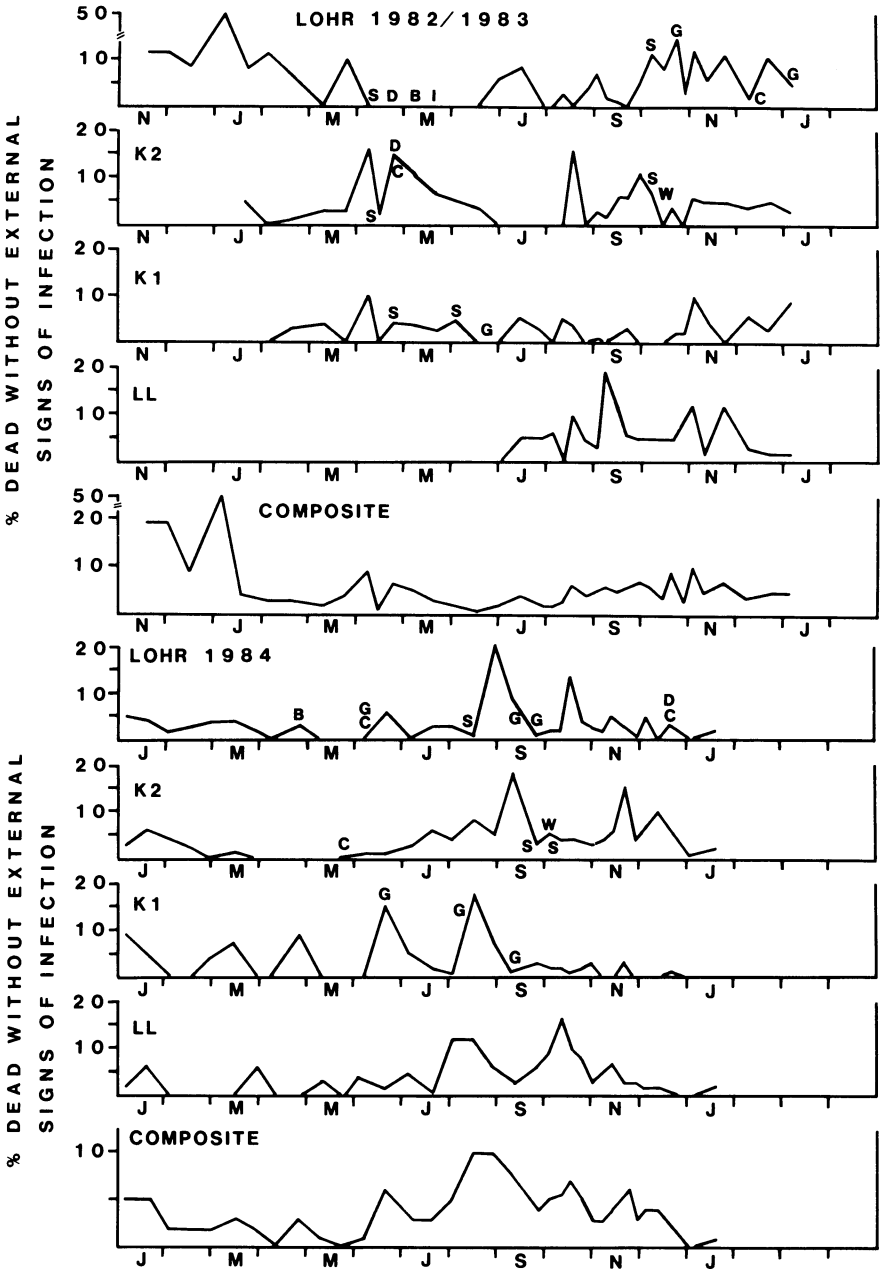

Fig. 5. Percentage of *M. domestica* that died of unknown causes in collections at four poultry ranches. Letters designate producer management practices as follows: S = known insecticidal spray dates (dimethoate at Lohr, naled or nicotine sulfate at K1 and K2), D = birds removed, B = feathers burned from cages and structures, I = new birds into house, C = manure clean-out, G = methomyl bait applied, W = weeds mowed. See figure 2 for site designations.

General Seasonal Trends in Infection Rates

The muscoid fly species collected and categorized as live or infected (patent infections only) were as follows: *M. domestica,* 33,691; *F. canicularis,* 8,297; *O. aenescens,* 3,793; *Muscina stabulans,* 638; *F. femoralis,* 532; and *S. calcitrans,* 49. None of the *M. stabulans* or *S. calcitrans* was infected. Of the *F. femoralis,* 8/308 (2.6 percent) were infected in 1983, and the only infections were found in March, April, and June. No infected *F. femoralis* (0/224) were collected in 1984. Of the many thousands of flies (all species) collected on the sticky tapes during the course of the study, less than five had died with patent signs of *E. muscae* infection.

Of the four poultry operations, the Lohr ranch was the only one that regularly had large numbers of *O. aenescens,* and most of the *F. femoralis* also were captured there. Coupled with large reproducing populations of *M. domestica* and *F. canicularis,* this site was the best for examining *E. muscae* in all four muscoid fly hosts. Even here, however, collections of *F. femoralis* were small; thus, we have concentrated on *M. domestica, F. canicularis,* and *O. aenescens.*

Infections in *M. domestica* were at a fairly high level (40 to 50 percent) when studies were initiated at Lohr in fall 1982 (fig. 6*a*). Adult populations were declining by the time sampling was initiated, and infections dropped below 10 percent by January 1983. With the exception of a small June peak (the single large Lohr peak in late May likely was an artifact due to a small sample size), infections remained at a low, but detectable, level until mid-September. Infection levels were moderate (15 to 35 percent) until November, when they began to decline. *Musca domestica* populations were high from July through October, when they began to decline as well. This basic pattern was similar for all four sites in both years (fig. 6*a* and *b,* 7*a-d,* 8*a-d*). Patent infections were detectable, but remained below 10 percent, during most of the year. *Musca domestica* populations developed to high levels in mid-late summer, 1 to 3 months before the fungus became active. In general, *E. muscae* was present, but at infection levels well under 1 percent during the hot summer months. Infection levels were maximal in October, November, and December. The average infection level at the four poultry ranches reached a maximum of 45 percent for both years (fig. 8*c, d*).

The situation for *F. canicularis* differed markedly. This species was most prevalent in late spring (April and May); *M. domestica* was distinctly a summer and fall pest (fig. 8*c, d*). Populations of *F. canicularis* were at very low levels in summer and fall, but began to increase during winter (January and February). Infection with *E. muscae* was common in 1983, and the average for the four sites reached a maximum of 40 to 45 percent in May (fig. 8*c*). This was 3 to 4 weeks later than the average peak adult population levels for that year. In 1984, infections in *F. canicularis* were practically nonexistent—only two infected flies were found. Populations in 1984 were markedly less than in the previous year, as was rainfall. This will be discussed later.

Both *E. muscae* infections and adult tape counts fluctuated considerably for *O. aenescens,* which were captured regularly only at Lohr (fig. 6*c, d*). In general, populations of this species were highest in spring and fall, lowest in late summer. Both adult populations and infections with *E. muscae* showed three peaks—one in mid- to late winter (February and March), one in early summer (June), and one in fall (October and November). Infection prevalence was high at times for this species, and all of the individuals in a few collections died with patent signs of *E. muscae.* As was the

(Continued on page 21)

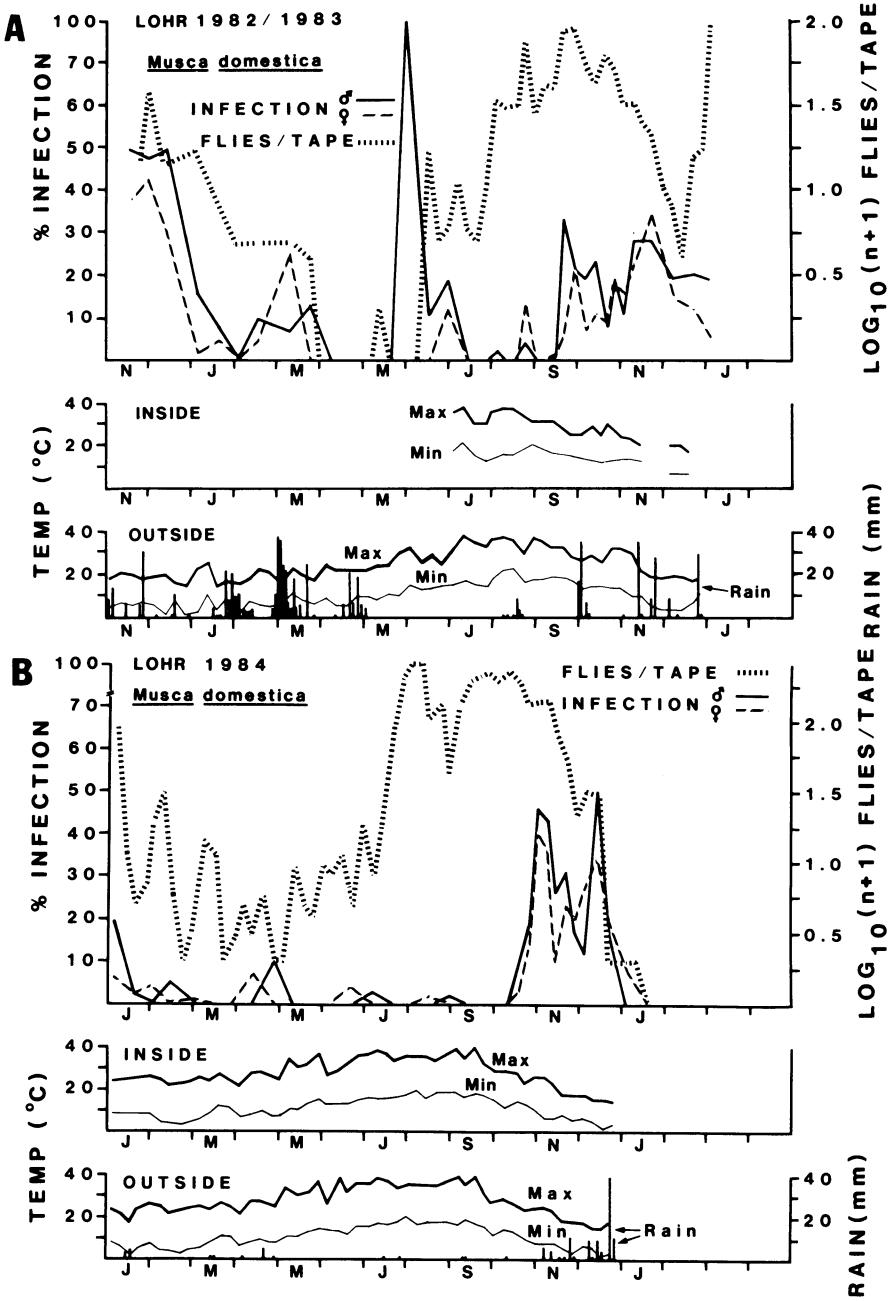

Fig. 6. Seasonal sticky tape counts and *E. muscae* infection data for *M. domestica* at the Lohr egg ranch for 1983(*A*) and 1984(*B*), with weekly average inside hygrothermograph temperatures and outside temperature and rainfall data from the fire station (see figure 1). This figure continues on page 16.

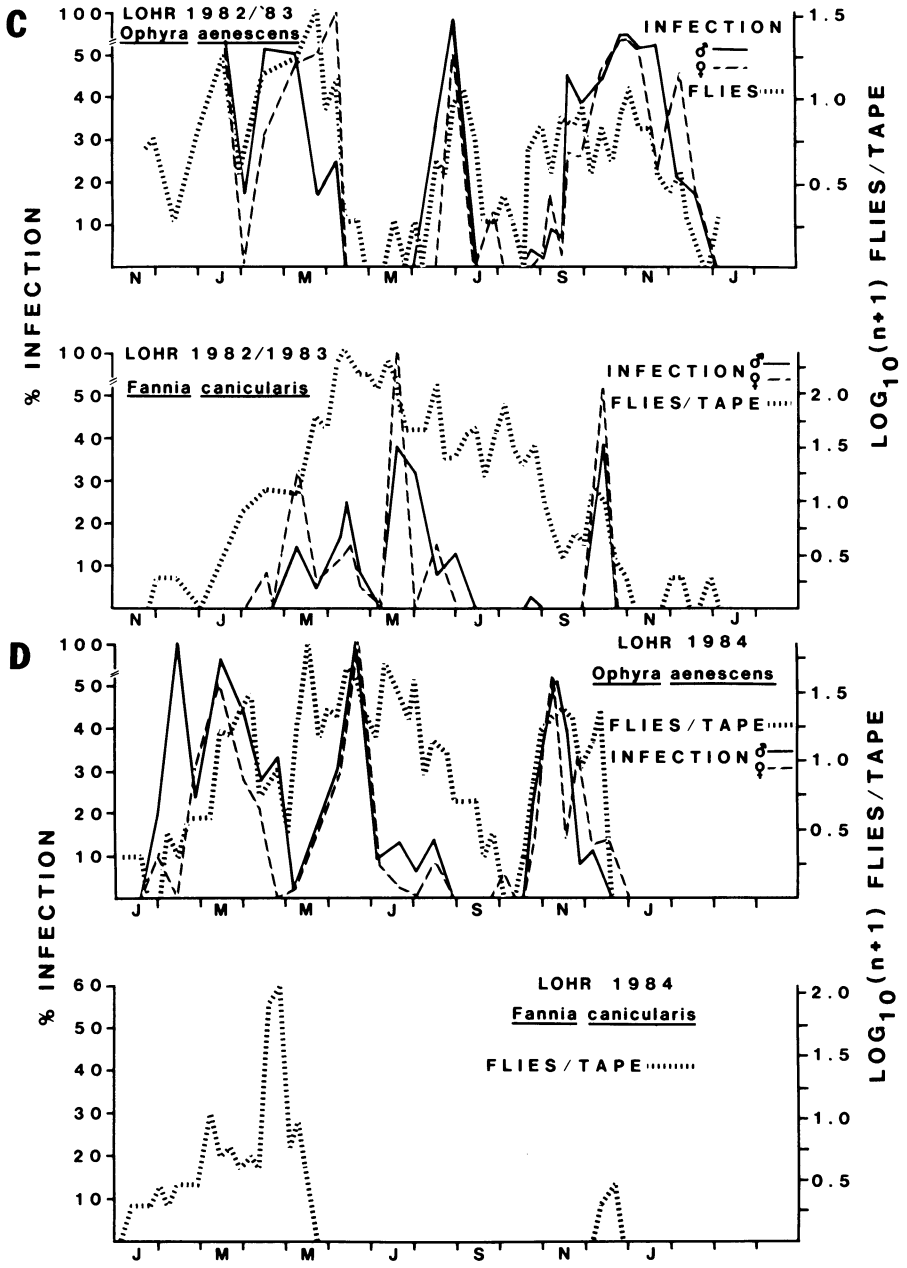

Fig. 6 (cont.). Seasonal sticky tape counts and *E. muscae* infection data for *O. aenescens* and *F. canicularis* for 1983(*C*) and 1984(*D*) at the Lohr egg ranch. Refer to prior page for temperature and rainfall data.

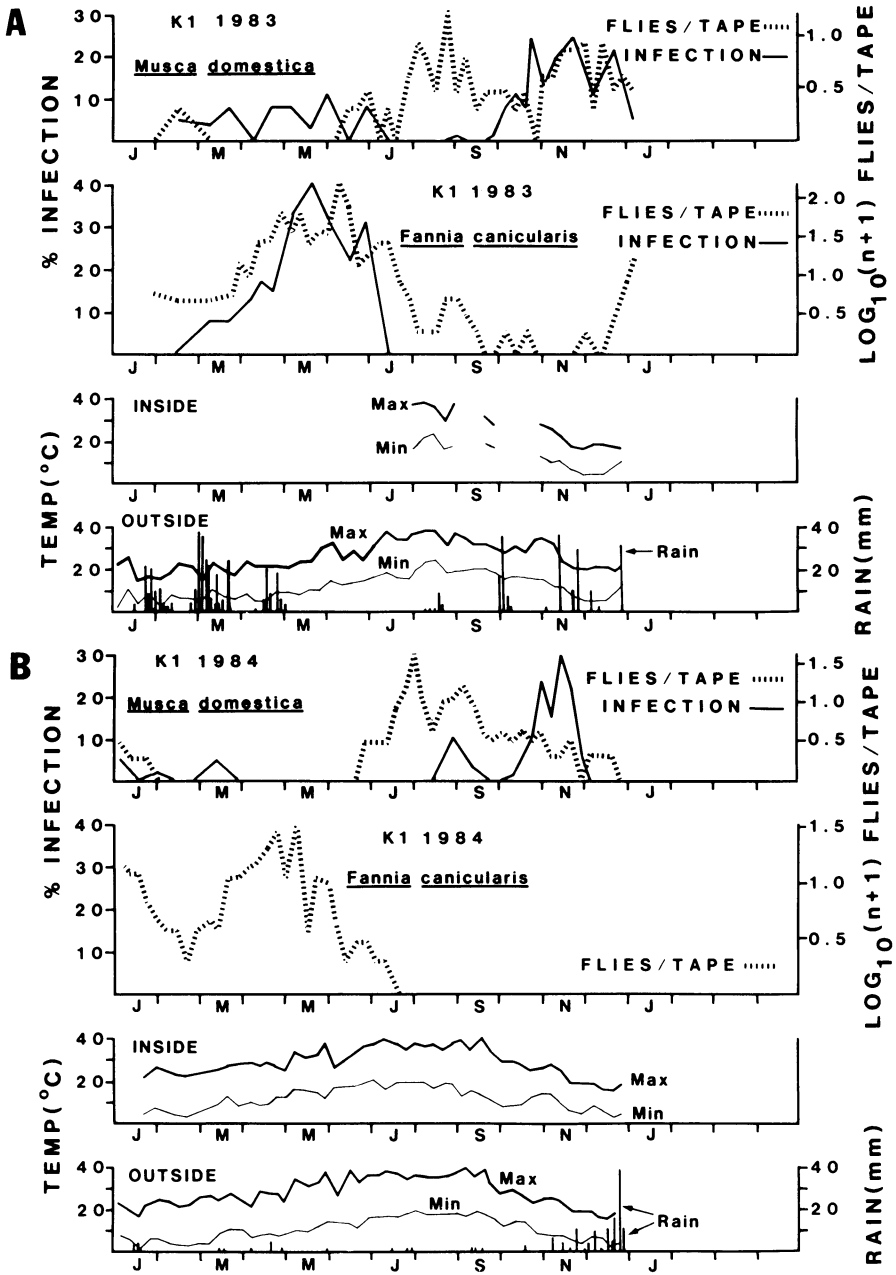

Fig. 7. Seasonal sticky tape counts and *E. muscae* infection data for *M. domestica* and *F. canicularis* for 1983(*A*) and 1984(*B*) at the Kramer 1 egg ranch, with weekly average hygrothermograph temperatures and outside temperature and rainfall data from the fire station. This figure continues on page 18.

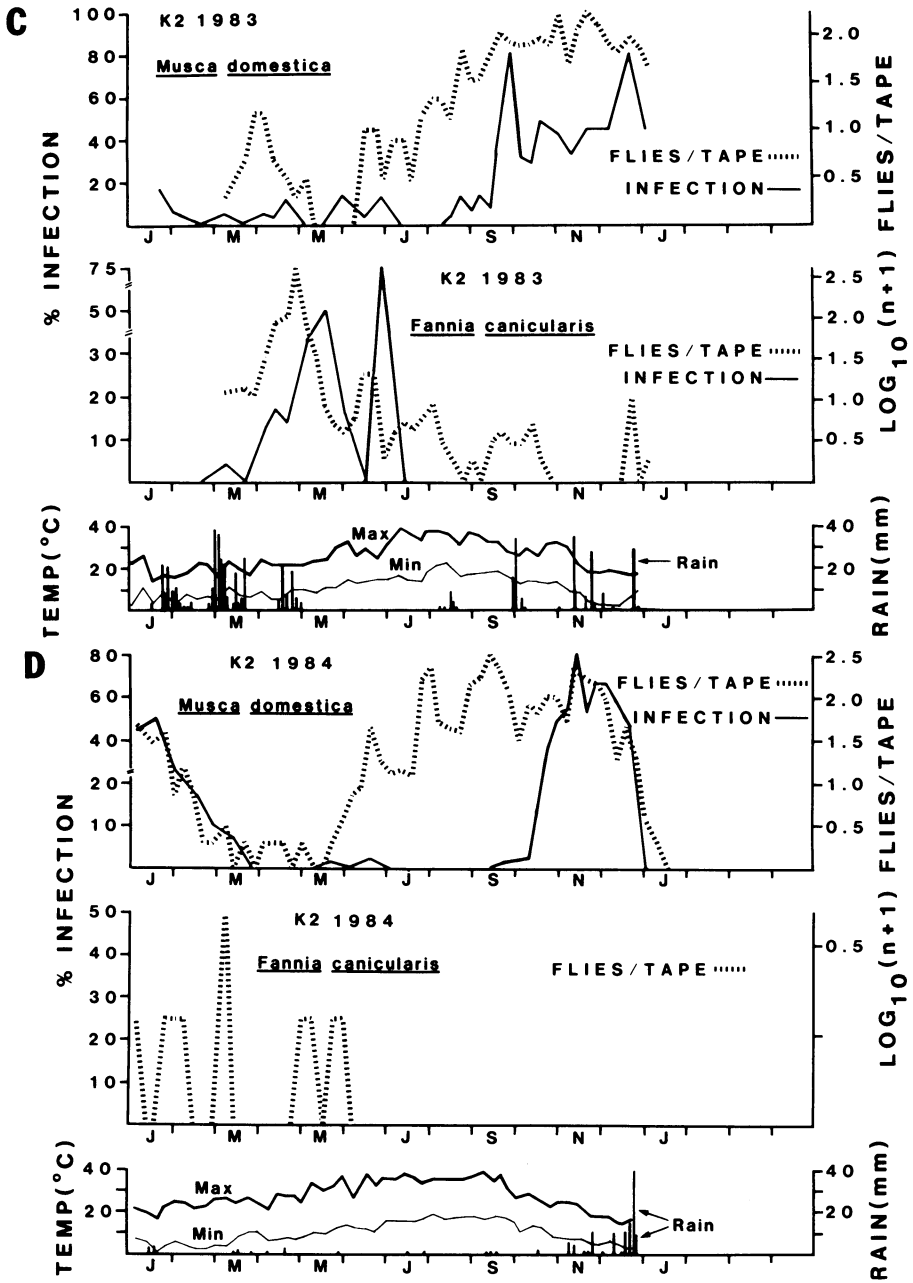

Fig. 7 (cont.). Seasonal sticky tape counts and *E. muscae* infection data for *M. domestica* and *F. canicularis* for 1983(*C*) and 1984(*D*) at the Kramer 2 egg ranch, with outside temperature and rainfall data from the fire station.

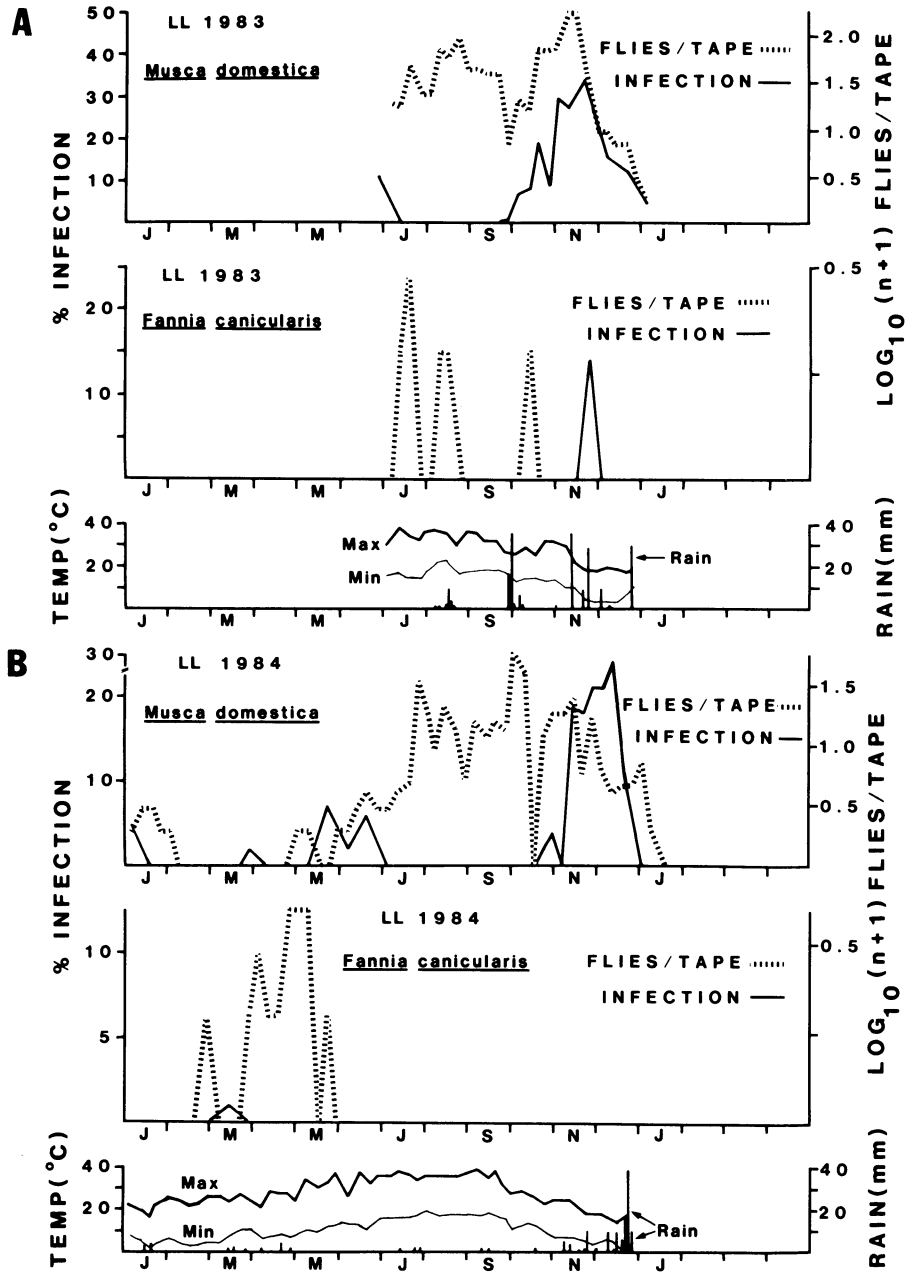

Fig. 8. Seasonal sticky tape counts and *E. muscae* infection data for *M. domestica* and *F. canicularis* for 1983(*A*) and 1984(*B*) at the Loma Linda University egg ranch, with outside temperature and rainfall data from the fire station. This figure continues on page 20.

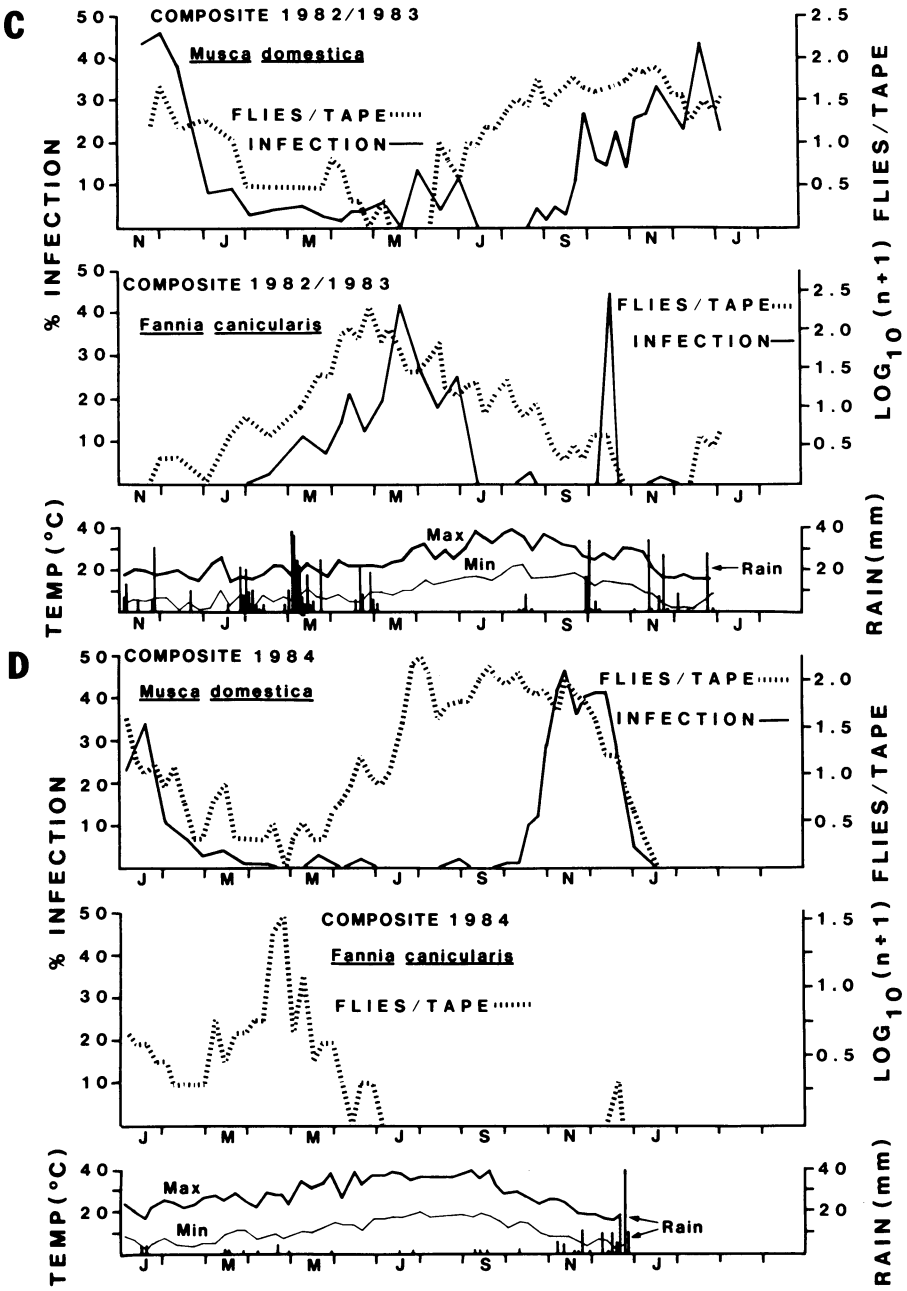

Fig. 8 (cont.). Seasonal sticky tape counts and *E. muscae* infection data for *M. domestica* and *F. canicularis* in 1983(*C*) and 1984(*D*) from the four ranches combined, with outside temperature and rainfall data from the fire station.

(Continued from page 14)
situation with *M. domestica,* infected individuals of *O. aenescens* could be found at any time of the year, but were rarely found in mid- to late summer.

The numbers of flies examined for each host species (by sex) at each study site, and the yearly average infection percentages are presented in table 2. Several general aspects of *E. muscae* infection merit separate consideration.

TABLE 2. YEARLY AVERAGE *ENTOMOPHTHORA MUSCAE* INFECTION LEVELS IN MUSCOID FLY POPULATIONS ON SOUTHERN CALIFORNIA POULTRY RANCHES

	Poultry ranch (no. collected/% infected)				
Host	Lohr	K1	K2	LL*	Total
1983 (33 collecting dates)					
M. domestica	♂1543/12.5	2303/ 8.1	3228/33.5	1986/12.0	9060/18.8
	♀1436/10.8	843/ 8.9	1738/30.0	2076/ 8.6	6093/15.3
Total	2979/11.7	3146/ 8.3	4966/32.3	4062/10.3	15153/17.3
F. canicularis	♂1501/15.1	2475/16.8	902/20.6	61/16.4	4939/17.0
	♀ 818/ 5.7	383/ 8.6	103/13.6	2/ 0.0	1306/ 7.2
Total	2319/11.8	2858/15.7	1005/19.9	63/15.9	6245/14.9
O. aenescens	♂ 675/29.6	189/25.9	130/34.6	4/ 0.0	998/29.5
	♀ 690/31.2	60/28.3	75/10.7	6/16.7	831/29.0
Total	1365/30.4	249/26.5	205/25.9	10/10.0	1829/29.3
1984 (31 collecting dates)					
M. domestica	♂2235/14.9	2083/ 5.6	4624/30.3	2048/ 8.1	10990/18.4
	♀2604/11.3	957/ 5.9	2355/25.9	1632/ 4.6	7548/14.2
Total	4839/13.0	3040/ 5.7	6979/28.8	3680/ 6.5	18538/16.5
F. canicularis	♂ 319/ 0.0	1615/ 0.0	112/ 0.9	419/ 0.2	2465/ 0.1
	♀ 205/ 0.0	478/ 0.0	35/ 0.0	69/ 0.0	787/ 0.0
Total	524/ 0.0	2093/ 0.0	147/ 0.6	488/ 0.2	3252/ 0.1
O. aenescens	♂ 789/24.7	114/10.5	77/11.7	7/ 0.0	987/21.9
	♀ 899/17.0	25/20.0	49/ 2.0	4/ 0.0	997/16.2
Total	1688/20.6	139/12.2	126/ 7.9	11/ 0.0	1964/19.1

*Not monitored until June 1983.

Infection Levels among Sites and between Years

Table 3 presents the pairwise comparisons of *E. muscae* infection data for the four poultry ranches. Average *E. muscae* infection levels in *M. domestica* were higher at K2 than at any other site in both 1983 (32 percent) and 1984 (29 percent). In descending order, *M. domestica* infections were next most prevalent at Lohr, LL, and K2 during both years. Infections in *F. canicularis* also were highest in 1983 at K2 (20 percent), followed by K1 (16 percent) and Lohr (12 percent). Too few *F. canicularis* or *O. aenescens* were captured at LL to include that ranch in the individual site comparisons for these species. In 1983 infections in *O. aenescens* were not significantly different for Lohr, K1, or K2. In 1984, however, Lohr had the highest average *O. aenescens* infection rate (21 percent); K1 and K2 did not differ significantly.

Overall, 1983 had considerably more *E. muscae* activity than did 1984. With the exception of infections in *M. domestica* at Lohr, average yearly *E. muscae* infection

rates were significantly higher in 1983 in both *M. domestica* and *O. aenescens* populations at each site. Differences were much less marked in *M. domestica* (3 to 5 percent at all sites except Lohr) than in *O. aenescens* (10 to 18 percent at all sites except LL. See table 2). In *F. canicularis,* yearly differences were extreme, with an average 1984 infection rate of only 0.1 percent (versus 15 percent in 1983).

TABLE 3. YEARLY AVERAGE *E. MUSCAE* INFECTION LEVELS AND STICKY TAPE COUNTS AT FOUR POULTRY RANCHES IN SOUTHERN CALIFORNIA

	Lohr		K1		K2		LL	
Host species	% Infection	(flies/tape)	% Infection	(flies/tape)	% Infection	(flies/tape)	% Infection	(flies/tape)
1983								
M. domestica	11.7 b*	(18.2)	8.3 c	(10.9)	32.3 a	(42.9)	10.3 b	(39.8)
F. canicularis	11.8 c	(45.0)	15.7 b	(13.6)	19.9 a	(19.2)	NC†	
O. aenescens	30.4 a	(5.5)	26.5 a	(1.4)	25.9 a	(3.8)	NC	
1984								
M. domestica	13.0 b	(68.3)	5.7 c	(2.4)	28.8 a	(52.8)	6.5 c	(8.2)
O. aenescens	20.6 a	(13.0)	12.2 b	(0.4)	7.9 b	(1.7)	NC	

*Infection percentages within the same row followed by the same letter are not significantly different by pairwise χ^2 comparisons ($P>.05$).

†NC = not calculated due to low numbers (occurred rarely on tapes).

Infection Levels among Species

Pairwise comparison statistics of yearly average infections in the three fly species are presented in table 4. In 1983, yearly average infections in *M. domestica* were not significantly different from *F. canicularis* overall, and at Lohr the two species had nearly identical average levels of infection (12 percent). At K1 *M. domestica* had a significantly lower infection level (8 percent) than did *F. canicularis* (16 percent); at K2 *M. domestica* had a significantly higher level of infection (32 percent) than did *F. canicularis* (20 percent).

In 1983 *O. aenescens* had a higher overall infection level (29 percent) than *M. domestica* (17 percent) or *F. canicularis* (15 percent). This trend persisted in 1984;

TABLE 4. χ^2 VALUES FOR PAIRWISE COMPARISONS OF *E. MUSCAE* INFECTION LEVELS (YEARLY AVERAGE—SEE TABLE 2) BETWEEN MUSCOID FLY SPECIES

Year	Species comparison	Lohr	K1	K2	LL	Total
1983	M.d. versus F.c.*	1.74 ns†	76.77	60.95	NC‡	1.02 ns
	M.d. versus O.a.	191.34	76.29	3.76 ns	NC	210.35
	O.a. versus F.c.	209.49	15.44	3.65 ns	NC	183.30
1984	M.d. versus O.a.	58.50	10.12	26.54	NC	6.99

*M.d. = *M. domestica,* F.c. = *F. canicularis,* O.a. = *O. aenescens.*

†All values significantly different (χ^2, $P<0.05$) unless marked ns (not significant).

‡NC = not counted due to low numbers captured.

overall differences were significant for *O. aenescens* (19 percent) versus *M. domestica* (17 percent), especially at Lohr (21 percent for *O. aenescens* and 13 percent for *M. domestica*), which had the largest and most stable population of *O. aenescens.*

For dates when both species were captured at Lohr, infection levels for *M. domestica* and *O. aenescens* were significantly correlated in both 1983 ($r = .657$, $n = 22$, $P < .01$) and 1984 ($r = .469$, $n = 27$, $P < .02$). Conversely, infection rates in *F. canicularis* in 1983 were positively but not significantly correlated with infections in *M. domestica* ($r = .384$, $n = 18$, $P > .05$) or *O. aenescens* ($r = .453$, $n = 13$, $P > .05$).

Infection Levels for Males versus Females

There was a general trend for infection rates to be higher for males of *M. domestica* and *F. canicularis;* infections for *O. aenescens* tended to be similar for both sexes (table 2 and fig. 6*a-d*). K1 actually had slightly higher infection rates in females of *M. domestica,* but the differences were not significant (table 5). In general, differences were significant in *M. domestica* at the other sites, and males had apparent infection rates 3 to 4 percent higher overall than did females of this species. Comparisons for *F. canicularis* could be done only for 1983 (due to the absence of infections in 1984), but also generally showed significantly higher (10 percent overall) prevalence of infection in males. In contrast, male infection rates for *O. aenescens* generally were not different from female infection rates in 1983 at any of the sites. In 1984 infections were significantly more prevalent (6 percent overall) in males of *O. aenescens.*

TABLE 5. χ^2 VALUES FOR PAIRWISE COMPARISONS (MALE VERSUS FEMALE HOSTS) OF *E. MUSCAE* INFECTION LEVELS (YEARLY AVERAGE—SEE TABLE 2) IN MUSCOID FLY POPULATIONS

Year	Host species	Lohr	K1	K2	LL	Total
1983	*M. domestica*	2.12 ns*	1.25 ns	6.27	12.80	31.08
	F. canicularis	44.69	16.67	2.86 ns	NC†	78.15
	O. aenescens	0.38 ns	0.07 ns	14.23	NC	0.05 ns
1984	*M. domestica*	13.88	0.07 ns	14.84	18.28	56.66
	O. aenescens	15.21	1.71 ns	3.81 ns	NC	10.39

*Not significant ($P > 0.05$). See table 2 for explanation.

†NC = not counted due to low numbers captured.

Factors Influencing Seasonality of Infection Rates

Multivariable regression statistics for seasonal changes in the infection levels in the three fly species at Lohr are presented in table 6. None of the independent variables examined contributed significantly to infection prediction for *M. domestica,* as will be discussed later. Infection in *O. aenescens,* however, was influenced significantly by several variables. As can be seen in figure 6*c* and *d,* infections in this species tended to be highest when fly densities also were high. This relationship was reflected in the positive coefficient for Tape. Infection in *O. aenescens* was negatively related to MinRH and Maxt; that is, when temperatures were high and minimum humidities were high, infection rates were low in this host. With *F. canicularis* humidity data were not

available for spring 1983, when nearly all infections occurred. The MinRH variable thus was not included in the analysis. No single variable contributed significantly to infection prediction for *F. canicularis.* However, when both maximum and average weekly temperatures were included, infection was less when maximum temperatures were high and average temperatures were low. Obviously, however, these variables were not independent.

Infections in *F. canicularis* were reduced for 1984 versus 1983. Rainfall in 1984 was sparse; 1983 was an exceptionally wet year (fig. 6*c,d*). Lack of rainfall and presence of dry winds resulted in unusually rapid manure drying, and *F. canicularis* populations were 5 to 10 times lower in 1984 (fig. 8*c,d*). Humidity data, unfortunately, were not available for spring 1983, but humidities were likely relatively high and related to rainfall during that period. Rainfall did not appear to be as important in *E. muscae* infections in *M. domestica* and *O. aenescens,* though 1984 infection rates were lower overall than in 1983 for these species (tables 2 and 3).

With regard to *M. domestica* infection, it was useful to examine the infection trends visually, as in figure 8*c* and *d.* Infections with *E. muscae* followed declining fly populations closely from November through April in both 1983 and 1984. Fly populations built rapidly in early summer, reaching high levels from July through October. Average maximum temperatures during summer, however, often exceeded 35°C. Onset of substantial levels of *E. muscae* infection was associated with moderating temperatures (average maximum <30°C) in the early to mid fall months. Infections reached high levels as average maximum temperatures dipped below 25°C.

TABLE 6. MULTIVARIABLE REGRESSION STATISTICS EXAMINED FOR PREDICTION OF PERCENT *E. MUSCAE* INFECTION IN THREE MUSCOID FLY SPECIES AT THE LOHR POULTRY RANCH FOR JANUARY 1983—DECEMBER 1984

Host	Variable*	Variable F when added†	Model r^2	Variable coefficient (b) when added
M. domestica	Mint	2.47 ns	0.066 ns	−0.760
	add Maxt	1.28 ns	0.070 ns	0.333
	add Tape	0.83 ns	0.070 ns	−0.007
	add MinRH	0.00 ns	0.070 ns	−0.005
O. aenescens	MinRH	11.64 (P<.01)	0.250 (P<.01)	−1.086
	add Maxt	8.34 (P<.01)	0.397 (P<.01)	−1.510
	add Tape	4.60 (P<.05)	0.471 (P<.001)	0.618
	add Avet	0.52 ns	0.480 (P<.001)	2.056
	y = 163.8 − 1.313 Maxt + 0.618 Tape − 0.919 MinRH			
F. canicularis‡	Tape	1.64 ns	0.045 ns	0.191
	add Maxt	1.80 ns	0.093 ns	−0.608
	add Avet	6.37 (P<.05)	0.240 (P<.05)	5.496
	y = 94.3 − 5.350 Maxt + 5.496 Avet			

*Mint = average weekly minimum temperature, Maxt = average weekly maximum temperature, Avet = average weekly temperature (Max + Min/2), MinRH = average weekly minimum RH, Tape = average weekly tape count.

†ns = not significant (P>.05).

‡MinRH data not available for spring 1983 and not included in analysis.

Relationship of Other Variables to Infection Rates

Infections at certain sites were influenced by factors other than the biotic and abiotic factors mentioned previously. Insecticide application appeared to affect *E. muscae* infection in a few cases. Use of a residual dimethoate spray and methomyl fly bait at Lohr in October 1983 (fig. 5) may have caused the drop in infection in *M. domestica* (fig. 6*a*), though *O. aenescens* infections were not impacted (fig. 6*c*). At LL, where regular, heavy use of insecticides was employed, infection rates in *M. domestica* did not reach high levels (fig. 8*a,b*) in spite of high host densities.

The most definite response in infection due to pesticide use and habitat alteration was at K2 in early October of 1983. In fall 1983, *M. domestica* infections at most sites were low until early October (fig. 6*a,* 7*a,* 8*a*). At K2, however, infections began to be detected regularly by late August, and an epizootic rapidly developed (fig. 7*c*). By late September, infection rates were in excess of 80 percent. This site was unique in that a lush growth of weeds had developed along the south wall of the house and was over 1 m high in some areas (fig. 9). Growth was due to excessive use of the roof

Fig. 9. K2 egg ranch south wall in August 1983. Note water accumulation at drip line and weed growth.

sprinkler system, which led to standing water under the eaves. Flies aggregated in high numbers in this moist weed growth during the day. In very early October, the producer applied a nicotine sulfate spray inside the house for the northern fowl mite, *Ornithonyssus sylviarum* (Canestrini and Fanzago). Some of this fine spray drifted to the outside of the house, and fly populations were depressed for a few days. This did not significantly affect tape counts, but the infection rate immediately was reduced from more than 80 percent to about 30 percent. About 1 week later the producer also mowed this weed growth, and infection rates did not rise appreciably until mid-December, when fly populations were declining.

Cadaver Distribution during Epizootics

Cadavers of *M. domestica* killed by *E. muscae* could be found in many areas in and around the poultry ranches. At some sites it was difficult to find enough cadavers to explain the high incidence of infection, especially when epizootics were developing. Cadavers were usually found near the houses, but were also found in vegetation at least 30 m away. They were usually on surfaces with a vertical orientation and were most noticeable on suspension wires for the cages, wall surfaces, the tops of grassy vegetation, and strings used to raise and lower the wall curtains. If they were found on nonvertical surfaces, they invariably were attached to the underside, hanging on the bottom of leaves or from rafters or eaves outside, and rarely inside, the houses. As mentioned earlier, cadavers generally were not found on sticky tapes. A study was conducted at K2 in fall 1984 to determine cadaver distribution.

At K2 most *M. domestica* cadavers were found on the south side of the house; east, west, and north sides had few cadavers (table 7). The south side wall had the majority of cadavers, and most were found on the upper region (above 2 m high). Many old cadavers were found (9.1 cadavers/m) along the inside wall (upper and lower portions) of the house, but the majority of fresh cadavers (2.3/m on November 1 and 3.4/m on

TABLE 7. DISTRIBUTION OF *M. DOMESTICA* CADAVERS KILLED BY *E. MUSCAE* AT THE KRAMER 2 POULTRY HOUSE IN NOVEMBER 1984; NUMBERS ARE MEANS OF 10 1-METER SECTIONS (11/14, 11/16) OR 15 1-M SECTIONS (11/1) ALONG WALLS OF HOUSE

		$\bar{x}$ no. of fresh (old) cadavers/linear m			
Date	Wall orientation	Vegetation*	Wall-upper†	Wall-lower†	Rafters‡
11/1	South-outside	0.9 (1.6)	2.0 (2.5)	0.3 (0.8)	Not searched
11/14	South-inside	Not present	0.8 (4.6)	0.5 (4.5)	0.0 (0.2)
	South-outside	0.8 (0.1)	3.0 (2.0)	0.4 (0.5)	Not searched
	North-outside	0.0 (0.1)	0.1 (0.3)	0.1 (0.1)	Not searched
11/16	East-outside	Not present	0.0 (0.3)	0.1 (0.2)	Not searched
	West-outside	Not present	0.0 (0.2)	0.4 (0.1)	Not searched

*Vegetation searched from base of wall to 1.5 m from wall over the 1 linear m span.

†Walls divided into lower (<2 m) and upper (2-3.5 m) regions.

‡Rafters searched from wall to first beam over 1 linear m span (inside only).

November 11) were found on the outside wall, with most on the upper portion of the wall and chicken wire (table 7, fig. 10). Though relatively few *O. aenescens* cadavers were found at K2, they were all in nearby vegetation, attached to stems of grasses or to the undersides of leaves of citrus and avocado trees. They sometimes were found at Lohr or K1 attached to vertical structures on the outside of the houses or to plants.

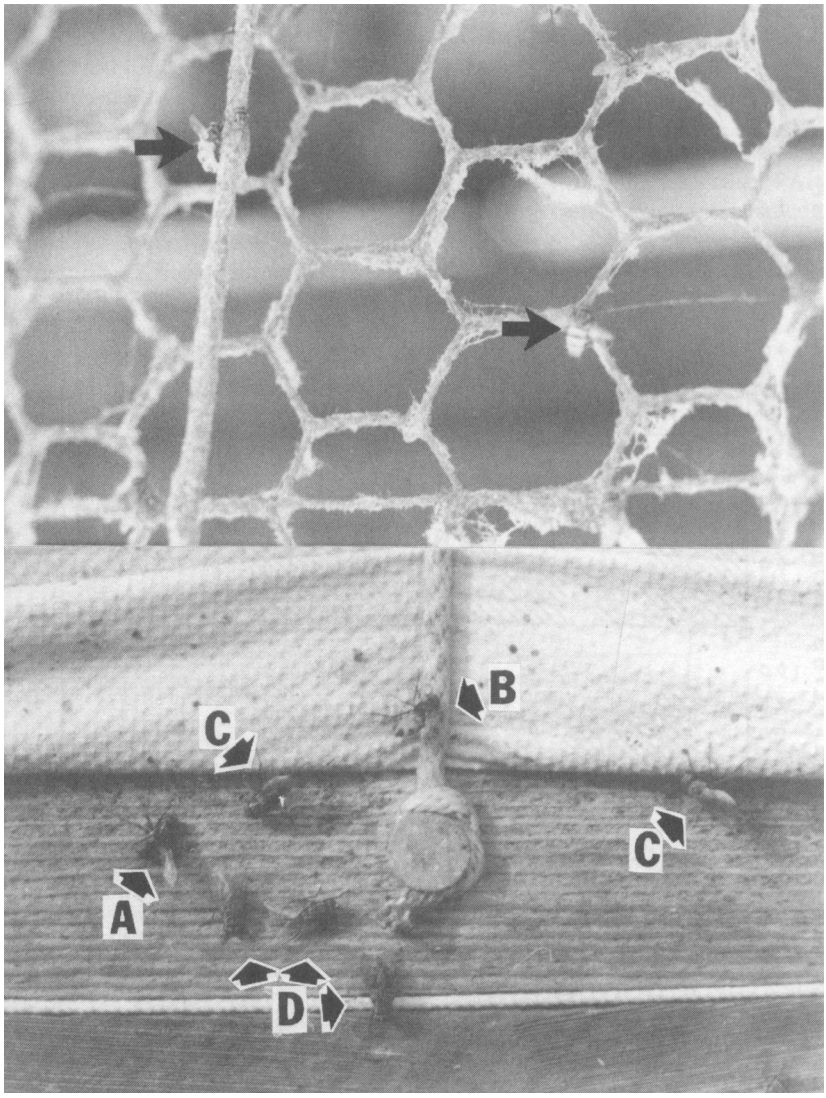

Fig. 10. *Musca domestica* cadavers killed by *E. muscae* at K2 egg ranch during 1984 epizootic. *Upper photo* shows fresh cadavers on curtain rope and chicken wire at top of south wall. *Lower photo* shows old cadaver (*A*), 1-day-old cadaver (*B*), two critically ill flies about to die (*C*) and three normally active flies (*D*) on south wall in late afternoon.

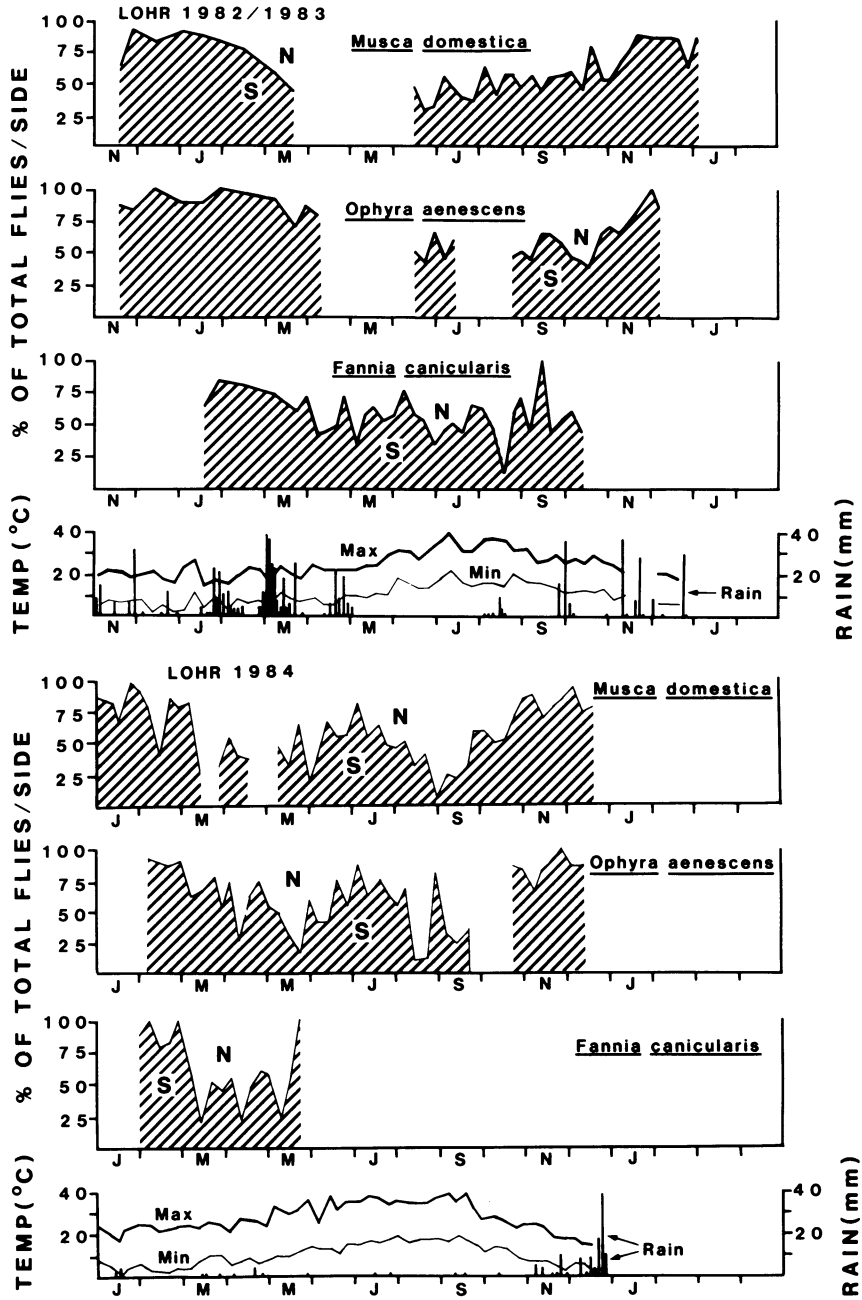

Fig. 11. Seasonal proportion of total flies taken on sticky tapes on south side versus north side of the Lohr poultry house, with outside temperature and rainfall data from the fire station (see figure 1).

Seasonal and Daily Host Aggregation Patterns

Seasonal sticky tape catches at Lohr were plotted according to the percentage of total flies caught on the north or south sides of the house (fig. 11). During the cooler fall and winter months, most flies were caught on the south side. Aggregation on this side began with the onset of cool weather (nocturnal temperatures 10° to 15°C) in October and November. During hot weather, catches were approximately equal on the north and south sides.

Daily movement studies showed that *M. domestica* became active in early daylight hours. They aggregated in sunlight on the south side curtains of K2 for 1 to 2 hours in the morning after air temperatures warmed to 15°C (fig. 12*a,b*). Sunrise and sunset during these studies were approximately 6:30 a.m., and 4:30 p.m., respectively. After this morning aggregation period, flies dispersed and were seen in many areas, including the manure surface, broken eggs, etc. Another aggregation period on the south wall occurred 1 to 2 hours before sunset. After this time flies moved into the chicken houses and spent the night on the undersides of the roof and rafters.

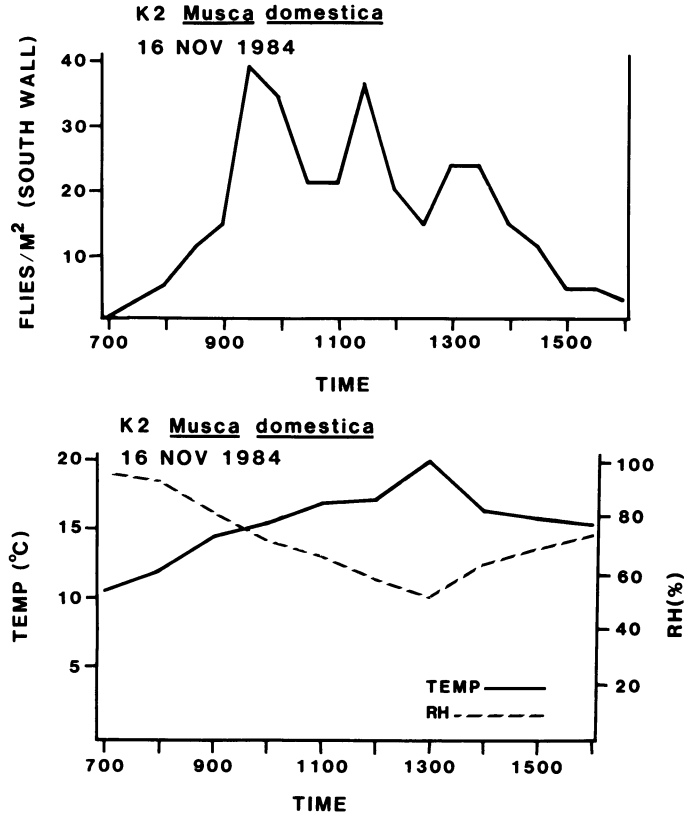

Fig. 12(*a*). *M. domestica*/m² of south wall surface at K2 egg ranch during November 16, 1984 *E. muscae* epizootic.

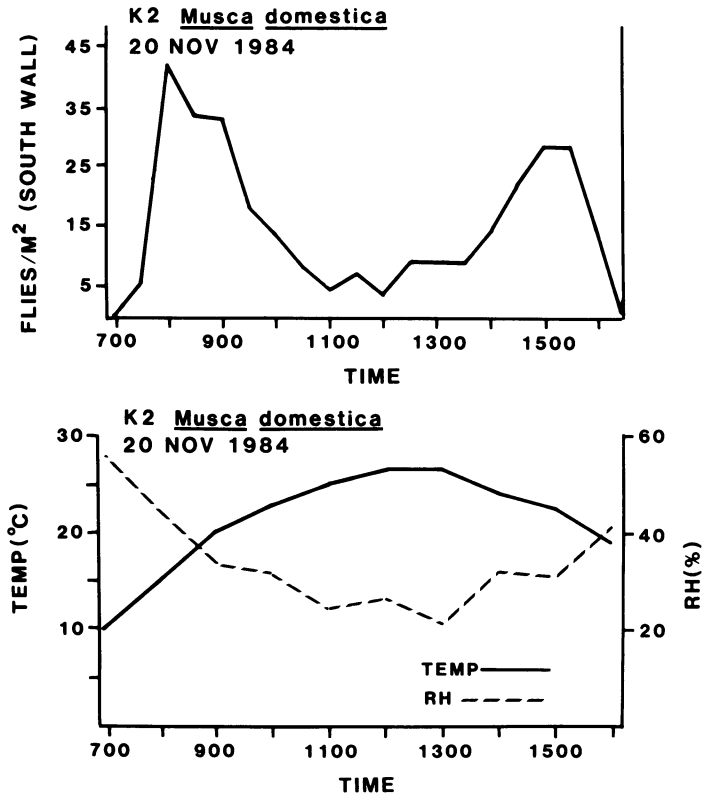

Fig. 12(*b*). *M. domestica*/m² of south wall surface at K2 egg ranch during November 20, 1984 *E. muscae* epizootic.

Infection of Larvae or Emerging Adults

Larvae of *M. domestica* could not be infected with *E. muscae* in these trials (table 8). Larvae were exposed for 24 hours to levels of *E. muscae* conidia well in excess of 50/mm², the exposure required to kill 95 to 100 percent of adults (Mullens 1985). Larvae which likely ingested conidia (and perhaps fed on the moist cadavers) or were merely exposed to high numbers of conidia on the cuticle pupated normally and emerged well (93 percent emergence overall). No adults showed any signs of mycosis, and survival was excellent (99 percent) over the 14-day holding period.

Flies also apparently did not become infected with *E. muscae* while emerging from manure habitats. As can be seen in table 9, emerging *M. domestica* and *Fannia* spp. collected during natural epizootic periods at Lohr did not show any signs of infection after holding for 12 or 19 days. For *M. domestica* and *F. canicularis* the difference in infection between flies collected in traps and by sweep net was highly significant ($P<.01$), with field infection rates 13 to 16 percent for the test period. The difference for *F. femoralis* also was significant ($P<.05$).

TABLE 8. EMERGENCE AND SURVIVAL OF *M. DOMESTICA* EXPOSED AS LARVAE TO *E. MUSCAE*

Larval age at exposure (days before pupation)	Treatment	n	No. pupating*	No. emerging	No. surviving†
1-2	Control	30	30	29	28
	E. muscae	40	39	38	38
	E. muscae + surface sterilization	40	40	33	32
2-3	Control	30	28	27	27
	E. muscae	30	29	29	29

*Total of four larvae escaped before pupation.

†Survival of adult flies for 14 days after emergence.

TABLE 9. FLIES COLLECTED EMERGING FROM CHICKEN MANURE AT LOHR RANCH AND HELD TO ASSESS *E. MUSCAE* INFECTION, 1983

Host	Dates collected	No. flies held (no. days)	No. dead	No. *E. muscae*	% Field infection*	No. infected/ total	χ^2 Emergence vs. field
M. domestica	Oct 12-31	163 (12)	9	0	16.1	(80/498)	29.8†
F. canicularis	April 7-22	791 (19)	154	0	13.3	(109/822)	112.5
F. femoralis	April 7-22	91 (19)	18	0	7.0	(3/43)	6.0

*Assessed by weekly sweep-net collections (see text) for dates shown.

†All χ^2 values significant ($P<.05$).

DISCUSSION

The collection and holding methods reported here to assess *E. muscae* infection were both simple and accurate. Data presented by Wilding and Lauckner (1974) suggested that *L. coarctata* in the later stages of *E. muscae* infection were easier to capture. The authors further stated that the proportion of flies that died later, after a collection, was likely a more accurate index of pathogen activity. Our method of collecting only flies that actually flew when disturbed, and were caught by a rapidly passing sweep net, emphasized this aspect. Critically ill flies were not captured, and, from that viewpoint, our figures may be conservative. The 7-day holding period allowed most infections to be expressed, but also was by necessity conservative to prevent misinterpretation caused by pathogen transmission within the container.

Infection Levels in Males versus Females

Mullens (1985) showed that female *M. domestica,* due to their larger size, took longer than males to succumb to *E. muscae* infection, a fact also suggested by this study (fig. 4). Thus, in light of the conservative holding period, the generally higher rates of *E. muscae* infection in male flies reported here may be an artifact of the differ-

ence in incubation period. The two sexes are not inherently different in susceptibility to *E. muscae* in *M. domestica* (Mullens 1985), though different sexual behaviors may have contributed to a real difference in infection. For example, we often have observed male *M. domestica* attempting to mate with cadavers killed by *E. muscae* in the laboratory (fig. 13). Whether the cues responsible for this behavior are visual, chemical, or tactile have not been determined. Cadavers (male and female) have the legs and wings extended, and the body (including the abdomen) is somewhat elevated. This position is no doubt conducive to the distribution of conidia from the abdomen after host death. Visual stimuli are important in male mating orientation, however, and the "wing-out" position of the female, whether achieved by the male or female, is a prerequisite to copulation (Tobin and Stoffolano 1973); female extrusion of the ovipositor and some elevation of the abdomen also must occur. Female *M. domestica* cadavers killed by *E. muscae* seldom have the ovipositor fully extended, but the elevated abdomen and spread wings of the cadavers may be visually attractive to males. If so, this also would expose males to higher numbers of conidia, which would help explain the higher prevalence of *E. muscae* in males.

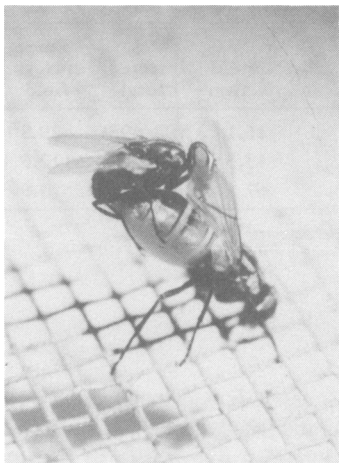

Fig. 13. Male of *M. domestica* copulating with female *M. domestica* cadaver killed by *E. muscae* in the laboratory.

Infections in Immature Flies and the Potential Role of Resting Spores

Larvae in the present experiments were subjected to extreme levels of *E. muscae* exposure, but the pathogen had no effect on pupation, emergence, or adult longevity. Brefeld (1871) correctly determined that larvae were not infected, but confirming experimental data have been lacking. From our studies it was apparent that flies could not be infected by exposure to conidia of *E. muscae* as larvae. Larval and adult cuticle differ markedly. If conidia were able to attach and to penetrate the relatively smooth and moist larval cuticle, it is likely that they were not able to survive. The pathogen resides in the fat body in the early stages of incubation. This tissue is radically altered

during metamorphosis; *Musca* fat body is essentially destroyed and reformed from mesenchyme cells on the inside of the imaginal discs (Chapman 1971).

Though *D. antiqua* (Meigen) can become infected with *E. muscae* while emerging from soil (Carruthers, Haynes, and MacLeod 1985), muscoid flies were not infected when emerging from chicken manure in this study. Manure at these ranches usually is cleaned out at least every 6 months in California. Thus, *E. muscae* would have limited periods to accumulate. The continuous addition of manure to the piles below the chickens also would fairly rapidly bury any conidia or resting spores that landed on the surface, perhaps below the level where they would be contacted by an emerging fly. Most importantly, most of the fly cadavers killed by *E. muscae* in November were found on the outside surfaces of the houses or in outside vegetation, with relatively few cadavers positioned above or near the breeding medium. Thus, the lack of host infection either as larvae or during emergence was not surprising.

Descriptions of *E. muscae* resting spores vary tremendously, at least in terms of their measurements (MacLeod, Müller-Kögler, and Wilding 1976). As mentioned earlier, some workers with experience with this pathogen, notably Brefeld (1871) and Thaxter (1888), did not find forms they considered to be resting spores; others have reported finding them under varying environmental conditions. We did not find forms we considered to be resting spores, though some flies that died without external signs of infection did have mycelial growth and forms that resembled conidia inside their abdomens. The seasonal pattern of host death in the "unknown causes" category (fig. 5) might have been expected to have a late fall "peak" if the numbers of flies producing resting spores increased significantly then. Wilding and Lauckner (1974) documented increased resting spore formation in *L. coarctata* as the population age structure increased late in the season. Also, it has been shown that older *M. domestica* tend to die after exposure to *E. muscae* without producing primary conidia, which implies possible resting spore formation (Mullens 1985). We know of no year-round studies of seasonal fluctuations in *M. domestica* age structure, but data presented by Krafsur (1985) suggest that it increases in very late season, as would perhaps be expected. Though we cannot comment specifically on their role, the lack of circumstantial evidence for seasonal resting spore formation does not eliminate the possible importance of these forms in *E. muscae* epizootiology in southern California. Carruthers, Haynes, and MacLeod (1985) demonstrated that *E. muscae* resting spores in the soil infected considerable numbers of emerging *D. antiqua* and *D. platura* (Meigen), but these authors never observed resting spore production in more than 10 percent of cadavers, regardless of time of year. Resting spores may be significant at times in *E. muscae* epizootiology on poultry ranches, particularly in cases where fly larvae migrate considerable distances, e.g. to the debris below the walls at the margins of the house, to pupate. Nevertheless, our studies have demonstrated that patent *E. muscae* infections occurred year-round in *M. domestica* and *O. aenescens.* It was entirely possible that direct conidial transmission maintained the infection throughout the year in these hosts and that infections increased from this reservoir when favorable conditions prevailed. Brefeld (1908) noted that *E. muscae* infections persisted year-round in Italy and Brazil, which, like southern California, are regions without temperatures low enough to stop fly activity. The situation may have differed in *F. canicularis,* which was far more seasonal in its occurrence. Resting spores may have had a vital role in allowing the pathogen to survive periods when *Fannia* spp. adults were rare.

Interspecific Transmission of *E. muscae*

Interspecific transmission of *E. muscae* could also have a role in the epizootiology. We have shown that four hosts, *M. domestica, F. canicularis, F. femoralis,* and *O. aenescens,* naturally were infected with *E. muscae* on poultry ranches in southern California. However, Keller (1984) has suggested that *E. muscae* is a species complex, and it is known that various muscoid fly species may differ in susceptibility to a particular *E. muscae* strain (Kramer and Steinkraus 1981). In the present study, the lack of infections in *M. stabulans* and *S. calcitrans,* which sometimes were collected in areas of considerable *E. muscae* activity in other Diptera, suggests that they were not susceptible to these strains. Preliminary data suggest that *E. muscae* from the two *Fannia* spp. (subfamily Fanniinae) is not very infective for *M. domestica* and *O. aenescens;* the latter two species (subfamily Muscinae) can transmit the pathogen back and forth to some extent (Mullens, unpublished). Likewise, transmission trials from *M. domestica* to either *Fannia* sp. have failed (Mullens, unpublished). The hypothesis of cross transmission in the field between *M. domestica* and *O. aenescens,* but not between those hosts and *F. canicularis,* was supported by the significant seasonal correlation of infection rates between *M. domestica* and *O. aenescens* and the insignificant correlation between either of these two hosts and *F. canicularis. Fannia* spp. are also active at a different time of year. Chances for interspecific transmission with Muscinae would be minor, and the possibility of *E. muscae* strains specifically adapted to these hosts and environmental conditions seems likely. Too few cases of *E. muscae* infection were noted in *F. femoralis* to conduct statistical analyses, but the general seasonal correlation (spring) in 1983 was good for the two *Fannia* spp. In fact, the infection passes readily between these two hosts in the laboratory (Mullens, unpublished).

Factors Governing Infection in *F. canicularis*

Seasonal infection in *F. canicularis* in 1983 strongly suggested a lagged, density-dependent response (fig. 6*c*). Such a response is common to many Entomophthoraceae (Shands, Simpson, and Hall 1963; Robert, Robasse and Scheltes 1973; Vandenberg and Soper 1978; Soper and MacLeod 1981), including *E. muscae* (Wilding and Lauckner 1974; Carruthers, Haynes, and MacLeod 1985). In this host, abiotic conditions were probably suitable from late fall through late spring, and we feel that population density was the most important single factor governing the dynamics of *E. muscae* infection in *F. canicularis.* This relationship did not emerge significantly from the multiple regression analysis at Lohr ranch, but visual examination of the composite ranch situation (fig. 8*c*) seemed clear. Declining host numbers and rapid onset of hot summer weather drastically reduced the prevalence of *E. muscae* in *F. canicularis,* and the negative influence of high temperatures was reflected in the negative coefficient for Maxt in the analysis (table 6).

The almost complete lack of *E. muscae* infections in *F. canicularis* in 1984 stood in marked contrast to the previous year. It was difficult to positively attribute this to either weather conditions or host density. Both may have been involved. Rainfall was considerably less in 1984, and the drier manure conditions led to adult population densities 5 to 10 times less than existed in 1983. It is possible that *F. canicularis* populations remained below the epizootic threshold in 1984. Average infection rates

also were generally lower in 1984 for *M. domestica* and especially *O. aenescens.* This trend likely reflected the later onset of infections in fall 1984 due to high temperatures that September. Maximum infection rates in these hosts were actually slightly higher in fall 1984 relative to 1983 (figs. 6, 7, and 8).

Factors Governing Infection in *O. aenescens*

Infections in *O. aenescens* were well correlated with population density and negatively related to periods of high minimum humidity and high temperature. The negative relationship with MinRH may be spurious, but suggests that high humidities by day are not needed for transmission. Nocturnal humidities were nearly always above 95 percent RH for a few hours, when primary conidial discharge likely was high. The very high infection rates observed repeatedly for *E. muscae* in *O. aenescens* indicate that this species was highly susceptible to infection. It is known that *O. aenescens* and *O. leucostoma* are facultative predators on other fly larvae in the manure community (Anderson and Poorbaugh 1964a; Müller 1982; Nolan and Kissam 1985) and that *Ophyra* adults spend the night primarily in vegetation, as opposed to the inside roof areas of the poultry house for *M. domestica* and *F. canicularis* (Anderson and Poorbaugh 1964b). *Ophyra aenescens* is seldom reported as a nuisance in California and thus is seen as either a neutral or beneficial member of the manure taxa. Notably, *O. aenescens* cadavers at K2 were found mainly in vegetation; *M. domestica* cadavers were less common in vegetation there. This differential in cadaver and host distribution must have tended to minimize direct *E. muscae* transmission between the two hosts. Though seasonal infection rates were significantly correlated statistically, this may have been partly due to general similarities in seasonal occurrence. Adults of both species were relatively rare in spring, but *O. aenescens* also was uncommon in late summer (fig. 6*a-d*). The generally higher levels of infection in *O. aenescens* (table 3) may reflect the fact that the overnight resting areas (vegetation) were the same areas in which most cadavers were found. This would possibly place healthy flies in a good position to be contacted directly by large numbers of primary conidia discharged at night. The situation differed from *M. domestica,* as discussed below.

Factors Governing Infection in *M. domestica*

Infection rates in *M. domestica* could not be directly related statistically to any of the variables examined. Such regressions are difficult, however, with infection data collected only at 1- to 2-week intervals, as in the present study. Other studies with *E. muscae* also generally have failed to relate infection to abiotic variables, but generally have noted a relationship with host densities (Berisford and Tsao 1974; Wilding and Lauckner 1974; Carruthers, Haynes, and MacLeod 1985). In the present case it is preferable to examine the patterns of infection visually (fig. 6*c,d*). Population density was important in the dynamics of *E. muscae* infection in *M. domestica,* and was related to infection prevalence between late fall and early summer. In summer, however, *M. domestica* populations built rapidly, unimpeded by the fungal pathogen. The abrupt drop in *E. muscae* infection in all three hosts in early July 1983 (figs. 6*a,* 6*c,* 8*c,*) likely was related to the rapid onset of extremely hot weather. Average maximum temperatures increased over this 2-week period from 25° to 38°C. Conidial production from the *M. domestica* strain of *E. muscae* in this area is curtailed at mean temperatures in excess

of 27°C, and flies held at 30°C tend to die without producing conidia (Mullens, unpublished). Other studies with *E. muscae* have shown that the pathogen functions poorly at high temperatures (Carruthers and Haynes 1985), and recent work with *E. muscae* in Denmark has shown that even fairly brief exposures to temperatures in the 35° to 40°C range could eliminate that strain of the pathogen from infected *M. domestica* (U. S. Olesen, personal communication). Because it is known that the *M. domestica* strain of *E. muscae* from southern California can produce substantial numbers of primary and secondary conidia at moderate or even low humidities (Mullens and Rodriguez 1985), it is likely that high temperature was the primary limiting factor for *E. muscae* in *M. domestica* in summer. In fall, as average maximum temperatures dropped below 25° to 30°C, the existing *M. domestica* population density was more than sufficient to allow a rapidly building epizootic to occur. Additionally, rapidly reproducing populations would be expected to contain many young individuals, which are more susceptible to *E. muscae,* have a shorter incubation period, and produce many conidia (Mullens 1985).

In light of the similarities in onset and duration of substantial numbers of *E. muscae* infections in a particular host captured at different sites, we feel justified in using yearly average infections as a basis for rough comparisons. The sites varied in terms of prevalence of infections in *M. domestica,* but the highest infection levels were found at the two sites (Lohr and K2) with the highest population densities. As mentioned earlier, the K2 ranch, with the highest level of infection, did not often have breeding populations of *M. domestica* due to the weekly manure cleanout schedule. However, fresh chicken manure accumulated for a week at a time and, together with the adjacent thin-bed manure drying field, was highly attractive to flies. Several other poultry facilities were close to K2. Additionally, the lush weed growth that occurred here in late summer 1983 was both an aggregation site for flies and an excellent focus of *E. muscae* infection. Intense host aggregation and evaporative cooling in the moist weeds probably contributed to conditions favorable to *E. muscae* several weeks before the other sites in 1983. The cutting of this weed growth likely was responsible for holding down the increase in *E. muscae* prevalence after September 1983. The K2 site thus could be regarded as a natural "infection arena," which was attractive to flies in the area and conducive to *E. muscae* activity. Weed growth adjacent to poultry facilities is not recommended because it impedes air flow (and manure drying) and can serve as a haven for rodents. There may be potential in certain situations, however, for encouraging strictly seasonal weed growth near, but not directly adjacent to, poultry houses for the purpose of increasing disease impact on fly populations. Carruthers (1981) showed that *Delia* spp. adults and cadavers killed by *E. muscae* were aggregated in weedy border areas at the edges of carrot and onion fields, a situation that encouraged disease transmission.

Studies conducted during the 1984 epizootic at K2 showed that *M. domestica* cadavers killed by *E. muscae* often were found both inside and outside the poultry house, but were far more prevalent on the south side. That cadavers were commonly seen inside, however, likely was due to the fact that they were relatively protected from weather (especially wind) and perhaps diurnal scavengers and thus accumulated through time. The data clearly showed (table 7) that most fresh cadavers occurred on the outside of the house, especially the upper curtain. These cadavers persisted well during the calm hours of night and early morning, but often apparently were dislodged by winds by midday. We did not conduct experiments to determine this, but the failure

of cadavers to accumulate outside through time shows that they were dislodged or removed in some way.

We found an exceedingly small number *E. muscae* cadavers on the sticky tapes. This was expected due to the behavior of infected flies. Tapes inside the houses would be expected to have very few *E. muscae* cadavers due to the fact that most critically ill flies were outside. Whether the tapes were inside or outside, however, the behavior of infected flies is to crawl upward. Most crawled up to the upper curtain area, and a few then moved out to the undersides of the eaves. To be captured on the tapes, however, they would have had to crawl downwards. This is counter to the typical behavior of critically ill flies. Flies captured on the tapes earlier than the last day of pathogen incubation would have died before the fungus could exit, and thus would not have been noticed.

Daily fly movements were critical to the pathogen's success. Mullens and Rodriguez (1985) showed that primary conidial discharge reaches peak levels 10 to 12 hours after host death, and that infective secondary conidia are discharged from these conidia 3 to 6 hours later. Host death has a distinctive diurnal cycle, occurring in late afternoon and early evening (Mullens 1985). A host dying at this time will produce most primary conidia during the night, and these adhere to surfaces near the cadaver. Peak production of secondary conidia, on the other hand, would be expected to occur in early morning hours. *Musca domestica* spent the night in the inside roof region of the house, but generally it was unusual to find cadavers there. As the sun warmed the poultry house structures in the morning, however, flies aggregated on the exterior south wall when some primary conidia and especially large numbers of secondary conidia were being discharged. The bimodal activity pattern we have observed in *M. domestica* also was documented by Parker (1962) in the hot, dry climate of the Sudan, and Holway, Mitchell, and Salah (1951) noted a variable aggregation activity peak by *M. domestica* that often occurred in the morning in spring and early fall in Egypt. Maier et al. (1952) and Kilpatrick and Quarterman (1952) also noted *M. domestica* morning aggregation on house and barn walls in Texas and Georgia, respectively. Additionally, Willson and Mulla (1975) documented the same bimodal fly aggregation patterns at bait stations at the perimeter of poultry houses in southern California, with increased seasonal aggregation on the south sides during cool weather. We observed this increased south side aggregation with cooler weather (fig. 11), and this was yet another means of increasing effective host density and the efficiency of transmission in the fall. Critically ill flies were present at the south wall in the afternoon, but such flies cannot fly (Mullens, personal observation) and likely were too sick to migrate to the inside roof area with the onset of darkness. Instead, they climbed the structure and were in an optimal position to expose flies to the pathogen the following morning.

Though secondary conidial discharge does not seem to be greatly influenced by humidity, at least in the range of 20 to 80 percent RH (Mullens and Rodriguez 1985), there may be potential for early morning operation of the cooling mister systems. Where these are located on the eaves, as at K2, operation during the morning period might enhance *E. muscae* transmission.

As mentioned earlier, young flies are superior hosts for the pathogen, and, of course, it would be desirable from a control standpoint to infect females as early in adult life as possible to reduce the potential for progeny production. The south side aggregation and its significance for *E. muscae* transmission becomes more important in light of the work of Willson and Mulla (1973) on southern California poultry ranches. These

authors documented that activity of male and virgin female *M. domestica* at bait stations was greatest in the peripheral poultry house regions. Further, young nulliparous females were most common at bait stations with a southeastern orientation. Unfortunately, the authors did not provide data on time of day in this paper, but subsequent work indicated that this activity was characterized by morning and early evening aggregation (Willson and Mulla 1975). If these bait station data are assumed generally to reflect fly aggregation trends, it would suggest that nulliparous females do tend to be most prevalent in areas of poultry houses, at the appropriate time, with the best potential for *E. muscae* transmission.

Compatibility of *E. muscae* and Insecticides

Our studies have shown that *E. muscae* was a significant mortality factor and that secondary conidial discharge was important in disease epizootiology. Mullens and Rodriguez (1986) have shown that insecticide residues can reduce secondary conidial discharge in *E. muscae.* In this area and habitat, therefore, it should be advantageous at least to avoid treating the south side of poultry houses in fall. These residues would not only reduce secondary conidial discharge in the short term but would also intoxicate diseased flies crawling up these surfaces. Such flies produce conidia on the ground and thus would be relatively ineffective in furthering transmission, a situation also observed with *D. antiqua* infected with *E. muscae* in Michigan (Carruthers, Whitfield, and Haynes 1985). Generally speaking, insecticides probably are not very compatible with this pathogen. Insecticidal baits, or sprays applied to the inside rafter areas (nighttime fly resting sites), as recommended by the University of California (Loomis 1981), would preserve the integrity of the transmission foci (outer walls and vegetation on the south side). It probably would kill both healthy flies and those in early or intermediate stages of infection, however, assuming the feeding and nighttime resting behavior of infected flies is not altered until the later stages of *E. muscae* incubation. This would reduce the reservoir of infected hosts for future *E. muscae* transmission. Still, if insecticides must be used, baits or inside rafter sprays probably would be preferable to direct treatment of the transmission areas.

CONCLUSION

The infection levels documented in this study indicate that *E. muscae* is an important seasonal mortality factor in fly populations. We feel that the rapid population declines in *M. domestica* in late fall cannot be attributed solely to the effect of lower temperatures on the biotic potential of the insect. Winter daytime temperatures in this region often reach or exceed 25°C, which should have allowed *M. domestica* populations to persist at higher densities than we have observed. Carruthers (1981) showed that fecundity of *D. antiqua* females was reduced 50 percent by the pathogen, with no oviposition by infected flies after 3 to 4 days incubation (21°C). Preliminary studies on *M. domestica* (Mullens, unpublished) also suggest that *E. muscae* dramatically reduces fecundity in *M. domestica.* We feel the pathogen is thus a primary factor in cool-season regulation of fly populations in this habitat and region.

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