JOURNAL OF INVERTEBRATE PATHOLOGY 43, 133–139 (1984)

Entomopathogenic Species of the Hyphomycete Genus *Tolypocladium*

ROBERT A. SAMSON

Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, The Netherlands

AND

GEORGE G. SOARES, JR.

University of Florida, AREC Lake Alfred, Florida 33850

Received January 18, 1983; May 23, 1983

Two entomopathogenic species of the hyphomycete genus *Tolypocladium* are described in detail. *Tolypocladium extinguens* sp. nov. was found on larvae of *Arachnocampa luminosa* (Diptera; Mycetophilidae) in caves in New Zealand. *Tolypocladium cylindrosporum* W. Gams is shown to be pathogenic to *Aedes sierrensis* and *A. australis* and is reported from *Plecia nearctica* (Diptera; Bibionidae). The occurrence of entomopathogenic species in this normally soil-borne genus is discussed. KEY WORDS: Fungal pathogens, *Tolypocladium cylindrosporum, T. extinguens,* sp. nov., Diptera, *Aedes*.

INTRODUCTION

The fungal genus *Tolypocladium* was erected by Gams (1971) with the type species *T. inflatum*. The genus contained three soil-borne species resembling those of the genus *Beauveria*. The species are characterized by white slow-growing cottony colonies. The conidiophores are usually short and bear lateral or terminal whorls of phialides, which have a swollen, sometimes cylindrical base and thin, often bent necks. The conidia are one celled, hyaline, and formed in slimy heads. In addition to the soil-borne species, Barron (1980, 1981) described two new species, *T. parasiticum* and *T. trigonosporum,* from rotifers. Recently Bissett (1983) revised the genus and added three species to it.

During the last years several *Tolypocladium* isolates from insects have become available. In a study of the natural enemies of the western treehole mosquito, *Aedes sierrensis,* in California, a fungal pathogen was observed (Sanders, 1972; Pinnock et al., 1973) and tentatively identified as *Beauveria tenella*. However, later studies showed that this fungus is *Tolypocladium cylindrosporum* (Soares et al., 1979; Soares, 1979). Weiser and Pillai (1982) have described a similar fungus from *Aedes australis* from New Zealand. Kish et al. (1974, 1977) isolated *T. cylindrosporum* from the love bug, *Plecia nearctica* (Diptera; Bibionidae), in Florida, and observed larval mortality after reinfectivity tests. In this paper a detailed description of this still little-known entomopathogenic fungus is given on the host and from pure cultures.

Recently another fungal pathogen on the glowworm, *Arachnocampa luminosa* (Diptera; Mycetophilidae), at Waitomo, New Zealand, was received at the Centraalbureau voor Schimmelcultures. This isolate also belongs to *Tolypocladium,* but could not be placed among the described taxa and is proposed as new.

Tolypocladium extinguens spec. nov. (Figs. 1,2)

Corpora hyphalia in hospite 10–25 × 4–5 μm. Coloniae in agaro malti (2%) post 14 dies, ad 1 cm diam, primum albae pulver-

0022-2011/84 $1.50

ulentae, deinde cremeae. Conidiophora ad 100 μm longa, stipites 3–5 μm lati, identidem verticillati, in verticillos phialidum exeuntes. Phialides e ventro inflato et collulo angusto constantes, 3.5–9 × 1.5–2.0 μm, nonnumquam subterminales collulum laterale ferentes vel semel sympodialiter proliferentes; conidia in capitulis mucidis congregata, continua, elipsoidea, hyalina, levia, 2.0–2.5 × 1.5–2.0 μm; in vitro majora ellipsoidae ad cylindrica, nonnumquan reniformia, 3.0–5.5 × 1.5–2.0 μm.

Holotypus in *Arachnocampa luminosa*, Morin Cave, Waitomo in Nova Zelandia, lectus a D. Williams et P. Johnston, in Herb. CBS praeservatus, cultus CBS 345.77, isolatus a G.J. Samuels.

Etymology. Lat. extinquere = to extinguish (the light of the glowworms).

Description on the Host

Host filled with hyaline hyphae, 1.5–4 μm wide and cylindrical hyphal bodies, 10–25 × 4–6 μm; surface covered by a dense felt of hyaline hyphae, 2.5–4 μm wide, bearing numerous complex branched conidiophores. Conidiophores up to 100 μm long, with stipe, 3–5 μm wide, bearing several regularly verticillate branches, which terminate in whorls of phialides. Phialides small, consisting of a swollen base and a thin neck, 3.5–9 × 1.5–2 μm, or sometimes reduced to a lateral conidioferous peg or with one sympodial proliferation, giving rise to conidia in slimy heads. Conidia one celled, ellipsoidal, hyaline, smooth walled, 2–2.5 × 1.5–2 μm; sometimes larger, subglobose to ellipsoidal conidia are also formed, 3.5–4 × 3–3.5 μm.

Description in Vitro

Colonies on agar media grow very slowly but sporulate abundantly within 7 days. On 2% malt extract agar reaches a diameter of 1 cm within 14 days, at first white powdery, later changing to cream. On YpSs and Sabouraud glucose agar similar growth but colony color changing to pale avellaneous. Conidiogenous structures as on the host but conidia larger and ellipsoidal to cylindrical, sometimes slightly curved, 3–5.5 × 1.5–2.3 μm. On mealworm agar conidia are both subglobose to ellipsoidal and more elongated and curved. On this medium the

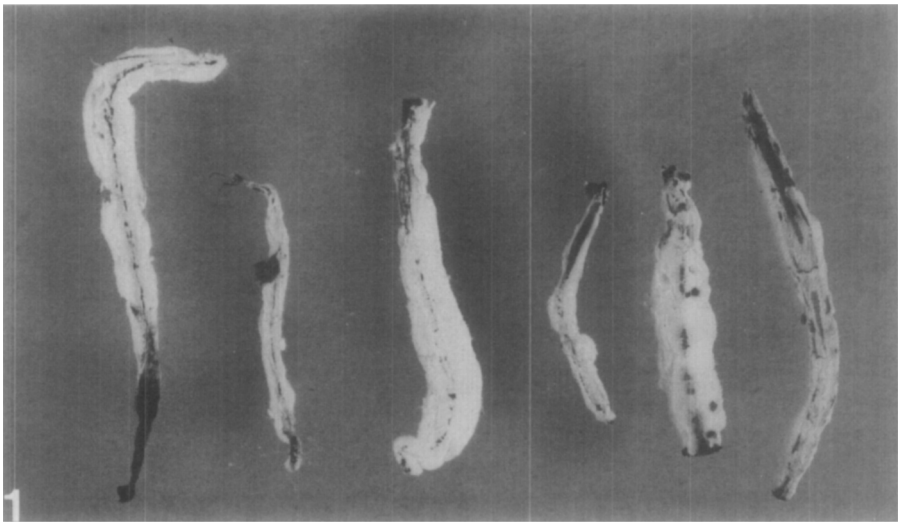

FIG. 1. Diseased larvae of *Arachnocampa luminosa* by *Tolypocladium extinguens* (×2.5)

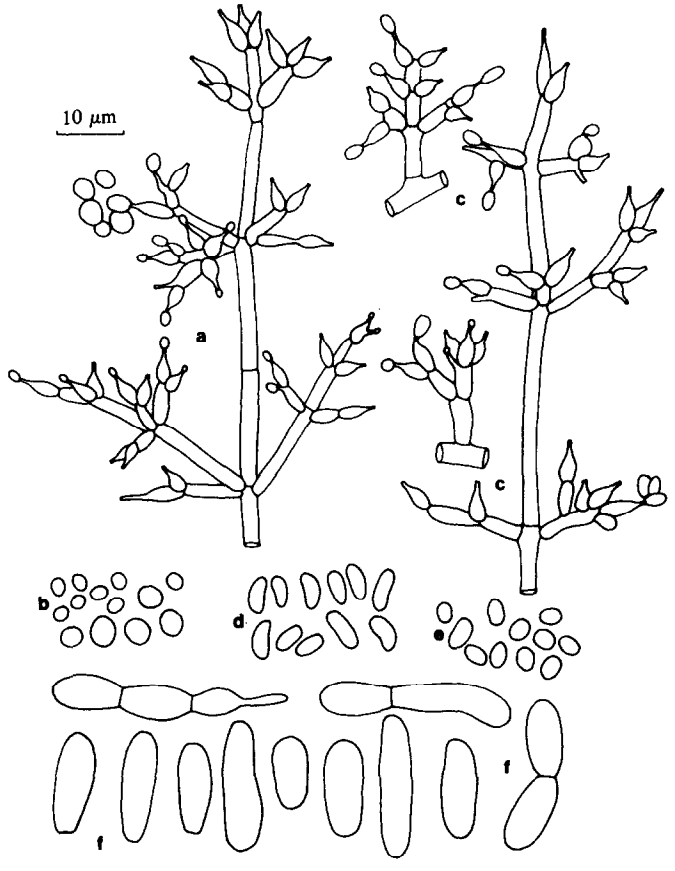

FIG. 2. Camera lucida drawing of *T. extinguens:* (a) conidiophore on host, (b) conidia on host, (c) conidiophore on mealwormagar, (d, e) conidia on mealwormagar, (f) hyphal bodies within *Arachnocampa* host.

colonies change from white to pale yellow-brown. Teleomorph not observed.

Material Examined

Holotype on glowworm, *Arachnocampa luminosa* (Skuse), Morin Cave, Waitomo, New Zealand. Leg. D. Williams and P. Johnston. 27.01.82, in herb. CBS; CBS 345.77, isolated from glowworm by G. J. Samuels.

The fungus seems to be endemic in glowworm populations, which normally occur in large numbers in the caves of Waitomo, New Zealand (Richards, 1964). Mortality of the larval and worm stages is usually less than 5%, but it may reach up to 15–20% (G. J. Samuels, pers. commun.).

T. extinguens differs from the described taxa by its very slow growth in pure culture and by its subglobose to ellipsoidal, sometimes kidney-shaped, conidia. Only one isolate was available for study.

Tolypocladium cylindrosporum W. Gams (Figs. 3–7)

Description on the Host

In mosquito larvae infection usually occurs through the external cuticle or the alimentary canal, most commonly the midgut. Following infection, hyphae grow and ramify first locally, then throughout the hemocoel. Ellipsoidal to cylindrical hyphal bodies variable in size and shape, 9–25 ×

3.5–5 μm, budding from hyphae on short pegs (Fig. 3). Hyphal bodies or "blastospores" (Fig. 4) carried to other parts of the hemocoel where they initiate new infection foci. Larvae (fourth instar *Aedes sierrensis*) begin to die 3–7 days after exposure to conidia when they have been extensively colonized by the fungus (Fig. 5). Larvae become packed with mycelia, giving the cadavers a pale pink to cream color and cheese-like consistency. Hyphae penetrate out of all parts of the body of the cadaver, growing outward in all directions, ramifying. On cadavers at the surface of the water, hyphae form a dense tuft of mycelia, usually at the tip of the siphon tube of culicine larvae (Fig. 6) and over the entire dorsal surface of anopheline larvae. Such an aerial mycelium produces conidiophores and conidia in slimy heads as on solid synthetic media. No sporulation occurs under water. For more detailed data on infectivity and pathogenesis see Soares (1982).

Description in Vitro

Colonies grow well on 2% malt extract or oatmeal agar attaining a diameter of 1.5–2.7 cm at 25°C in 10 days, forming a white, cottony felt. Conidiophores short, hyaline, relatively complex, consisting of verticillate branches bearing lateral or terminal whorls of phialides. Phialides consisting of a swollen cylindrical base. Conidia one celled, hyaline, smooth walled, cylindrical with rounded ends, often slightly bent, mostly 4–5 × 1.2–2.0 μm, occasionally longer, up to 8 μm. Teleomorph not observed.

Material Examined

CBS 989.73 isolated from *Plecia nearctica* by L. P. Kish, IFAS, University of Florida, Gainesville. CBS 612.80 = N8-35; 613.80 = N8-29III; 614.80 = N2-B.t.1 isolated from larvae of *Aedes sierrensis* in California by G. G. Soares. CBS 276.82, isolated from Larvae of *Aedes australis*, Qoint Point, Dunedin Otago Coast, New Zealand, by J. S. Pillai.

The morphology of the strains examined is similar to soil-borne isolates (see Gams, 1971) except the phialides and conidia are occasionally longer.

DISCUSSION

The isolation of *T. extinguens* from *Arachnocampa* represents an additional example of a *Tolypocladium* species infecting an insect host. The realization that species within the genus *Tolypocladium* may be entomopathogens is a recent one and to date natural infections by these fungi have been documented in only four insect species.

When Gams (1971) established the genus he examined mostly soil isolates. However, one strain of *T. inflatum* (CBS 716.70) was isolated from *Aradus cinnamomius* (Hemiptera; Aradidae). Whether this was acting as a true pathogen or as a saprophyte could not be established.

The paucity of isolates raises the question of why *Tolypocladium* species have not been described more often from insects in the past. One possibility is that *Tolypocladium* infections are rare in nature. However, several *Tolypocladium* spp. are relatively common inhabitants of soil (Bissett, 1983). Lundgren et al. (1978) found inhibitory effects of three *Tolypocladium* spp. to a number of other fungi. They suggested that this inhibitory ability may be of considerable importance for the survival of these fungi in the harsh environment of soil. The common occurrence in soil may also indicate that *Tolypocladium* spp. have an alternate host in the soil, e.g., nematodes. The morphological resemblance of *T. cylindrosporum* to the nematophagous species *Harposporium baculiforme* and *H. sicyodes* (Drechsler, 1959), for example, is very striking and the susceptibility of nematodes to *Tolypocladium* is worthwhile examining.

Another possible explanation for this scarcity of isolates may be the confusion of *Tolypocladium* spp. with other similar and better known fungal entomopathogens. How often in the past, for example, has in-

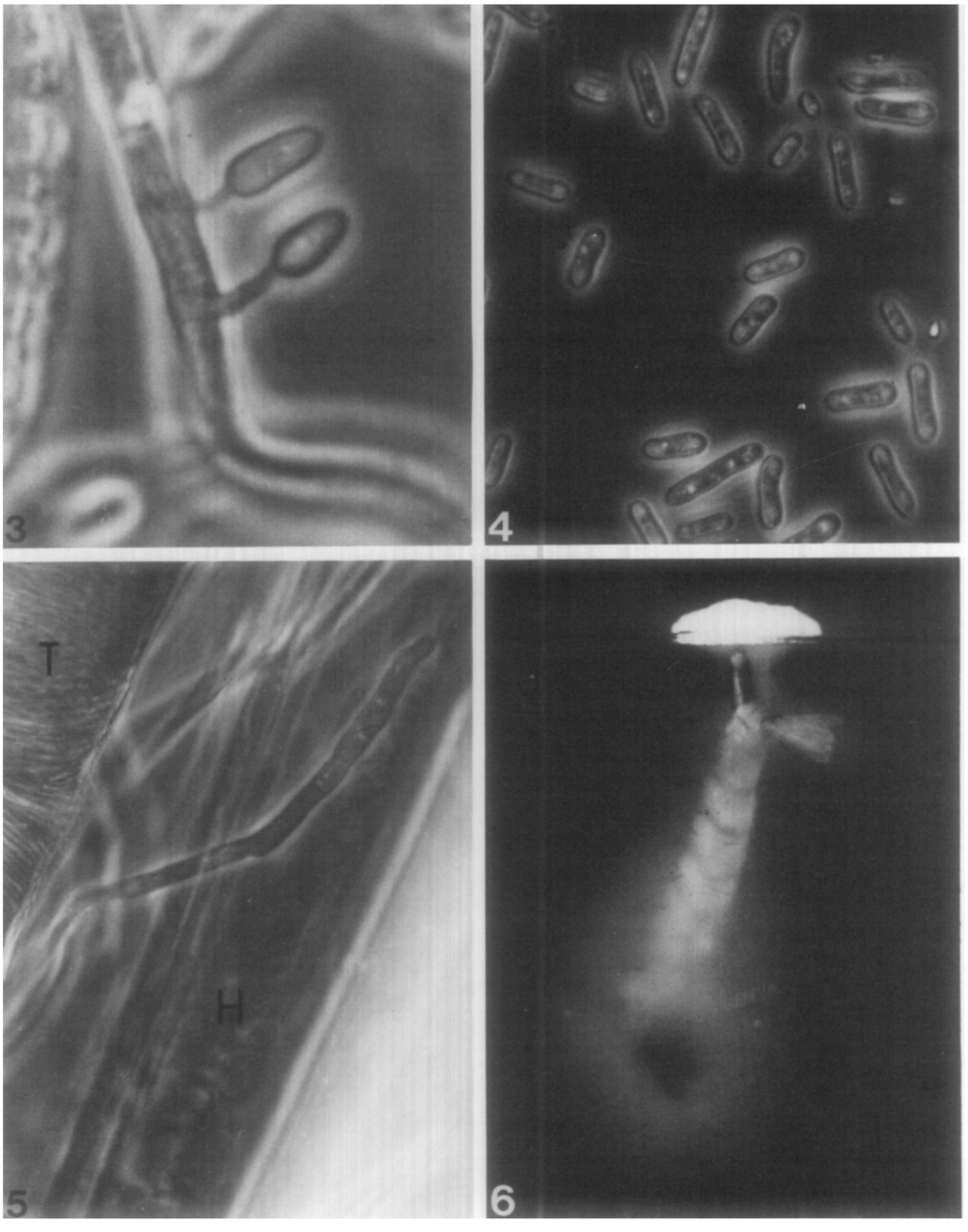

FIG. 3. Hyphal bodies being budded from hyphae of *Tolypocladium cylindrosporum* within the hemocoele of an infected *Aedes sierrensis* L2 larva. Phase contrast 640×.

FIG. 4. Hyphal bodies from the hemocoele of an infected *Aedes sierrensis* larva (L4). Phase contrast, 440×.

FIG. 5. Hyphae of *Tolypocladium cylindrosporum* within the siphon tube hemocoele of an *Aedes sierrensis* L2 larva. H, hemocoele; T, tracheal trunk within siphon tube. Phase contrast, 640×.

FIG. 6. *Aedes sierrensis* L4 larva killed by *Tolypocladium cylindrosporum*. Note sporulating surface tuft of mycelium arising from siphon and anal papillae of host.

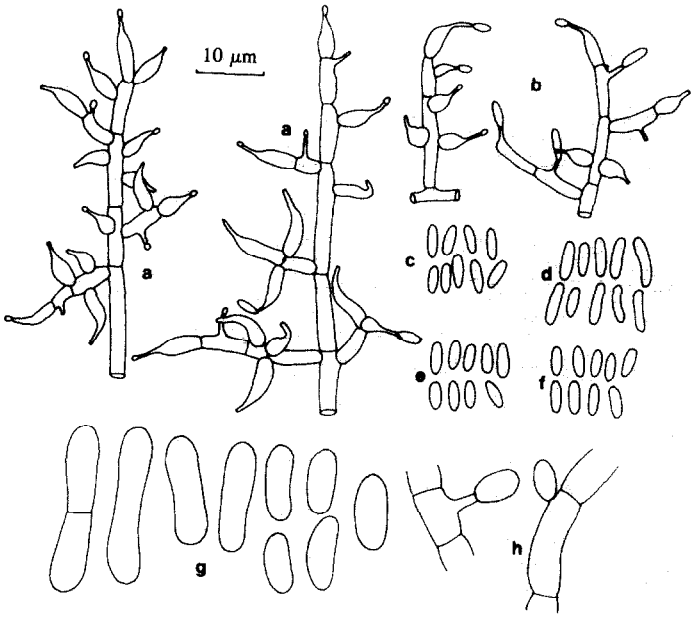

FIG. 7. Camera lucida drawings of *Tolypocladium cylindrosporum:* (a, b) conidiophores, (c–f) conidia, (c) CBS 614.80 from *A. sierrensis*, (d) CBS 613.80 from *A. sierrensis*, (e) 983.73 from *Plecia nearctica*, (f) 276.82 from *A. australis*, (g) Hyphal bodies within fourth instar larvae of *A. sierrensis*, (h) hyphal bodies budding from mycelium within host body.

fection caused by a *Tolypocladium* sp. been attributed to a *Beauveria* sp. or other hyphomycetous fungi of similar morphology? The similarities of *T. cylindrosporum* and *T. inflatum* to *Beauveria brongniartii* and *B. bassiana*, respectively, are particularly striking and it seems very possible that *Tolypocladium* isolates may have been misidentified as *Beauveria* species in the past (Soares et al., 1979). Culture morphology of all four species is very similar, with all four producing compact hyaline mycelia. All of these fungi also have flask-shaped conidiogenous cells with thin necks. Although *Beauveria* spp. produce sympodial conidia on an elongating rachis, while *Tolypocladium* spp. produce conidia on phialides, these differences are less evident in young cultures. Moreover, this distinction is not always as clear as might be supposed. In any event, it is important to point out the similarities of these fungi to *Tolypocladium* and stress the importance of careful examination of insect specimens infected by fungi producing a compact hyaline mycelium.

It is interesting to note that all cases of *Tolypocladium* infections recorded to date have been reported from Diptera of the same suborder, Nematocera, including the glowworm, *Arachnocampa*. This suggests a somewhat greater host specificity than is normally associated with the hyphomycetous entomopathogens such as *Beauveria* and *Metarhizium*. This is important if these fungi are eventually to be used in insect pest management since they would be less likely to infect certain beneficial insect species present in a given ecosystem. However, preliminary host range studies have shown certain nondipteran hosts to be susceptible to *T. cylindrosporum*. Notably, 100% of *Galleria mellonella* larvae were killed by this fungus 10 days after contamination of the integument with conidia (Soares, 1979).

All entomopathogenic strains of *Tolypocladium* have also been isolated only from

arthropod hosts occupying cryptic habitats, which are not easily sampled for pathogens. Thus casual isolations are less likely here. Indeed, the isolations from mosquitoes were made as part of long-term ecological studies of the host species involved. In the case of *A. sierrensis* surveys were conducted specifically for the purpose of isolating entomopathogens.

This underscores the importance of long-term field surveys of pest populations for the purpose of isolating pathogens. A greater emphasis on such surveys would no doubt result in the isolation of many new pathogen species and strains, and it seems reasonable to assume that such surveys, particularly of soil and aquatic habitats, would also produce new records of *Tolypocladium* species infecting insects.

ACKNOWLEDGMENTS

The authors thank Dr. G. J. Samuels and P. R. Johnston (DSIR, Auckland New Zealand) for sending the specimens of the killed glowworms and the data about the disease. Dr. W. Gams kindly provided the Latin translation, while Mr. Keith Seifert made linguistic corrections.

REFERENCES

BARRON, G. L. (1980). Fungal parasites of rotifers, a new *Tolypocladium* with underwater conidiation. *Canad. J. Bot.*, **58**, 439–442.

BARRON, G. L. (1981). Two new fungal parasites of bdelloid rotifers. *Canad. J. Bot.*, **59**, 1449–1455.

BISSETT, J. (1983). Notes on *Tolypocladium* and related genera. *Canad. J. Bot.* **61**, 1311–1329.

DRECHSLER, C. (1959). Two new species of *Harposporium* parasitic on nematodes. *J. Washington Acad. Sci.*, **49**, 106–112.

GAMS, W. (1971). *Tolypocladium*, eine Hyphomycetengattung mit geschwollene Phialiden. *Persoonia*, **6**, 185–191.

KISH, L. P., ALLEN, G. E., KIMBROUGH, J. W., AND KUITERT, L. C. (1974). A survey of fungi associated with the love bug, *Plecia nearctica* in Florida. *Fla. Entomol.*, **57** (3), 281–284.

KISH, L. P., TERRY, I., AND ALLEN, G. E. (1977). Three fungi tested against the love bug, *Plecia nearctica* in Florida. *Fla. Entomol.*, **60** (4), 291–295.

LUNDGREN, B. BAATH, E., AND SÖDERSTRÖM, B. E. (1978) Antagonistic effects of *Tolypocladium* species. *Trans. Brit. Mycol. Soc.*, **70**, 305–307.

PINNOCK, D. E., GARCIA, R., AND CUBBIN, C. M. (1973). *Beauveria tenella* as a control agent for mosquito larvae. *J. Invertebr. Pathol.*, **22**, 143–147.

RICHARDS, A. M. (1964). The New Zealand glowworm. *Stud. Speleol.*, **1**, 38–41.

SANDERS, R. D. (1972). Microbial mortality factors in *Aedes sierrensis* populations. *Proc. Calif. Mosq. Control Assoc.*, **40**, 66–68.

SOARES, G. G., JR., (1979). "Study of *Tolypocladium cylindrosporum* Gams, a New Naturally Occurring Fungal Pathogen of Mosquitoes (with Notes on *Beauveria bassiana* (Bals.) Vuill.)." Ph D. thesis, University of California, Berkeley.

SOARES, G. G., JR., (1982). Pathogenesis of infection by the hyphomycetous fungus, *Tolypocladium cylindrosporum* Gams in *Aedes sierrensis* and *Culex tarsalis* (Diptera: Culicidae). *Entomophaga*, **27** (3), in press.

SOARES, G. G. JR., PINNOCK, D. E., AND SAMSON, R. A. (1979). *Tolypocladium*, a new fungal pathogen of mosquito larvae with promise for use in microbial control. *Proc. Mosq. Control Assoc.*, **47**, 51–54.

WEISER, J., AND PILLAI, J. S. (1981) *Tolypocladium cylindrosporum* Gams (Deuteromycetes, Moniliales), a new pathogen of mosquito larvae. *Entomophaga*, **26** (4), 357–361.