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# Entomophthora muscae — moisture as a factor affecting its transmission and conidial germination

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The role played by moisture in the transmission of *Entomophthora muscae* and in the germination of its conidia was investigated. A majority of adult house flies exposed to conidial showers that fell upon surfaces covered with droplets of condensation acquired the parasite, while no flies exposed to conidial showers that fell upon dry surfaces did so. A microscopical study of conidial showers showed that germination was practically non-existent on dry surfaces while a vast majority of conidia that fell upon a droplet--covered surface germinated. A method for the *in vivo* culture of *E. muscae* was developed and 11 serial passages of the fungus were achieved. Resting spores rather than conidia became the dominant form produced in the cadavers, and flies in a twelfth group remained unifected.

## INTRODUCTION

*Entomophthora muscae* (Cohn) Fres. is a pathogen of the house fly *Musca domestica* and related dipterans. It does on occasion cause epizootics in populations of flies and accounts of these epizootics have been compiled by Greenberg (1973) and West and Peters (1973). No really satisfactory method for its culture *in vitro* has been found (MacLeod et al. 1976) and reliable reports of its successful *in vivo* culture under laboratory conditions are quite limited in scope (Baird 1957; Brefeld 1977; Güssow 1913; Kramer 1971). Hence experimental investigations of the *E. muscae*-fly relationship continue to be hampered by unresolved technical problems relating to the culture of the fungus. The present study, however, provides some new information concerning: 1 — the role played by moisture in the transmission of *E. muscae* and in the germination of its conidia and 2 — a method for the *in vivo* culture of *E. muscae*.

## MATERIALS AND METHODS

To find how moisture influences the transmission of *E. muscae*, I tied three fresh cadavers of field-collected cluster flies *Pollenia rudis* that had died of the mycosis to the moist cotton wick of a water bottle of a type commonly used as a source of drink for laboratory cultures of muscoid flies. An additional set of three cadavers was tied to the wick of a second bottle. Each bottle was placed in a glass battery jar. These were numbered 1 and 2. Jar 1 contained a layer of dry sand while Jar 2 contained a layer of wet sand. Both jars were covered a layer of clear polyethylene wrap. About 40 well-fed disease-free laboratory-reared house flies were added to each jar. Jar 1 was placed inmediately on a bench in the laboratory. Jar 2 was initially held in a dark and cold room overnight to promote the formation of condensation within this closed chamber. Jar 2 was then placed on the laboratory bench next to Jar 1 where all of the flies received some artificial light from overhead fluorescent lamps during the day. To sustain the flies during the course of the experiment, small lumps of powdered milk and sugar were added to each jar every day. The dead flies in each jar were removed at daily intervals and examined for sings of disease. Flies that died of causes other than *E. muscae* infections have been omitted from the data presented in Table 1. On post-exposure days 1 and 2 the abdomens of the *P. rudis* cadavers on the wicks were bloated and produced conidial showers that fell onto the aluminium collars of the water bottles. Within the next two days, however, the abdomens resumed normal dimensions, indicating that the conidial bloom had probably run its course. Throughout the test period the house flies in both jars readily landed on the wicks and their collars to drink or to rest, often on or near the *P. rudis* cadavers. Some flies also explored the cadavers with their proboscises.

To determine the effects of moisture on the germination of *E. muscae* conidia, I placed on clean glass slides fresh cadavers of fieldcollected *P. rudis* that had succumbed to *E. muscae*. One series of slides was held overnight in dry glass petri dishes placed in the drawer of a laboratory bench at room conditions. The other series of slides was also placed in petri dishes but these were held in a desiccator containing a layer of wet sand. The desiccator was placed in a dark and cold room overnight. By the following morning all of the cadavers in both series had produced conidial showers, visible to the unaided eye as distinct white halos around the abdomens. While the surfaces of slides held at room conditions were quite dry, those of slides held in the cold room had developed innumerable minute droplets of condensation. All of the

cadavers from both series were removed from the slides and these slides with their halos were held in their respective chambers for an additional 24 hours to provide the conidia with ample time for germination: at the end of this period the halos of conidia on the slides were stained with Colley's solution (C o l l e y 1925). These preparations, protected by coverslips, were observed under a microscope to determine the nature and extent of conidial germination. These findings are summarized in Table 2.

To test the reliability of the regimen described for Jar 2 in Table 1, I attempted to maintain continuous cadaver-to-fly passages of *E. muscae*. The procedures were not changed but the numbers of cadavers employed as inoculum sources were varied as indicated in Table 3.

## RESULTS AND DISCUSSION

Not a single fly in Jar 1 developed the mycosis by day 10. In contrast, several flies in Jar 2 died with *E. muscae* infections on days 6 and 7, with additional deaths due to the fungus occurring on days 8 through 10. The accumulative mortalities in the transmission tests are summarized in Table 1. These results suggest that the parasite's ability

Table 1

Transmission of *Entomophthora muscae* to adult *Musca domestica* held in glass battery jars

| Number of jar | Substrate * for conidia | Conditions of temperature | Relative humidity | % of flies dying with *E. muscae* infections ** |
|---|---|---|---|---|
| 1 | dry surface | 21-27°C | 30-40% | 0 (0/34) |
| 2 | with condensation droplets | 14-16°C then 21-27°C *** | 100% | 73 (22/30) |

* Aluminium collar of water bottle
** At post-exposure day 10
*** At lower range for 16-18 hours at start of test

to invade the host is quite good when the conidia are discharged onto a substrate containing minute droplets of condensation. In the absence of such droplets, however, transmission of the parasite did not occur. Death due to the mycosis was not observed in any *M. domestica* that lived beyond day 10 in either of the jars.

The results presented in Table 2 show that germination of conidia on the dry surface was practically non-existent. Slight protrusion from

Table 2

Germination of *Entomophthora muscae* conidia about 24 to 40 hours after their discharge from cadavers of *Pollenia rudis*

| Substrate for conidia | Conditions of temperature | Relative humidity | Percentages of stage present * | | | | Total % germinated |
|---|---|---|---|---|---|---|---|
| | | | primary conidia | | secondary conidia | | |
| | | | intact | with bud | intact | with bud | |
| Dry slide | 21-27°C | 30-40% | 98 | 2 | 0 | 0 | 2 |
| Slide with condensation droplets | 14-16°C then 21-27°C ** | 100% | 18 | 46 | 12 | 24 | 82 |

* Average of four replicates, 100 conidia per replication from each substrate
** At lower range for 16-18 hours at start of test

the spore wall were detectable in only a few conidia. In contrast, a vast majority of the conidia on the surface containing minute droplets of condensation not only germinated, but produced secondary conidia that also germinated. No long germ tubes grew from any of the primary conidia. The germinated secondary conidia, however, produced very long bifurcate or trifurcate germ tubes. No tertiary conidia were produced by the secondary conidia. These observations suggest that the invasive germ tubes arise from secondary rather than primary conidia. Clearly the germination patterns observed in these experiments complement the results of the transmission tests. In both cases the presence of minute droplets of condensation was necessary for conidial activity. The question as to whether *E. muscae* conidia can also germinate in a near-saturated atmosphere was not pursued since truly reliable methods for such investigations are wanting (see Shein 1964; Rotem et al. 1978).

As indicated in Table 3, I obtained 11 consecutive cadaver-tofly passages of *E. muscae*. The number of cadavers used as sources of inoculum varied from one to nine, and the percentage of house flies dying of the phycomycosis varied from 58 to 100%. A study of Table 3 reveals no quantitative relationships between the number of cadavers used and the percentages of flies that acquired the parasite. Flies dying with *E. muscae* infections in Passages 1 through 9 produced only conidia. In Passage 10 the picture changed markedly: while a majority of the cadavers bore only conidia, some contained only resting spores, and still others produced a mixture of resting spores and a small number of conidia. In Passage 11 no cadavers produced conidia exclusively, and the conidial showers from the two cadavers that contained a mixture

Table 3

Cadaver-to-fly passages of *Entomophthora muscae* in adult *Musca domestica* during a fall-winter period under laboratory conditions

| Number of passage | Date trial started | No. cadavers as inoculum sources * | Per cent of flies dying with *E. muscae* ** | Per cent infected flies producing | | |
|---|---|---|---|---|---|---|
| | | | | conidia only | resting spores | resting spores + conidia |
| 1 | 30 X 75 | 2 | 83 (10/12) | 100 | 0 | 0 |
| 2 | 12 XI 75 | 9 | 92 (11/12) | 100 | 0 | 0 |
| 3 | 17 XI 75 | 2 | 88 (14/16) | 100 | 0 | 0 |
| 4 | 23 XI 75 | 1 | 69 (11/16) | 100 | 0 | 0 |
| 5 | 7 XII 75 | 4 | 58 (11/19) | 100 | 0 | 0 |
| 6 | 20 XII 75 | 4 | 93 (13/14) | 100 | 0 | 0 |
| 7 | 27 XII 75 | 5 | 81 (26/32) | 100 | 0 | 0 |
| 8 | 2 I 76 | 7 | 75 (21/28) | 100 | 0 | 0 |
| 9 | 10 I 76 | 5 | 100 (13/13) | 100 | 0 | 0 |
| 10 | 24 I 76 | 7 | 86 (24/28) | 78 | 9 | 13 |
| 11 | 31 I 76 | 1 | 75 (12/16) | 0 | 87 | 13 |
| 12 | 14 II 76 | 2 | 0 (0/20) | — | — | — |

* Sources for Passage 1 were field-collected *Pollenia rudis*; sources for subsequent passages were *M. domestica* cadavers from the previous trial

** By post-exposure day 10

of resting spores and conidia did not provide an amount of inoculum adequate to infect another group of flies. The reasons for this shift from the production of conidia to that of resting spores in *M. domestica* held in a favorable environment is unknown. All of the house flies used in these tests were two to six days old, and the sexes were represented in about equal numbers in all of the passages. Wilding and Lauckner (1974) suggest that resting-spores formation occurs more frequently in older flies, particulary females. The results reported by Kramer (1979) for black blow flies (*Phormia regina*) infected with *E. bullata* support this contention. He found that conidia alone were formed in more than 80% of flies that were 1/2 to three days old at the time of infection. In flies four to five days old at infection resting spores alone were formed in about 50% of them. Whether resting-spore formation is exclusively a response of the fungus to the age of the fly or whether it is a form of formancy governed by intrinsic characteristics of the fungus remains an open question.

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## Wpływ wilgoci na przekazywanie i kiełkowanie konidiów *Entomophthora muscae*

Streszczenie

Badano wpływ wilgoci na przekazywanie i kiełkowanie konidiów *Entomophthora muscae*. Większość dorosłych much domowych wystawionych na działanie konidiów, które padały na powierzchnie pokryte kropelkami kondensacji, zarażało się pasożytem, podczas gdy muchy wystawione na działanie konidiów, które padały na suche powierzchnie, nie ulegały zakażeniu. Badania mikroskopowe opadów konidiów wykazały, że na suchych powierzchniach praktycznie rzecz biorąc nie zachodziło kiełkowanie, podczas gdy większość konidiów, które padały na mokrą powierzchnię kiełkowało. Opracowano metodę hodowli *E. muscae in vitro* i uzyskano 11 kolejnych pasaży tego grzyba. Przetrwalniki raczej niż konidia były dominującą formą wytwarzaną na martwych muchach. Muchy w 12 kolejnej grupie nie uległy zakażeniu.