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# Interaction between *Paranosema locustae* and *Metarhizium anisopliae* var. *acridum,* two pathogens of the desert locust, *Schistocerca gregaria* under laboratory conditions

A.-K. Tounou [a,b,*], C. Kooyman [a], O.-K. Douro-Kpindou [a], H.-M. Poehling [b]

[a] *International Institute of Tropical Agriculture (IITA), Biological Control Centre for Africa, 08 B.P. 0932 Tri postal Cotonou, Benin*
[b] *Leibniz University of Hanover, Institute of Plant Diseases and Plant Protection, Herrenhäuser Str. 2, 30419 Hanover, Germany*

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**Abstract**

The interaction between two pathogens, the microsporidian *Paranosema locustae* Canning and the fungus *Metarhizium anisopliae* var. *acridum* Driver and Milner was studied under laboratory conditions in an attempt to develop an improved method of microbial control for the desert locust, *Schistocerca gregaria* Forskål. Fifth-instar locust nymphs, reared in the laboratory, were treated with various concentrations of one of the two pathogens or with both pathogens. The numbers of locusts killed were recorded each day and the production of pathogen spores within the dead locusts was assessed at the end (day 21) of each experiment. Locust nymphs treated with both *P. locustae* and *M. anisopliae* died sooner than nymphs infected with only one of the pathogens. At the lower concentrations of pathogen tested, the effects of the two pathogens were additive. At the higher concentrations the combined effects were synergistic. In terms of locust mortality, there was no evidence of any antagonistic effects between the two pathogens. However, the production of spores by *P. locustae* was reduced considerably when the host insects were infected also with *M. anisopliae*. For example, nymphs treated initially with *P. locustae* and then treated 3 and 10 days later with *M. anisopliae* produced 3–20 times and 2.5–8 times fewer spores, respectively, than nymphs treated only with *P. locustae*. Hence, in areas where *M. anisopliae* is applied, the natural persistence of *P. locustae* in the local grasshopper and locust populations may be diminished.


*Keywords:* Additive and synergistic effects; Biological control; Desert locust; *Metarhizium anisopliae*; *Paranosema locustae*; Spore production; Survival time

## 1. Introduction

The desert locust, *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) causes extensive damage to food crops in Africa and western Asia (Steedman, 1988; Showler, 1993) particularly during years with locust outbreaks. The current method for locust control is based on applying synthetic insecticides, a situation that is unlikely to change during outbreak years. Nevertheless, concerns about pesticide tolerance, environmental contamination and human safety have enabled researchers to look for alternative methods of control. Such methods include the use of biopesticides based on spores of fungi and microsporidia (Johnson et al., 1992; Johnson and Goettel, 1993; Inglis et al., 1995; Lomer et al., 1999; Lange et al., 2000; Lange, 2005). The LUBILOSA (Lutte Biologique contre les Locusts et Sauteriaux) programme has developed an oil formulation of the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* Driver and Milner (Deuteromycotina: Hyphomycetes) to control locusts and grasshoppers (Bateman et al., 1997; Lomer et al., 1999; Lomer et al., 2001).

*Paranosema locustae* (Canning) (Microsporidia: Nosematidae) was isolated from the African migratory locust,

* Corresponding author. Address: Leibniz University of Hanover, Institute of Plant Diseases and Plant Protection, Herrenhäuser Str. 2, 30419 Hanover, Germany. Fax: +49 511 7623015.
*E-mail address:* ktounou@gmail.com (A.-K. Tounou).

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*Locusta migratoria migratorioides* Reiche and Fairmaire (Orthoptera: Acrididae) by Canning in 1955 in a laboratory locust colony in England, and was originally described as *Nosema locustae* (Canning, 1955). *P. locustae,* as it is now known, has been shown to infect more than 100 species of the Orthoptera found in North and South America, Africa, Australia, China and India (Lange, 2005). Both laboratory and field studies indicate that younger grasshopper nymphs are more susceptible to *P. locustae* infections than older nymphs (Henry et al., 1973; Lockwood et al., 1999; Lange et al., 2000). In an earlier study, first-, second- and third-instar nymphs of *S. gregaria* were more susceptible to infections of *P. locustae* than fifth-instar nymphs (Tounou, 2007). Therefore, there is still a need to improve the speed and level of kill by these pathogens, particularly against late-instar nymphs.

The pathogens *P. locustae* and *M. anisopliae* do not kill insects as rapidly as synthetic insecticides. Nevertheless, combinations of microsporidian and fungal pathogens could help to improve the overall efficacy of locust and grasshopper control. A perceived advantage of such biopesticides is that the pathogens applied could recycle through new generation of acridids via vertical and/or horizontal transmission (Raina et al., 1995; Langewald et al., 1999). Such recycling might be hampered, however, by several factors, including other control agents (Solter et al., 2002; Pilarska et al., 2006). In the past, studies of host–pathogen interactions tended to focus on the direct interaction between one pathogen and one host. Under natural conditions, however, infections involving more than one pathogen are common (Hochberg and Holt, 1990; Koppenhöfer and Kaya, 1997; Ishii et al., 2002; Solter et al., 2002; Pilarska et al., 2006). Therefore, there is a need to understand not only how pathogens interact with the host insect but also how they interact with each other. In mixed infections, it is possible that the efficacy of one or both pathogens may be improved, suppressed or enhanced (Cox, 2001; Solter et al., 2002; Pilarska et al., 2006). The objective of the present study is to examine the levels of control that can be achieved by using combinations of two microbial entomopathogens, *P. locustae* and *M. anisopliae,* against nymphs of the desert locust *S. gregaria*. To do this, we studied under laboratory conditions (1) the compatibility and interactions between *P. locustae* and *M. anisopliae* var. *acridum* and (2) the production of spores of both *P. locustae* and *M. anisopliae* within locusts when the two pathogens were applied either on their own or together.

## 2. Materials and methods

### 2.1. Schistocerca gregaria

*Schistocerca gregaria*, obtained from the laboratory culture at IITA-Benin, were reared continuously from egg to adult at $31 \pm 1$ °C, with a 12:12 (L:D) photoperiod and at 85–90% RH (Bateman et al., 1997). The locust adults were provided with cassava leaves (*Manihot esculenta* Crantz (Euphorbiales: Euphorbiaceae)) as food. Fifth-instar nymphs (21 day-old) were used as the test insects in all experiments.

### 2.2. Paranosema locustae and Metarhizium anisopliae

Water suspensions of spores of *P. locustae* (isolated originally from *Melanoplus differentialis* Thomas (Orthoptera: Acrididae) in Dr. John Henry's laboratory at Montana State University, Bozeman) were provided by Dr. Lee Anne Merrill (M and R Durango, Inc. P. O. Box 886 Bayfield, CO 81122, USA). Generally the spores were stored for about a week at −20 °C before being used in the bioassays.

The isolate of *M. anisopliae* var. *acridum* (strain IMI 330189), that we used was isolated originally in Niger from *Ornithacris cavroisi* Finot (Orthoptera: Acrididae) and is the standard isolate used in locust biocontrol in Africa (Lomer et al., 2001). Conidia were produced in vitro at IITA-Benin, West Africa (Cherry et al., 1999). The viability of the spores within the *Metarhizium anisopliae* preparation was determined at the start of each experiment. This was done by stirring samples of conidia into a mixture (50:50) of ondina oil (Shell Pty, Ltd.) and pure paraffin oil (Jet A1 grade) and then streaking out a sample of the solution onto agar plates maintained at 25 °C. The numbers of conidia that germinated were counted 24 h later. Germination was >90% .

The experiments were replicated three times during a three week period. Fresh suspensions were prepared for each replicate. The numbers of spores within the various preparations were quantified using a Neubauer haematocytometer. Serial dilutions were then made with distilled water for the *P. locustae* preparation, and with the mixture (50:50) of ondina oil and pure paraffin for the *M. anisopliae* preparation, to produce the spore concentrations needed for the bioassays (Bateman et al., 1997).

### 2.3. Inoculation of hosts

Fifth-instar nymphs of *S. gregaria* were placed into 30 cm × 30 cm × 30 cm wooden-framed cages covered with wire mesh. The nymphs were then starved for 48 h before the experimental treatments were applied. The test insects were treated either with one pathogen (single treatment) or with both (mixed pathogen). As *P. locustae* requires considerable time to invade host tissue and reproduce (Henry, 1972) we did not apply the two pathogens on the same day. Instead, we allowed *P. locustae* to establish for either 3 or 10 days before applying the *M. anisopliae* treatments.

For the treatment with *P. locustae* alone, groups of locust nymphs, each containing 30 individuals, were provided with 10 g of wheat bran infused with one of three concentrations ($1 \times 10^4$, $1 \times 10^5$ and $1 \times 10^6$) of spores. The insects were allowed to feed on the spore-treated bran