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Susceptibility of immature stages of the locusts *Schistocerca gregaria* and *Locusta migratoria migratorioides* to the microsporidium *Johenrea locustae* and effects of infection on feeding and fertility in the laboratory

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Second instar nymphs of African migratory locust, *Locusta migratoria migratorioides*, and desert locust, *Schistocerca gregaria*, were tested for their susceptibility to the microsporidium pathogen *Johenrea locustae* (Lange et al. 1996, *Journal of Invertebrate Pathology*, 68, 28) in the laboratory. Spores of *J. locustae* were produced from live *L.m. migratorioides*, a conspecific to *L. migratoria capito*. *Locusta m. migratorioides* and *S. gregaria* were exposed to wheat seedlings sprayed with 20 mL of three concentrations (10^6, 10^7 and 10^8 spores mL^{-1}). Both second-instar nymphs of *L.m. migratorioides* and *S. gregaria* were susceptible to *J. locustae* infection at the three concentrations. There was no effect of concentration of the microsporidium on mortalities of *S. gregaria* (92–98%) 19 days postinfection. Adjusted mortality in *L.m. migratorioides* at 19 days post-exposure was 24, 43 and 80% at the corresponding treatment concentrations. The effect of infection on fecundity was tested on both *L.m. migratorioides* and *S. gregaria.* Spinach leaf discs were treated with different concentrations (0, 10^4, 10^5 and 10^6 spores mL^{-1}) of *J. locustae* and presented to female insects for 24 h. Female *L.m. migratorioides* surviving infection as nymphs laid significantly fewer egg pods than untreated controls at all levels of exposure. The number of eggs per female was also significantly lower in treated lots than in the controls. Higher spore concentrations also adversely affected egg hatching rate. The effect of *J. locustae* infection on feeding was tested on *S. gregaria*. There was a significant decrease in food intake among *S. gregaria* nymphs treated only at the high concentration (10^6 spores mL^{-1}).

Keywords: *Locusta migratoria migratorioides*; *Schistocerca gregaria*; *Johenrea locustae*; acridids; microsporidium; biological control; fecundity; feeding

Introduction

The microsporidium *Johenrea locustae* was first described by Lange, Becnel, Razafindratiana, Przybyszewski, and Razafindrafara (1996) from the migratory locust, *Locusta migratoria capito* (Sauss), from Madagascar. *Johenrea locustae* produces polysporophorous vesicles resulting in xenomas of fat body tissue associated with the digestive tract (Lange et al. 1996). Several species of microsporidia isolated from different acridids were investigated for use as potential control agents of locusts and grasshoppers (Bidochka and Khachatourians 1991; Johnson 1997). Microsporidian infections can cause mortality (Ewen and Mukerji 1980;

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Erlandson, Mukrji, Ewen, and Gillott 1985; Erlandson, Ewen, Mukrji, and Gillott 1986), reduce the rate of feeding (Oma and Hewitt 1984; Johnson and Pavlikova 1986), and reduce female fecundity (Henry, Tiahrt, and Oma 1973; Ewen and Mukerji 1980; Erlandson et al. 1986). Microsporidian infections also interfere with host metabolism and growth, and compete for vital energy supplies (Brooks 1988; Becnel and Andreadis 1999). *Paranosema (=Nosema) locustae* (Canning 1953) Slamovits, Williams, and Keeling 2003 (Lange 2005), for instance causes a debilitative, mostly chronic (i.e. prolonged course of infection) rather than an acute disease (cited by Lange and Cigliano 2005).

The potential of *J. locustae* for biological control of locusts and grasshoppers has not yet been fully investigated (Lange et al. 1996). The present study was therefore, carried out to determine the susceptibility of *Locusta migratoria migratorioides* (Reiche & Farmaire), conspecific host, and *Schistocerca gregaria* (Forskal), (non-host) to *J. locustae* and the effects of infection on feeding and fecundity.

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Materials and methods

Insects

Schistocerca gregaria and *L.m. migratorioides* were obtained from the ICIPE Animal Rearing and Quarantine Unit (ARQU). Insects were reared in groups in $50 \times 50 \times 50$-cm cages and maintained at $30 \pm 2^{\circ}$C, $50 \pm 10\%$ RH under a photoperiod of L12:D12. Insects were fed on wheat seedlings supplemented with wheat bran.

Microsporidium

Johenrea locustae inoculum was derived from infected, dessicated cadavers of *L.m. capito* stored at the insect pathology laboratory at Ambatobe, Antananarivo, Madagascar. Cadavers were surface-sterilized with sodium hypochlorite, rinsed thrice in sterile distilled water, and ground in a mortar. The mixture was then suspended in sterile distilled water and used to inoculate locust nymphs for pathogen multiplication. The inoculum was multiplied by passing it through approximately 100 second-instar *L. migratoriaoides* by feeding them with wheat seedlings sprayed with spore suspension. Spores were harvested by crushing dead insects using a mortar, adding sterile distilled water, and mixing with a magnetic stirrer. The suspension was sieved using fine netting, and the cadaver debris discarded. The recovered supernatant was centrifuged at 6000 rpm for 30 min. The pellet was washed with sterile distilled water and resuspended in this manner three times. The resulting inoculum was refrigerated at 4–6°C for less than 1 month before being used in bioassays. Viability of spores was tested using the sugar assay (Undeen and Vávra 1997).

Bioassays

Susceptibility of S. gregaria *and* L.m. migratorioides *to* J. locustae

Insects were exposed to concentrations of 0, 10^6, 10^7 or 10^8 spores mL^{-1}. Wheat seedlings were sprayed with 20 mL of *J. locustae* suspension using a Burgerjon's spray tower (1956). Treated wheat seedlings were allowed to dry for 20 min under a laminar flow cabinet and transferred into aluminum cages with sliding glass doors. Insects were then introduced into the cages and offered the food for 24 h. After 24 h, these seedlings were replaced with

another batch of similarly treated wheat seedlings for a total exposure of 48 h. Treatments were arranged in complete randomized blocks and consisted of 15 insects per replicate and the experiment was repeated six times. For the control group, wheat seedlings were treated with sterile distilled water. Test insects were maintained under room conditions (27–33°C) and 12D:12L. Following treatment, wheat seedlings were replaced with fresh ones every day. Mortality was recorded daily for up to 21 days. Five cadavers per replicate were selected at random, crushed and examined as fresh preparations in order to confirm microsporidian infection.

Fecundity experiment

The effect of *J. locustae* on the reproductive potential of female survivors' total egg production and egg hatching rate was investigated in both *L.m. migratorioides* and *S. gregaria*. Wheat seedlings were sprayed once with 20 mL of either 10^6, 10^7 or 10^8 spores mL^{-1} of *J. locustae* using a Burgerjon's spray tower and were then placed in rearing cages ($50 \times 50 \times 50$ cm). One hundred fifth-instar nymphs were introduced to each cage and maintained until they reached the adult stage, after which they were sexed. Twenty male/female pairs were transferred to clean rearing cages. Treatments were also arranged in complete randomized blocks and repeated four times. Control treatments consisted of wheat seedlings treated with sterile distilled water. Food was changed every day as described above.

Steel oviposition cups were filled with sand and sterilized for 24 h at 100°C. They were then placed in each cage after the first mating was observed. Egg pods were recovered every 2 days for 3 weeks and were divided into two groups. Eggs in the first group were incubated at room temperature and the number of hatchings recorded. Twenty nymphs per treatment were selected at random, transferred to aluminum cages ($20 \times 20 \times 20$ cm) and raised to the adult stage. Mortality was recorded. Five of the nymphs that emerged from these eggs were randomly selected and examined for the presence of spores after dissection. In the second group, destructive sampling was carried out to determine the number of eggs per pod. The experiment was replicated three times.

Effect of infection by J. locustae *on feeding*

This experiment was carried out only on *S. gregaria* nymphs. Both sides of spinach leaf discs (3 cm diameter) were sprayed once with 10 mL of *J. locustae* spore suspension using a Burgerjon's spray tower at one of the following concentrations: 10^4, 10^5 and 10^6 spores mL^{-1}. In the control treatments, leaf discs treated with sterile distilled water. Leaf discs were allowed to dry for 20 min under a laminar flow cabinet. Twenty insects were used for each treatment concentration. Insects were placed individually in plastic containers ($15.0 \times 10.5 \times 6.0$ cm) and maintained under the same conditions as described above. After 24 h exposure to treated leaf discs, they were replaced with fresh, untreated spinach leaf discs. Leaf discs were weighed before and after offering them to insects. Ten leaf discs were kept in similar rearing boxes in the absence of locusts under the same conditions to estimate moisture loss. Discs were weighed before and after being dried in an oven at 80–100°C for 24 h. The percentage of food eaten was calculated using the following formula: % food eaten = (dry weight eaten/(initial fresh weight of disc × proportion dry matter of disc)) × 100 (Schroeder 2004).

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Statistical analysis

Data on mortality were rank-transformed before ANOVA analysis. Lethal time mortality values (LT_{50} and LT_{90}) were estimated with repeated measures logistic regression using generalized estimating equations (GEE) (Stokes, Davis, and Koch 2000). All analyses were carried out using GENMOD procedure of SAS (1999–2001). Data on feeding and egg production were transformed to $\log_{10}(x+1)$ and subjected to analysis of variance and means were separated by Student–Newman–Keuls (SNK) test ($P=0.05$) using the ANOVA procedure of SAS (1999–2001). Data on percentage of egg hatching rate were arc sin-transformed prior to ANOVA analysis.

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Results and discussion

Susceptibility of S. gregaria *and* L.m. migratorioides *to* J. locustae

Mortality in the control treatments was less than 5% in both *L.m. migratorioides* and *S. gregaria*. There was no block effect between the experiments as revealed by a two-way analysis of variance (ANOVA): *L.m. migratorioides* ($F=0.87$; df $=3,15$; $P=0.5216$) and *S. gregaria* ($F=2.08$; df $=3,15$; $P=0.1246$). Both *S. gregaria* and *L.m. migratorioides* were susceptible to *J. locustae* infection at the three concentrations used. In the case of *S. gregaria*, there was no significant difference in mortality between the three concentrations of the microsporidium (Figure 1). There was, however, a dose–mortality response ($F=22.02$; df $=3,15$; $P=0.0001$) in the case of *L.m. migratorioides.* Mortality was 24, 43 and 80% at the concentrations of 10^6, 10^7 and 10^8 spores mL^{-1}, respectively, 19 days post-exposure (Figure 2). Fat body tissues of dead insects, taken at random and examined as fresh preparations, showed the presence of spores, thereby confirming *J. locustae* as the cause of mortality. For *S. gregaria,* the LT_{50} and LT_{90} at the highest concentration of 10^8 spores mL^{-1} were 12.7 and 15.1 days. The corresponding LT_{50} and LT_{90} for *L.m. migratoriodes* were 15.1 and 21.6 days, indicating lower virulence of *J. locustae* in its host species. Susceptibility of non-host insects to particular pathogens of related taxa has been reported by many workers. Several species of Orthoptera belonging to the families Melanoplinae, Oedipodinae, and Gomphocerinae have been reported to be susceptible to *Paranosema (=Nosema) locustae*, although originally described from *L.m. migratorioides*

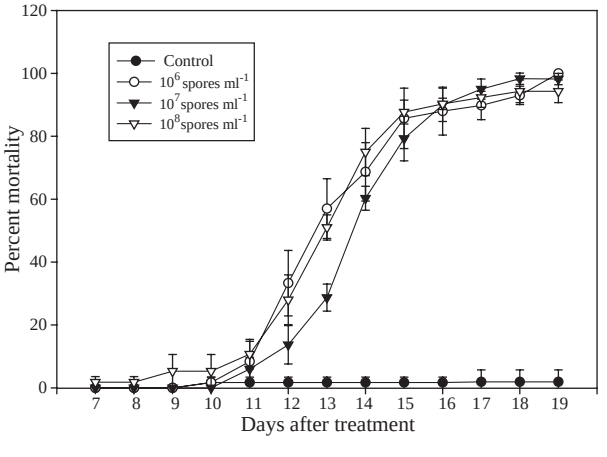

Figure 1. Mortality caused by the microsporidium *Johenrea locustae* to second-instar *Schistocerca gregaria.*

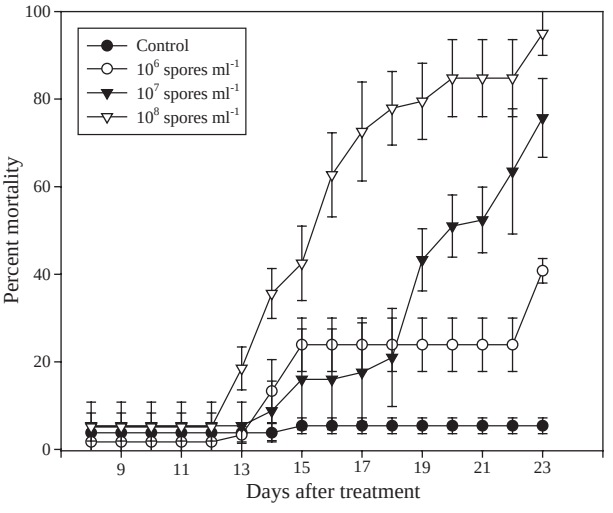

Figure 2. Mortality caused by the microsporidium *Johenrea locustae* to second-instar *Locusta migratoria migratorioides*.

(Henry 1971a). Immature stages of *M. sanguinipes*, *M. differentialis*, *M. bivittatus*, *M. femurrubrum* and *Schistocerca americana* have also been found to be susceptible to *Nosema cuneatum* that was isolated from *Melanoplus confusus* (Henry 1971b; Erlanson et al. 1986). However, Lange et al. (1996) tested two non-host Melanoplinae grasshoppers and found that they were not susceptible to *J. locustae* whose natural host is *L.m. capito* (Oedipodinae). No explanation could be found on why *S. gregaria* was more susceptible to *J. locustae* than *L.m. migratorioides*.

Effects of J. locustae *infection on reproduction potential of* L.m. migratorioides

All the *S. gregaria* treated as fifth-instar nymphs succumbed to infection by *J. locustae* at the adult stage at the three concentrations tested. No single mating was observed before their death. On the other hand, *L.m. migratorioides* survived infection up to adult stage and succeeded in mating although some individuals died during the mating. Female *L.m. migratorioides* in the control treatment produced more egg pods than did the treated ones ($F = 9.08$; df = 3,11; $P = 0.0091$); but there were no significant differences between the three concentrations (Table 1). The number of eggs laid per female was higher in the control than in the treated insects ($F = 13.36$; df = 3,15; $P = 0.0005$) but there was a significant difference between the concentration of 1.0×10^6 spores mL^{-1} and the other two concentrations (Table 1). The mean percentage egg hatching rate was higher in the controls (100%) than in the treated lots ($F = 10.81$; df = 3,15; $P = 0.001$); however, there was a significant difference between the treatments with the concentration of 1.0×10^8 spores mL^{-1} having the lowest egg hatching rate (Table 1). Reduction in fecundity following infection of acridid hosts by microsporidia has been documented elsewhere. Henry (1971a) noted reductions in numbers of egg pods in females of *M. bivittatus* and *M. differentialis* infected by a natural microsporidium-induced enzootic. Field studies have also shown that *A. locustae* infection reduced the number of eggs laid by *M. bivittatus* and *M. sanguinipes* (Henry 1972; Ewen and Mukerji 1980). However, Erlarndson et al. (1986) and Streett, Woods, and Onsager (1993) did not observe any effect of infection by microsporidia on fecundity of test-insects.

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Table 1. Reproductive potential of *Locusta migratoria migratorioides* following inoculation with *Johenrea locustae*. No *Schistocerca gregaria* females survived infection to reproduce.

Concentration (spores mL^{-1})	Pod/female (X ± SE)	Egg/female (X ± SE)	Mean % hatching rate (X ± SE)
Control	0.5 ± 0.1a	21.6 ± 0.5a	100a
1.0×10^6	0.2 ± 0.0b	8.6 ± 1.6b	65.2 ± 8.7b
1.0×10^7	0.1 ± 0.0b	3.6 ± 0.9c	78.9 ± 9.7b
1.0×10^8	0.2 ± 0.1b	3.3 ± 0.6c	34.5 ± 6.3c

Means within a column followed by the same letter are not significantly different by Student–Newman–Keuls ($P = 0.05$) test. Data were recorded over 20-day period and pooled together.

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No mortality was observed among insects that hatched from insects treated and maintained up to the adult stage. This could suggest that *J. locustae* is not transmitted transovarially. Infection of acridid progeny by microsporidia has been demonstrated under laboratory (Henry 1972; Erlandson et al. 1986; Raina, Das, Rai, and Khurad 1995) and field conditions (Ewen and Mukerji 1980).

Effects of J. locustae *infection on food consumption by* S. gregaria

The mean dry weight of food eaten by *S. gregaria* nymphs per day was 0.08 ± 0.01 mg in the control treatment, and 0.10 ± 0.02, 0.07 ± 0.01 and 0.04 ± 0.01 mg at the concentrations of 10^4, 10^5 and 10^6 spores mL^{-1}, respectively. The difference was only significant ($F = 9.94$; df = 3,27; $P = 0.0001$) at the higher concentration of 10^6 spores mL^{-1}. Reduction in food consumption following treatment with microsporidia has been reported in the case of *A. locustae* against *M. sanguinipes* (Johnson and Pavlikova 1986) and *M. differentialis* (Oma and Hewitt 1984).

The potential of *P. locustae* as an effective biological control agent of either short- or long-term suppression of grasshoppers was recently dismissed (Johnson and Dolinski, 1997); but should not be disregarded for long-term control in the classical biological control approach as demonstrated recently in Argentina (Lange and Cigliano 2005). *Johenrea locustae* is a recently discovered microsporidium from *L.m. capito* and therefore, its potential as biological control agent for acridids has yet to be evaluated. Our results indicate that it is sufficiently pathogenic to *S. gregaria* and merits further consideration. It causes substantial mortality of *S. gregaria* nymphs within 2 weeks, and killed all of them before they could reproduce.

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References

Becnel, J.J., and Andreadis, T.G. (1999), 'Microsporidia in Insects', in *The Microsporidia Andmicrosporidiosis*, ed. M. Wittner, Washington, DC: American Society of Microbiology, pp. 447–501.

Bidochka, M.J., and Khachatourians, G.G. (1991), 'Microbial and Protozoan Pathogens of Grasshoppers and Locusts as Potential Biocontrol Agents', *Biocontrol Science and Technology*, 1, 243–259.

Brooks, W.M. (1988), 'Entomogenous Protozoa', in *Microbial Insecticides, Vol. 5, Handbook of Naturally Occurring Pesticides*, ed. C.M. Ignoffo, Boca Raton, FL: CRC Press, pp. 1–149.

Burgerjon, A. (1956), 'Pulvérisation et poudrage au laboratoire par des préparations pathogenèses insecticides', *Annales Epiphytes*, 7, 675–684.

Erlandson, M.A., Mukrji, M.K. Ewen Al B., and Gillott, C. (1985), 'Comparative Pathogenicity of *Nosema acridophagus* Henry and *Nosema cuneatum* Henry (Microsporida: Nosematidae) for *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae)', *Canadian Entomologist*, 117, 1167–1175.

Erlandson M.A., EwenAl B., Mukrji, M.K., and Gillott, C. (1986), 'Susceptibility of Immature Stages of *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae) to *Nosema cuneatum* Henry (Microsporida: Nosematidae) and its Effects on Host Fecundity', *Canadian Entomologist,* 118, 29–35.

Ewen, A.B., and Mukerji, M.K. (1980), 'Evaluation of *Nosema locustae* (Microsporida) as a Control Agent of Grasshopper Populations in Saskatchewan', *Journal of Invertebrate Pathology*, 35, 295–303.

Henry, J.E. (1971a), 'Experimental Application of *Nosema locustae* for Control of Grasshoppers', *Journal of Invertebrate Pathology*, 18, 389–394.

Henry, J.E. (1971b), '*Nosema cuneatum* sp.n. (Microsporida: Nosematidae) in Grasshoppers', *Journal of Invertebrate Pathology*, 17, 164–171.

Henry, J.E. (1972), 'Epizootiology of Infection by *Nosema locustae* Canning (Microsporida: Nosematidae) in Grasshoppers', *Acrida*, 1, 111–120.

Henry, J.E., Tiahrt, K., and Oma, E.A. (1973), 'Importance of Timing, Spore Concentrations, and Level of Spore Carrier in Applications of *Nosema locustae* (Microporida: Nosematidae) for Control of Grasshoppers', *Journal of Invertebrate Pathology*, 21, 263–272.

Johnson, D.L. (1997), 'Nosematidae and other Protozoa as Agents for Control of Grasshoppers and Locusts: Current Status and Prospects', in *Microbial Control of Grasshoppers and Locusts*, eds. M.S. Goetteland D.L. Johnson, Memoirs of Entomological Society of Canada, 171, 375–389.

Johnson, D.L. and Dolinski, M.G. (1997), 'Attempts to increase the Prevalence and Severity of Infection of Grasshoppers with the Entomopathogen *Nosema locustae* Canning (Microporidia: Nosematidae) by Repeated Application', in *Microbial Control of Grasshoppers and Locusts*, eds. M.S. Goettel and D.L. Johnson, Memoirs of Entomological Society of Canada, 171, 391–400.

Johnson, D.L., and Pavlikova, E. (1986), 'Reduction in Consumption by Grasshoppers (Orthoptera: Acrididae) Infected with *Nosema locustae* Canning (Microspora: Nosematidae)', *Journal of Invertebrate Pathology*, 48, 232–238.

Lange, C.W. (2005), 'The Host and Geographical Range of the Grasshopper Pathogen *Paranosema* (Nosema) *locustae* Revisited', *Journal of Orthoptera Research*, 14, 137–141.

Lange, C.W., and Cigliano, M.M. (2005), 'Overview and Perspectives on the Introduction and Establishment of the Grasshopper (Orthoptera: Acridoidea) Biocontrol Agent *Paranosema locustae* (Canning) (Microsporidia) in the Western Pampas of Argentina', *Vedalia*, 12, 61–84.

Lange, C.E., Becnel, J.J., Razafindratiana, E., Przybyszewski, J., and Razafindrafara, H. (1996), '*Johenrea locustae* n.g., n.sp. (Microspora: Glugeidae): A Pathogen of Migratory Locusts (Ortrhoptera: Acrididae: Oedipodinae) from Madagascar', *Journal of Invertebrate Pathology*, 68, 28–40.

Oma, E.A., and Hewitt, G.B. (1984), 'Effect of *Nosema locustae* (Microsporida: Nosematidae) on Food Consumption in the Differential Grasshopper (Orthoptera: Acrididae)', *Journal of Economic Entomology*, 77, 500–501.

Raina, S.K., Das, S., Rai, M.M., and Khurad, A.M. (1995), 'Transovarial Transmission of *Nosema locustae* (Microsporida: Nosematidae) in the Migratory Locust *Locusta migratoria migratorioides*', *Parasitology Research*, 81, 38–44.

SAS Institute Inc. (1999–2001), *SAS/STAT User's Guide, Version 8.01,* Cary, NC: Author.

Schroeder, J.W. (2004), *Quality Forage-Storage, Sampling and Measuring*, NDSU Extension Service Circular AS-1255.

Stokes, M.E., Davis, C.S., and Koch, G.G. (2000), *Categorical Data Analysis Using the SAS System*, Cary, NC: SAS Institute, Inc.

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Streett, D.A., Woods, S.A., and Onsager, J.A. (1993), 'Vertical Transmission of a *Nosema* sp. (Microsporida: Nosematidae) Infecting a Grasshopper, *Chorthippus curtipennis* (Orthoptera: Acrididae)', *Environmental Entomology.*, 22, 1031–1034.

Undeen, A.H. and Vávra, J. (1997), 'Research Methods for Entomopathogenic Protozoa', in *Manual of Techniques in Insect Pathology*, ed. L.A. Lacey, London, UK: Academic Press Limited, pp. 117–151.

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