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IN VITRO PRODUCTION OF HYPHAE OF THE GRASHOPPER PATHOGEN *ENTOMOPHAGA GRYLLI* (ZYGOMYCOTA: ENTOMOPHTHORALES): POTENTIAL FOR PRODUCTION OF CONIDIA

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Fungi of the *Entomophaga grylli* pathotype or species complex (Zygomycota: Entomophthorales, Entomophthoraceae) are ecologically obligate parasites of grasshoppers and locusts (Orthoptera); their host range includes many economically important orthopteran species worldwide (Carruthers et al. 1994; Bidochka et al. 1995). The species of the *E. grylli* complex (heretofore collectively called *E. grylli*) are highly pathogenic and can cause spectacular field epizootics. They have attracted interest as insect control agents, in both classical and augmentative biological control of Orthoptera (Carruthers et al. 1994; Sawyer et al 1994; Bidochka et al. 1995). Penetration through insect cuticle by germinating conidia is the universal invasion route of entomopathogenic fungi, including *E. grylli*. In the *E. grylli* species complex, no production of walled cells (hyphae or conidia) has been reported to occur on the few artificial media that support its stable growth. These conidia must be produced on hyphal conidiophores.

Upon landing on a host, *E. grylli* conidia can penetrate the cuticle with germ tubes and reach the hemocoel; once there, the fungus produces amoeboid protoplasts. These fragile, wall-less, amoeboid vegetative cells are the invasive phase in the orthopteran haemocoel (Ramoska et al. 1988; Carruthers et al. 1994). The wall-less condition of protoplasts seems to allow them to remain undetected and escape the insect's immune system (Roberts & Humber 1982). After proliferating and killing the host, protoplasts produce cell walls. This results, in some members of the *E. grylli* species complex, in walled hyphae that emerge through the host cuticle and produce conidiophores and infective conidia externally on the insect. A latent intermediate stage (resting spores) inside the host can also result after production of cell walls by protoplasts. External, aerial spores (conidia), the only infective stage in nature, are responsible for the often-rapid horizontal transmission leading to epizootics (Carruthers et al. 1994).

Unfortunately, *E. grylli* is fastidious regarding its artificial culture; these fungi will rarely grow on solid media. In practice, they can only be propagated either *in vivo* in their orthopteran hosts, or in complex liquid media such as Graces's insect tissue culture medium (Ramoska et al. 1988; Bidochka et al. 1995). Further, in Grace's and similar media these fungi have so far been reported to grow only as protoplasts, not hyphae. Protoplasts cannot be distributed directly as a biological control tool. Their lack of cell wall renders them very fragile, and they are neither infective upon application to the insect's cuticle nor upon ingestion. Thus, manipulation of *E. grylli* usually requires that considerable amounts of appropriate live insect hosts are available.

Entomophaga grylli protoplasts are infective upon injection in their hosts, and they induce typical lethal mycoses. In classical biological control projects, live, protoplast-injected orthopterans have been released into populations free of these diseases (Carruthers et al. 1994; Bidochka et al. 1995; Sánchez Peña et al.1996). Production of *E. grylli* infective conidia on artificial media would facilitate its use as a bioinsecticide, analogous to other entomopathogenic fungi. Manipulation of fungi in biocontrol is possible if stable, resistant walled cells (such as infective conidia, or at least hyphae) can be produced. Hyphae of entomopathogenic fungi such as *Beauveria*, *Hirsutella,* and *Metarhizium* spp. (Deuteromycota: Hyphomycetes), and *Zoophthora* and *Pandora* (Zygomycota: Entomophthorales) can be massively produced in artificial media. These hyphae subsequently produce infective walled cells (conidia) that cause lethal disease in insects in the field (Rombach et al. 1986; Wraight et al. 1986; Sánchez-Peña and Thorvilson 1991; McCoy 1996).

In the *E. grylli* species complex, no production of walled cells (hyphae or conidia) has been reported to occur on the few artificial media that support its stable growth (i.e., Humber & Hansen 2004). Herein I report the observation of the *in vitro* transition from protoplasts to walled, myceliar (hyphal) vegetative phase in *E. grylli* pathotype I.

Entomophaga grylli pathotype I (informally called *E. macleodii*) was isolated from *Camnula pellucida* collected in Alpine, Arizona, passed through *Melanoplus bivittatus* (Orthoptera: Acrididae), and reisolated in Grace's insect tissue culture medium plus 1% fetal bovine calf serum (FBS) (Invitrogen-GIBCO, Carlsbad, CA) and stored in liquid nitrogen for 13 years as strain ARSEF 770 (Humber & Hansen 2004). The author passed ARSEF 770 through *Camnula pellucida* and reisolated it, originating ARSEF strains 4948, 4949, 4950, 4952, and 4953 (strains deposited at PPRU, USDA, ARS, Ithaca, NY). All *in vitro* cul-

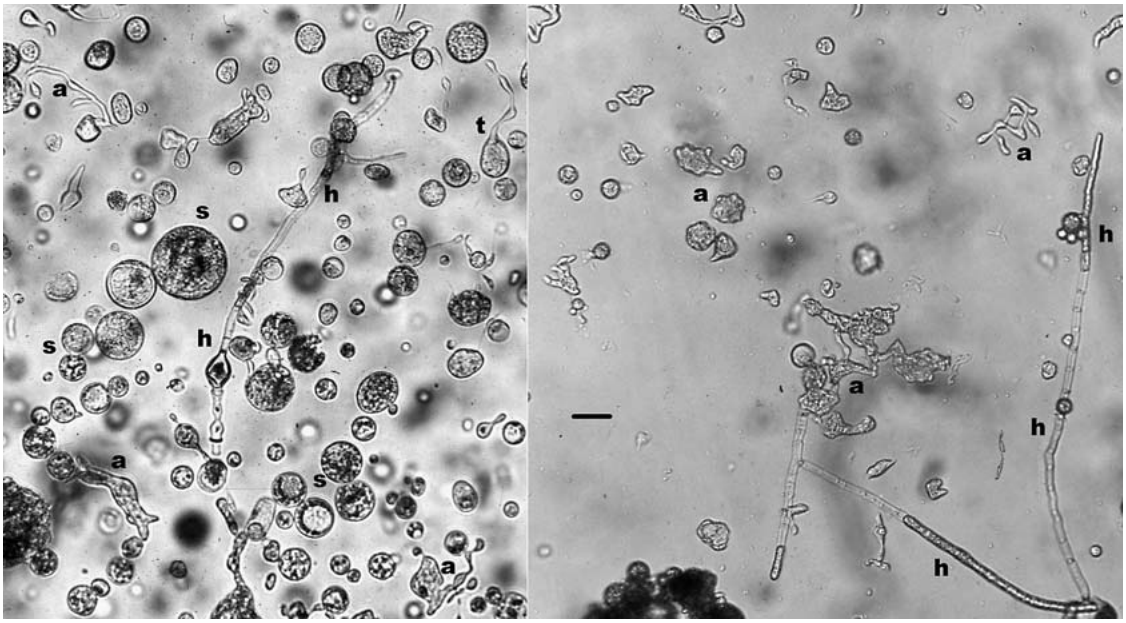

Fig. 1. *Entomophaga grylli* pathotype 1 cells from in vitro liquid culture: a, amoeboid and moniliform, motile protoplasts; s, spherical protoplasts; t, transition between amoeboid and spherical protoplasts; h, walled, septate hyphae. Notice typical entomophthoralean hyphae with apical cytoplasm and empty, septated spaced behind the tip. Bar = 20 microns.

tures were grown in 8-10 ml of Graces's medium plus FBS as described, in vented tissue culture flasks (9 × 5 × 2.5 cm) (TPP-MIDSCI, St. Louis, MO), under diffuse fluorescent and natural light.

After at least four weeks on artificial medium, numerous irregularly shaped, amoeboid as well as spherical protoplasts were observed in flasks from all these isolates (Fig. 1). Protoplasts are the propagules normally observed in such cultures of *E. grylli*. Some of the protoplasts assumed filamentous growth as walled hyphae. Hyphal growth was observed originating from both amoeboid and spherical protoplasts (Fig. 1 and 2). As described, *E. grylli* pathotype I changes from protoplasts to hyphae in its hosts. The hyphae then can produce the infective units, conidia, on conidiophores externally on the insect's surface. The transitions reported herein show that the first part of development leading to production of infective conidia (i.e., transition from protoplasts to hyphae) can be obtained in artificial media. It is possible that further differentiation to conidia can be obtained from artificial medium-grown biomass. This would facilitate the deployment of *E. grylli* fungi as biological control tools of Orthoptera. I expect that this report will stimulate searches towards completing the life cycle of these fungi *in vitro*, and towards the production of conidia from fungal biomass grown in artificial media.

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Summary

The species of the *Entomophaga grylli* complex that are fastidious grasshopper and locust pathogens have not been reported to complete their life

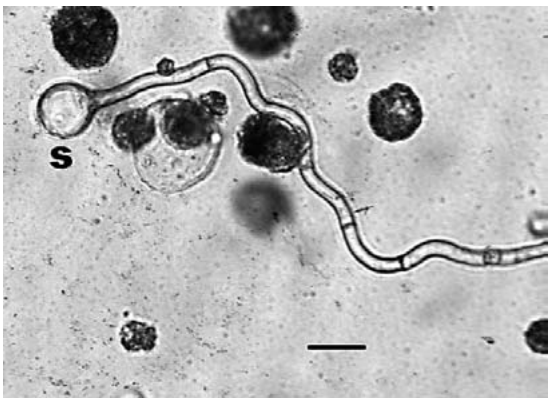

Fig. 2. *Entomophaga grylli* pathotype 1 walled, septate, empty hypha originating from now empty spherical cell. Bar = 35 microns.

cycles *in vitro*. The production of true hyphae in semisynthetic, liquid medium is described for *E. grylli* pathotype 1, a pathogen of banded-winged grasshoppers (Orthoptera: Oedipodinae). It is possible that induction of further differentiation of *E. grylli* hyphae grown on artificial medium might lead to production of infective conidia for biocontrol of pest Orthoptera.

References Cited

BIDOCHKA, M. J., S. R., WALSH, M. E. RAMOS, R. J. ST. LEGER, J. C. SILVER, AND D. W. ROBERTS. 1995. Fate of biological control introductions: Monitoring an Australian fungal pathogen of grasshoppers in North America. Proc. Nat. Acad. Sci. USA, 93 (2), 918-921.

CARRUTHERS, R. I., M. E. RAMOS, T. S. LARKIN, D. L. HOSTETTER, AND R. S. SOPER. 1997. The *Entomophaga grylli* (Fresenius) Batko species complex: Its biology, ecology, and use for biological control of pest grasshoppers. Memoirs Can. Entomol. Soc 171: 329-353.

HUMBER, R. A., AND K. S. HANSEN. 2004. Catalog of Strains. Plant Protection Research Unit, USDA-ARS, Ithaca, New York.

MCCOY, C. W. 1996. Pathogens of eriophyoids, pp. 481-490 *In* E. E. Lindquist, M. W. Sabelis, and J. Bruin [eds.], Eriophyoid Mites-Their Biology, Natural Enemies and Control. Elsevier Science B.V., Amsterdam.

RAMOSKA, W. A., A. E. HAJEK, M. E. RAMOS, AND R. S. SOPER. 1988. Infection of grasshoppers (Orthoptera: Acrididae) by members of the *Entomophaga grylli* species complex (Zygomycetes: Entomopthorales). J. Invertebr. Pathol. 52: 309-313.

ROBERTS, D. W., AND R. A. HUMBER. 1984. Entomopathogenic fungi, *In* D. W. Roberts, and J. R Aist [eds], Infection Processes of Fungi. Rockefeller Foundation. New York.

ROMBACH, M. C., R. M. AGUDA., B. M SHEPARD, AND D. W. ROBERTS. 1986. Entomopathogenic fungi (Deuteromycotina) in the control of the black bug of rice, *Scotinophara coarctata* (Hemiptera; Pentatomidae). J. Invertebr. Pathol. 48: 174-179.

SÁNCHEZ-PEÑA, S. R., R. CARRUTHERS, M-L. CUMMINS, J. CORREA, AND S. WRAIGHT. 1996. Entomopatógenos como agentes de control biológico clásico: experiencias con *Entomophaga grylli* en el control biológico de saltamontes y langostas. Memorias XIX Congreso, Sociedad Mexicana de Control Biológico, Culiacán, Sinaloa. p. 8-10.

SÁNCHEZ PEÑA, S. R., AND H. G. THORVILSON. 1991. The entomopathogenic fungus *Beauveria bassiana* for fire ant control in nursery stock, pp. 94-105 *In* M. Mispagel [ed.], Proc. 1991 Imported Fire Ant Conference, U. of Georgia, Atlanta, GA.

SAWYER, A. J., M. E. RAMOS, T. J. POPRAWSKI, R. S. SOPER, AND R. I. CARRUTHERS. 1997. Seasonal patterns of cadaver persistence and sporulation by the fungal pathogen *Entomophaga grylli* (Entomophthorales: Entomophthoraceae) infecting *Camnula Pellucida* (Orthoptera: Acrididae). Memoirs Can. Entomol. Soc. 171: 835-855.

WRAIGHT, S. P., S. GALAINI-WRAIGHT, R. I. CARRUTHERS, AND D. W. ROBERTS. 1986. Field transmission of *Erynia radicans* to *Empoasca* leafhoppers in alfalfa following application of a dry, mycelial preparation, p. 223 *In* R. A. Samson, J. M. Vlak, and D. Peters [eds.], Fundamental and Applied Aspects of Invertebrate Pathology. Proc. IV Int. Colloq. of Invertebrate Pathology. Wageninen, The Netherlands. 560 pp.