Introduction of *Entomophthora muscae* (Zygomycotina: Entomophthorales) to Caged House Fly Populations

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(Received 19 May, 1994; Accepted 13 June, 1994)

Experiments for the introduction of *Entomophthora muscae* to populations of the house fly adults were carried out. Rearing of 50 healthy flies in a 27-l cage with 1, 4, or 10 fresh cadavers attached to the inside top of the cage as conidial sources initiated death of flies from approximately 6 days after introduction. Including secondary infections, the fly populations finally collapsed within 11 days with the application of more than 4 cadavers. When 2, 4, or 10 living flies injected with *E. muscae* protoplasts were released into the population of 50 healthy flies in a 27-l cage, the 1st mortality peak of non-injected flies occurred 4 to 6 days after the death of the injected flies, and the 2nd peak of mortality detected several more days after this. The non-injected flies were totally killed within 17 days by releasing more than 4 living infected flies. The epizootics also occurred when more than 2 conidia-discharging cadavers were attached within a 0.8-m² cage, and the population of 50 healthy flies were killed within 14 days. Both the application of fresh fly cadavers and release of living-infected flies were thought to be effective means of introduction of *E. muscae* into house fly populations.

*Key words:* *Entomophthora muscae*, *Musca domestica*, epizootic, biological control, microbial control

INTRODUCTION

The house fly, *Musca domestica* is a well-known hygiene and nuisance pest in garbage disposal facilities, poultry and livestock houses. Biological control methods have been sought after to avoid the use of chemical insecticides. We have isolated *Entomophthora muscae* (Zygomycotina: Entomophthorales) and have conducted preliminary studies on this fungus; such as the discharge period of conidia, number of conidia produced per cadaver, and infectivity of conidia-discharging cadavers (SHIMAZU and KURAMOTO, 1994). These data show promise for the use of this fungus in poultry houses for the control of *M. domestica*. It is necessary to confirm the effectiveness of this fungus in small populations of flies before practical introduction. Therefore, *E. muscae* was introduced into fly populations in small and large cages and the prevalence of the disease observed.

MATERIALS AND METHODS

The fungus *Entomophthora muscae* F-1020, isolated from a naturally infected house
fly at Ibaraki Town, Ibaraki Prefecture, Japan was used in the experiment. The experimental insect, *M. domestica* was artificially reared in the laboratory of the Ibaraki Prefectural Poultry Experiment Station. Approximately 100 protoplasts of the fungus were suspended in 3 µl of CARLSON's solution (MITSUSHI, 1982), and injected to the adult flies to prepare the infected adults as a conidial source for the infection experiments. The details of fungus culture maintenance, rearing flies, and injection of protoplasts into flies were described in a previous paper (SHIMAZU and KURAMOTO, 1994).

Using these materials, the following infection experiments were carried out at the Forestry and Forest Products Research Institute, Ibaraki, Japan.

1. **Epizootics initiated from conidia-discharging cadavers.** The possibility of the introduction of *E. muscae* by conidial shower from injected flies was investigated. One, 4, or 10 fresh cadavers of *M. domestica* infected with *E. muscae* were attached to pieces of packing tape with vaseline, and the tapes were put on the inside top of a 27-l fly cage (30 × 30 × 30 cm, House Fly Cage, Shiga Insect Company). Fifty healthy adults of *M. domestica* were added to the cage to obtain contamination with the discharged conidia from the cadavers. The flies were fed a mixture of skim milk powder and sugar, and water. These cages were kept at 25°C, photophase of 16L–8D. Two cages were replicated for each plot. The flies were observed daily for mortality, and dead ones left to cause secondary and tertiary infections.

2. **Epizootics initiated from released living infected flies.** The possibility of the introduction of *E. muscae* by mixing living infected flies with healthy ones was investigated. Adults of *M. domestica* were injected with protoplasts and marked on the wings with a felt pen to distinguish them from the untreated flies. Twenty-four hours later, 50 healthy flies and 2, 4, or 10 injected flies were placed together in 27-l fly cages, and reared on skim milk-sugar mixture and water at 25°C, 16L–8D. Two cages were replicated for each treatment. The flies were observed daily for mortality, and dead ones left to cause higher orders of infection.

3. **Induction of epizootics in large cages.** Infection experiments using cadavers similar to those in small cages were carried out in large cages to investigate the possibilities of the introduction of *E. muscae* in larger spaces.

Two, 4, or 10 fresh cadavers infected with *E. muscae* were attached to pieces of packing tape with vaseline, and the tapes were put on the inside top of a 0.8-m³ fly cage (60 × 81 × 157 cm) made of wood frame and plastic screen. Fifty healthy adults of *M. domestica* were added to the cage and reared on the same diet as used in the experiments with small cages. The cages were kept in a room where neither temperature nor photophase were controlled. Temperatures and humidities in the room during the experiments were recorded by a self-registering thermo-hygrometer. The flies were checked for mortality daily, and dead flies left in the cage to cause higher orders of infection. As a control, the same lot of adult flies were reared in a small cage in the same room. The experiments were repeated twice.

**RESULTS**

1. **Epizootics initiated from conidia-discharging cadavers**

Most flies killed in the treatment plots showed typical symptoms of *E. muscae*. Though the individual causes of death were not checked microscopically because the cadavers were left untouched in cages, most flies seemed in appearance to be infected
with *Entomophthora*. Daily and accumulated mortalities in the 2 replicates are averaged and shown in Fig. 1. Some flies in the control plot died, and daily mortalities in the treatment plot were corrected using Abbott's formula (Abbott, 1925). Consequently, some daily mortalities became negative, and the accumulated mortality tended to decrease during some periods. In the plots applied with 4, and 10 cadavers, a major peak of mortality at 6 days and minor peaks at 10 to 11 days of rearing were detected. All the flies in these plots were killed within 11 days. There were also a small number of deaths in the plot with 1 cadaver on day 5, and the mortalities increased after day 11, however, the daily mortality curve is nearly flat after correction as shown in Fig. 1. The fly population in one replicate collapsed on day 19, however the mortality in another replicate stayed at 62%. Secondary and higher orders of infections were detected in both replicates.

2. Epizootics initiated from released living infected flies

Most flies in the treatment plots were killed by *E. muscae*, although there were also

![Fig. 1. Daily (dark line) and accumulated (gray line) mortalities of flies reared with various numbers of dead flies discharging conidia of *Entomophthora muscae* in small cages. Mortalities are average of 2 replicates after corrected with Abbott's formula.](image1)

![Fig. 2. Daily (dark line) and accumulated (gray line) mortalities of flies reared with various numbers of flies injected with *Entomophthora muscae* in small cages. Broken lines show the mortalities of injected flies as conidial sources. Mortalities are average of 2 replicates after corrected with Abbott's formula.](image2)
some deaths in the control plot, and consequently some daily mortality became negative (Fig. 2). The inoculated flies died 3 to 5 days after the mixing with the others which was 4 to 6 days after injection with protoplasts. The non-injected fly populations in plots with 4 and 10 infected flies finally collapsed due to the subsequent infections. The actual mortalities in the plots with 2 infected flies were finally around 30%. However, the Abbott-corrected mortality was about 9% on average. All flies in the plots with 10 infected flies died 8 to 10 days after mixing, and so there was only one peak of accumulated mortality. On the other hand, in the plot with 4 infected flies, the first peak of mortality was on day 8 to day 10 and the 2nd peak on day 14, and all of the flies were killed by both direct and secondary infection. Also in the plots with 2 infected flies, small peaks on day 13 to day 14 and on day 18 were found, however, the mortality curve is nearly flat owing to the correction.

3. Induction of epizootics in large cages

Most dead flies in the treatment plot were also infected with *E. muscae*. Since the room temperatures and humidities during the 1st and the 2nd trials differed from each other (Table 1), mortalities of each trial were not averaged, and are shown separately in Fig. 3. Temperatures during the 2nd trial were generally lower than those

<table>
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<th>Trial</th>
<th>Temperature (°C)</th>
<th>Humidity (%)</th>
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<tr>
<td></td>
<td>Minimum</td>
<td>Average</td>
</tr>
<tr>
<td>1</td>
<td>17.8</td>
<td>20.0</td>
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<tr>
<td>2</td>
<td>15.3</td>
<td>17.6</td>
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![Graphs](NII-Electronic Library Service)

Fig. 3. Daily (dark line) and accumulated (gray line) mortalities of flies reared with various numbers of dead flies discharging conidia of *Entomophthora muscae* in large cages. Mortalities in each trial are shown separately after corrected with Abbott's formula.
in the 1st trial, however, there were no conspicuous differences of the mortality curve between the trials. In the 1st trial, the flies started to die 3 days after the start of rearing, the major peak of mortality was on day 8, and a very small peak of the secondary infection occurred on around day 12 in each treatment. The curves of accumulated mortalities in plots with 2 cadavers and 4 cadavers progressed almost equally, and mortalities in the plot with 10 cadavers were always higher than the other plots. All of the flies were killed by day 13 in each plot. In the 2nd experiment, the major peaks of daily mortality curves appeared on day 7 for the plot with 10 cadavers, and on day 8 for the plots with 2 and 4 cadavers. Minor peaks were recognized on day 11 in every plot. The accumulated mortality tended to be higher in the plot with more cadavers, however, mortalities of those with 4 cadavers and 10 cadavers reversed after day 9. All of the flies were killed by day 13. Only one fly in the control plots in each replication died at the beginning of the experiments.

DISCUSSION

Although, our previous experiment (Shimazu and Kuramoto, 1994) revealed that conidia of *E. muscae* from 1 cadaver were able to kill more than 60% of healthy flies in 27-1 cage, the mortalities with small numbers of conidial sources in the present experiments in the 27-1 cages with living infected flies or conidia-discharging cadavers were not so high. Underestimation of mortality in the treatment plots due to the occurrence of deaths in the control plot, or scattering of the conidia discharged from the inocula are thought to be possible causes of these low mortalities. Also, in the experiments with living infected flies, it is not certain whether the inoculated flies discharged enough conidia after their death, even though they were forcibly injected with *E. muscae* protoplasts. This was indicated by the results of conidial numbers from individual cadavers which varied widely (Shimazu and Kuramoto, 1994). However, when more than 4 infected flies were released or attached, half of the flies were killed in several days (Table 2). These results show that by using more than 4 infected flies, the scattering of conidial numbers from cadavers could be covered during the primary infection. Including secondary infections, it is possible that the fly population in a 27-1 cage will certainly collapse when 4 or more infected flies are supplied. When conidia-discharging cadavers were applied in the large cages, the populations collapsed even with 2 cadavers, perhaps because the mortalities in the treatment plot were not affected by low mortalities in the controls in these experiments. Also, the number of discharged conidia from the conidial source might have been abundant in those cases.

The release of living infected flies among healthy ones has the demerit that the injected flies do not always discharge conidia abundantly, however, it also has the

<table>
<thead>
<tr>
<th>Table 2. Comparison of mortalities and lethal periods in each experiment</th>
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<tr>
<td><strong>Small cage</strong></td>
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<tr>
<td>with cadavers</td>
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<tr>
<td>No. of inocula</td>
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<td>Final mortality (%)</td>
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<td>LT50 (days)</td>
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<td><strong>Large cage</strong></td>
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following merits. The period for application which is from immediately after injection to a day before death is fairly long, and the injected flies can spread to the fly's habitat themselves and contaminate the natural population. On the other hand, the application of conidia discharging cadavers requires the rearing of flies from after injection until their death. Also, the conidia discharging cadavers should be applied quickly because the discharge period is limited. However, we can certainly select those cadavers which produce abundant conidia and apply them to any location. Since both release of infected flies and application of conidia discharging cadavers were found to be effective to introduce *E. muscae*, the appropriate method should be chosen according to the situation.

Mullens et al. (1987) observed natural epizootics of *E. muscae* in poultry houses, and reported that *M. domestica* populations were sometimes 100% infected with *E. muscae*. In Japan, *E. muscae*-infected flies are not so common in poultry houses. However, the artificial introduction of *E. muscae* to the house fly population in poultry houses is promising. Recently, poultry houses are being changed to a windowless type. Windowless poultry houses are a more suitable environment to utilize this fungus than open ones, because the temperatures and humidities which are important for the sporulation and germination of *E. muscae* conidia (Mullens and Rodríguez, 1985; Kramer, 1980) are fairly constant and at higher levels, and migrations of flies less.

There are few examples of the large scale artificial introduction of *E. muscae* into house fly populations. Kramer and Steinkraus (1987) released infected, and non-infected house flies in a small poultry building of 30 m³. The ratio of diseased and healthy flies were not investigated daily, however, the entire fly population was reported to have collapsed after 11 days. Since the initial ratio of infected flies per non-infected ones were 250: 400, which was far higher than ours, most flies of their experiment ought to have been killed by the primary infection and higher orders of infection probably occurred very little. Their experiment showed the possibility of an insecticidal use of *E. muscae* in poultry houses, however, the ratio of released infected insects was impractically high. On the other hand, the mixing ratio of infected flies in the present experiment was closer to a practical level. In a previous paper Kramer and Steinkraus (1981) successfully introduced *E. muscae* to adult house flies in a small carton also with higher density of cadavers. Our results in the present experiment confirmed that it is possible to introduce an *E. muscae* epizootic with less inocula. Even with 4% of initial infected flies, fly populations collapsed. However, since flies in actual poultry houses emerge successively, the effect of control must appear through a decrease in the total number of eggs. Steinkraus et al. (1993) introduced *E. muscae* to fly populations in dairy farms. In his experiment, the disease also occurred in control plots, however, the flies in the treatment plot were killed earlier and in greater numbers than in the control plot.

Compared with the natural population, the population density of 50 individuals in the small cage may be too high, however, those in the large cage which is of 0.8 m³ were comparable in number. Results of the present study are encouraging for the introduction of *E. muscae* and microbial control of *M. domestica* in poultry houses. Experiments in larger spaces should be carried out before practical use of this fungus is contemplated.
ACKNOWLEDGEMENTS

We thank Dr. Simon A. Lawson of the Forestry and Forest Products Research Institute, Japan, for reviewing the manuscript and correcting the English.

REFERENCES


