INFECTION OF ADULT MOSQUITOES BY THE ENTOMOPATHOGENIC FUNGUS *ERYNIA CONICA* (ENTOMOPHTHORALES: ENTOMOPHTHORACEAE)

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ABSTRACT. The infection of adult mosquitoes by the entomopathogenic fungus *Erynia conica* is presented for the first time. Adult *Aedes aegypti* were exposed to conidial showers from field-collected chaoborid, tipulid and chironomid cadavers for 24 h under conditions of 100% RH and 15°C. Up to 24% of the adults were killed by the mycosis. Cadavers of *Ae. aegypti* produced conidia that were infective to other adult *Ae. aegypti*; however, rates of infection were never more than 12%. Nevertheless, *Ae. aegypti* served as the laboratory host for *E. conica* via mosquito-to-mosquito serial passages for up to 6 months. Adult *Culex restuans* were also susceptible to infection by *E. conica*.

INTRODUCTION

Erynia conica (Nowakowski) Remaudière and Hennebert (Entomophthorales: Entomophthoraceae) is a naturally occurring fungal pathogen of adult nematocerous flies such as black flies (Simuliidae), crane flies (Tipulidae), moth flies (Psychodidae), midges (Chironomidae) and phantom midges (Chaoboridae) (Nowakowski 1883, Thaxter 1888, Gustafsson 1965, Gol'berg 1974, Descals et al. 1981). Although *E. conica* has been recorded in both Europe and North America for over a century, crucial information on its biology and ecology is still incomplete, particularly infection processes (Cuebas-Incle 1989[2]). While *E. conica* is not known to be pathogenic to adult mosquitoes, there are considerable morphological and ecological similarities mosquitoes share with susceptible Diptera. This paper presents the first report of the infection of adult mosquitoes by *E. conica*.

MATERIALS AND METHODS

Diseased adult Chaoboridae, Chironomidae and Tipulidae killed by *E. conica* were collected in Tompkins County, NY. Infected cadavers (Fig. 1) were removed from surfaces of damp wood, wet stones, and moist moss near lentic and lotic locations; identifications of cadavers beyond family levels were not possible due to fungal damage. These cadavers were placed onto 1% water agar surfaces inside small petri dishes and incubated overnight at 15°C. Under such conditions, infective conidia (Fig. 2) were produced and discharged. The dishes were then inverted and placed over clear, cylindrical, 30 × 35 mm, plastic infection cages (with 1 mm^2 mesh screens covering the top and bottom openings). One-week-old adult *Aedes aegypti* (Linn.) (NIH-"Rock" strain) were placed inside the infection cages to be subjected to conidial showers; mosquitoes were held at 15° ± 1°C for 24 h prior to their use. The infection cages were then placed inside clear plastic boxes (100% RH) and kept inside environmental-controlled cabinets (15°C and 12 h light:12 h dark photoperiod) for 24 h. Mosquitoes placed into similar infection cages topped only by water agar dishes served as controls. Cover glasses were placed under the cages to collect conidia that had fallen through the infection cages. The cover glasses were later mounted with modified Colley's stain (Gustafsson 1965, Cuebas-Incle[2]) and observed to determine whether conidia were collected; results were tabulated only for those trials in which conidia were found.

After exposure to conidia, mosquitoes were transferred to plastic-topped half-pint carton containers, provisioned with 5% sucrose solutions in cotton-plugged vials, and held at 15° ± 1°C and a 12 h:12 h photoperiod. Containers were observed daily for 21 days. Moribund and dead mosquitoes were removed and incubated in water agar dishes at 15°C for up to 3 days for signs of fungal growth from the cadavers; only those cadavers with typical post-mortem fungal emergence were scored as infected. These *E. conica*-killed mosquitoes served as the sources of conidia for subsequent infection trials.

Following initial infection, attempts were made to establish continuous *in vivo* laboratory cultures of *E. conica* via mosquito-to-mosquito serial passages. One-week-old adult *Ae. aegypti* were prepared and exposed to conidial showers from diseased mosquitoes as described above.

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[2] Cuebas-Incle, E. L. 1989. *Erynia conica* (Zygomycetes: Entomophthorales) as a pathogen of the Yellow Fever mosquito, *Aedes aegypti* (Diptera Culicidae). Unpublished Ph.D. dissertation. Cornell University, Ithaca, NY.

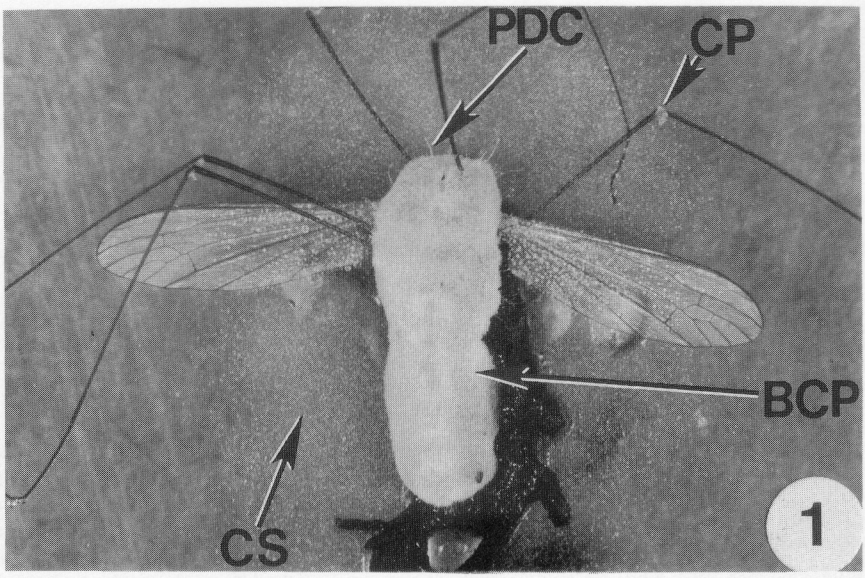

Fig. 1. Adult tipulid displaying post-mortem bloom typical of infection by *Erynia conica*. The entire cadaver, except for legs, is blanketed by a layer of conidiophores (BCP), and the area around the cadaver is littered by discharged conidia (CS). Conidiophores are also emerging from leg joints (CP); long pseudocystidia (PDC) are rising from the layer of conidiophores.

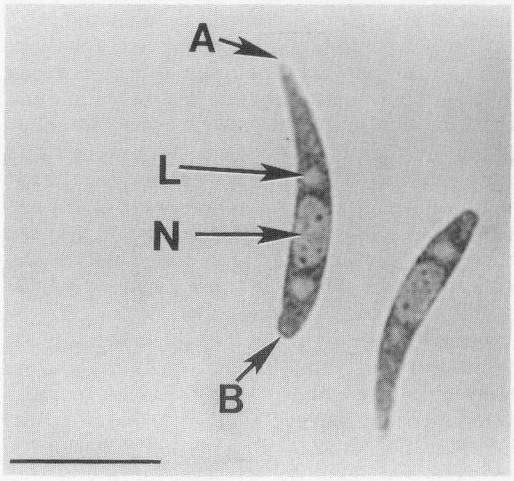

Fig. 2. Primary conidia of *Erynia conica*, discharged from chironomid cadavers. Nucleus indicated by (N), lipid droplet indicated by (L), and apex and base of conidium indicated by (A) and (B), respectively. Bar represents 25 μm.

Afterward, they were placed in holding containers and observed for 21 days with dead mosquitoes removed and held as described above. Cadavers that displayed typical post-mortem signs were then used to infect more mosquitoes (usually 20 mosquitoes were exposed to one conidia-discharging diseased cadaver).

In addition to a laboratory-maintained strain of mosquito, wild mosquitoes were also tested for their susceptibility to *E. conica*. *Culex restuans* Theobald, collected in Tompkins Co., NY as larvae, were reared to adults in the laboratory at 25°C, were held for a week at 15°C (to ensure they were free of fungal disease), and were then exposed to conidia from infected *Ae. aegypti* in the manner described above.

RESULTS

Initial data on transmission of *E. conica* to *Ae. aegypti* from cadavers of Chaoboridae, Tipulidae, and Chironomidae collected in the field were quite variable (Table 1). Nonetheless, *Ae. aegypti* were susceptible to all the tested strains of *E. conica*. Infected mosquitoes were almost always found on the bottoms of holding cages without any apparent signs of mycosis; only after post-mortem incubation did rhizoids, pseudocystidia and conidiophores emerge from the cadavers (Fig. 3). Formation and discharge of conidia from cadavers occurred usually within 12 h after emergence of fungal tissue.

The time required for all strains of *E. conica* to kill mosquitoes (i.e., incubation period) ranged from 3 to 14 days (mean = 7.3 days) after mosquitoes were exposed to conidia (Table 1). There were some differences in incubation periods among strains of *E. conica* tested but these

Table 1. Infections of *Aedes aegypti* by *Erynia conica* from different field-collected cadavers. Cumulative data presented for a 21-day observation period after exposure.

Year	Sources of conidia	Number of trials (total/ positive)	Total number of mosquitoes at risk	Percentage of mosquitoes with mycosis	Mean incubation period (range)
1984	Chaoboridae	3/2	29	24.1	8.0 (7–12)
1984	Chironomidae	3/2	104	6.7	8.4 (4–14)
1985	Tipulidae	4/4	34	20.6	10.0 (8–12)
1986	Tipulidae	3/3	72	23.6	6.5 (3–14)

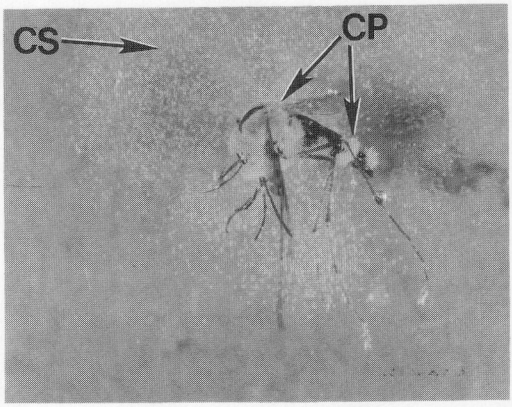

Fig. 3. Adult *Aedes aegypti*, killed by *Erynia conica*, 24 h after the death of the mosquito (head of cadaver pointing towards the left of the picture). Note the emergence of conidiophores (CP) from tissues between abdominal and thoracic segments and the abundant conidial shower (CS) around the cadaver.

differences were not significant to be attributed to different strains of *E. conica*.

Conidia from diseased *Ae. aegypti* were also shown infective to healthy mosquitoes (Table 2). The percentages of infection in these mosquitoes were, however, reduced as compared with those percentages in mosquitoes exposed to infected natural hosts. Prolonged maintenance of *E. conica* in adult mosquitoes was demonstrated when the pathogen came from both diseased Chironomidae and Tipulidae (Table 3; similar *in vivo* maintenance was not attempted with *E. conica* from diseased chironomids). The longest period *E. conica* was maintained in mosquitoes was 6 months (September 1986–February 1987) with the strain from Tipulidae.

Adult *Cx. restuans* also proved susceptible to *E. conica* (Table 2). Due to the unavailability of infected field cadavers, *Cx. restuans* were exposed to conidia discharged from *E. conica*-killed *Ae. aegypti*. None of the adult *Cx. restuans* held prior to exposure to conidia died of any entomophthoracean infection nor did any of the controls.

DISCUSSION

Although first identified over a century ago, little is known how *E. conica* infects its hosts and until now, no reports of laboratory-generated mycoses in insects by this pathogen have been published. Hywell-Jones and Webster (1986) exposed adult *Simulium variegatum* Meigen, *S. argyreatum* Meigen, and *S. equinum* Linn. (Simuliidae) to conidia from *E. conica*-killed black flies; they observed germinating conidia on cuticle and circulating hyphal bodies in hemolymph from several flies, but none of these flies died from mycoses. From the present study, *E. conica* has been shown to be infective to adult mosquitoes under laboratory conditions, and can be maintained *in vivo* as well.

Laboratory infections of adult mosquitoes by entomophthoracean fungi are rare. Gol'berg (1970) infected up to 100% adult *Culex pipiens* Linn. with an unidentified entomophthoracean from field-collected *Cx. pipiens*. Kramer (1982, 1983) infected 80% of adult *Ae. aegypti* and 100% of *Anopheles stephensi* Liston with *Entomophthora culicis* Braun from diseased adult midges, *Chironomus decorus* Johannsen. However, from my experience, no mycosis resulted when adult *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus* Say were repeatedly exposed to *Erynia rhizospora* (Thaxter) and *E. sepulchralis* (Thaxter) (pathogens of caddisflies and crane flies, respectively).

Although not clearly observed in the present study, the original host species may influence the pathogen's infectivity to mosquitoes. While Kramer (1982, 1983) obtained 100 and 80% infection by *En. culicis* from *Ch. decorus*, 4.8% *Ae. aegypti* were infected by *En. culicis* from another midge species, *Dicrotendipes nervosus* (Steiger), and no infections were obtained when *Ch. crassicadatus* Malloch, *Limnochironomus* sp. and *Tanytarsus* sp. (Chironomidae) were the sources of *En. culicis*. Kramer (1982) concluded that conidia of *En. culicis* from different host species were not equally infective. Similar effects have been observed in house flies infected by *En. muscae* (Cohn) isolated from different species of

Table 2. Infections of *Aedes aegypti* and *Culex restuans* by *Erynia conica* from laboratory-infected *Ae. aegypti*. Cumulative data presented for a 21-day observation period after exposure.

Year	Original sources of *E. conica*	Original percentage of infection	Mosquito species exposed	Number of trials (total/positive)	Total number of mosquitoes at risk	Percentage of mosquitoes with mycosis
1984	Chaoboridae	24.1	*Ae. aegypti*	9/3	101	5.9
1985	Tipulidae	20.6	*Ae. aegypti*	5/4	52	11.5
1986	Tipulidae	23.6	*Ae. aegypti*	4/3	81	11.1
1986	Tipulidae	23.6	*Cx. restuans*	2/2	34	17.6

Table 3. Maintenance of *Erynia conica* in adult *Aedes aegypti* through 2 sets of mosquito-to-mosquito serial passages under laboratory conditions. Cumulative data presented for a 21-day observation period after exposure.

Passage	Number of trials (total/positive)	Total number of mosquitoes at risk	Percentage of mosquitoes with mycosis
Chaoboridae to *Ae. aegypti*	3/2	29	24.1
Ae. aegypti to *Ae. aegypti*:			
I	9/3	101	5.9
II	12/4	154	3.9
III	4/0	44	0.0
Tipulidae to *Ae. aegypti*	3/3	72	23.6
Ae. aegypti to *Ae. aegypti*:			
I	4/3	81	11.1
II	3/2	78	9.0
III	5/4	134	6.0
IV	4/2	127	9.4
V	10/6	259	3.9
VI	7/2	170	7.1
VII	4/1	92	6.5
VIII	10/5	283	3.5
IX	4/1	107	1.9
X	2/0	65	0.0

muscoid flies (Steinkraus and Kramer 1987, Mullens 1989).

How mosquito morphology and physiology influence susceptibility is also not clearly understood. Gol'berg (1970) found male *Cx. pipiens* were more susceptible to infection than were females; she also concluded that *Cx. pipiens* was probably the culicid species most susceptible to fungal infections because the composition of its cuticular lipids was favorable for fungal penetration. The difference in susceptibility between males and females to infection by *E. conica* was not determined in the present study. Steinkraus and Kramer (1987) concluded that host susceptibility may be determined by interspecific morphological differences of the hosts. Ecological barriers may also limit the range of hosts available for infection (Gustafsson 1965, Kramer 1980), but again, more work is needed to elucidate the factors that govern host susceptibility.

The fact that infected mosquitoes required an average of 7.3 days to die was a surprise. Given their small size, infected mosquitoes were expected to die sooner than would infected larger robust insects. Mullens (1985) found that the differences in incubation periods of *En. muscae*-infected house flies depended on both the sizes of the flies, as well as the amount of conidia they received. However, the incubation period of *E. conica* in *Ae. aegypti* may also have been influenced by the low temperature employed (15°C) as well. This temperature was selected to maximize the rate of infection (by increasing both the rates of conidial germination and cuticular penetration) and to minimize the activity of mosquitoes. Gol'berg (1970) obtained infected mosquitoes when exposed to conidial showers and maintained under temperatures ranging from 12.5 to 26.6°C with the optimal temperature being 20°C for 100% mortality; 72% infection occurred when *Cx. pipiens* were infected and maintained at 15°C. Kramer (1982, 1983) found all adult *An. stephensi* infected by *En. culicis* died 6 days after infection, and the majority of *Ae. aegypti* and *Cx. quinquefasciatus* were dead within 5–7 days at 20°C. Gol'berg

(1970) reported death of infected adult *Cx. pipiens* as early as 48 h after initial infection, but the temperature was not recorded. However, Steinkraus and Kramer (1987) found the sole adult *Ae. aegypti* they infected with *En. muscae* died 33 days after infection at 18–20°C.

The role of entomophthoracean fungi in mosquito control has been given little attention (Roberts 1977); only one attempt to control mosquitoes with these fungi has been clearly documented. A naturally occurring culicid entomophthoracean, *Entomophaga destruens* (Weiser and Batko) was released in Czechoslovakian potato cellars in 1966 to control populations of adult *Cx. pipiens* (Novák 1977) and for the following 8 years, mortality of infected mosquitoes ranged between 5 and 38%. However, whether that original mosquito population was free of disease and whether immigration or emigration in that population was regulated after application of *En. destruens* was not discussed by Novák (1977). Undoubtedly, more study is required to correctly assess the potential of entomophthoraceans as a microbial control agent of mosquitoes.

The potential of *E. conica* for the control of medically important mosquitoes (or other groups) should be explored. Studies could include efforts to increase the pathogenicity of *E. conica*, to test the susceptibility of adults or immatures of other mosquito species or to integrate it with other biological or chemical control agents.

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REFERENCES CITED

Descals, E., J. Webster, M. Ladle and J. A. B. Bass. 1981. Variation in asexual reproduction in species of *Entomophthora* on aquatic insects. Trans. Br. Mycol. Soc. 77:85–102.

Gol'berg, A. 1970. (Experimental infection of the family Culicidae with entomophthorosis: I. Species specificity of the fungus *Entomophthora* sp. II. Susceptibility of entomophthorosis of preimaginal stages and adult *Culex pipiens* L. mosquitoes.) Med. Parazitol. Parazait. Bolezni 39:472–478; 694–698 (in Russian).

Gol'berg, A. 1974. (Fungi of the family Entomophthoraceae near Moscow.) Mikol. Fitopatol. 8:421–422 (in Russian).

Gustafsson, M. 1965. On species of the genus *Entomophthora* Fres. in Sweden: classification and distribution. Lantbruks. Ann. 31:103–212.

Hywell-Jones, N. L. and J. Webster. 1986. Mode of infection of *Simulium* by *Erynia conica*. Trans. Br. Mycol. Soc. 87:381–388.

Kramer, J. P. 1980. *Entomophthora sepulchralis* Thaxter (Entomophthoraceae): its rediscovery and experimentally induced infections in muscoid flies. Mycopathol. 70:163–167.

Kramer, J. P. 1982. *Entomophthora culicis* (Zygomycetes: Entomophthorales) as a pathogen of adult *Aedes aegypti* (Diptera: Culicidae). Aquatic Insects 4:73–79.

Kramer, J. P. 1983. Pathogenicity of the fungus *Entomophthora culicis* for adult mosquitoes: *Anopheles stephensi* and *Culex pipiens quinquefasciatus*. J.N.Y. Entomol. Soc. 91:177–182.

Mullens, B. A. 1985. Host age, sex and pathogen exposure levels as factors in the susceptibility of *Musca domestica* to *Entomophthora muscae*. Entomol. Exp. Appl. 37:33–39.

Mullens, B. A. 1989. Cross-transmission of *Entomophthora muscae* (Zygomycetes: Entomophthoraceae) among naturally infected muscoid fly (Diptera: Muscidae) hosts. J. Invertebr. Pathol. 53:272–275.

Novák, D. 1977. Ein Versuch mit Mykose in der Stechmüchen bekämysfung. Ceské Mykol. 31:38–40.

Nowakowski, L. 1883. (Contribution to the knowledge of parasitic fungi causing mass death of insects.) Akad. Wiss. Krakau 8:153–183 (in German).

Roberts, D. W. 1977. Fungal pathogens, except *Coelomomyces*, of Culicidae (mosquitoes), pp. 147–172. *In*: D. W. Roberts and M. A. Strand (eds.), Pathogens of medically important arthropods. Bull. W.H.O. 55 (Suppl.).

Steinkraus, D. and J. P. Kramer. 1987. Susceptibility of sixteen species of Diptera to the fungal pathogen *Entomophthora muscae* (Zygomycetes: Entomophthoraceae). Mycopathol. 100:55–64.

Thaxter, R. 1988. The Entomophthoraceae of the United States. Mem. Boston Soc. Nat. Hist. 4:133–201.