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Spore loads of *Paranosema locustae* (Microsporidia) in heavily infected grasshoppers (Orthoptera: Acridoidea) of the Argentine Pampas and Patagonia

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ABSTRACT

Paranosema locustae, an entomopathogen of grasshoppers and locusts, remains the only microsporidium registered as a biocontrol agent. After introductions from North America, it became established in grasshopper communities of Argentina. We measured the infection intensity of field collected, heavily infected male and female adults of individuals belonging to six grasshopper species, five melanoplines (Melanoplinae) (*Baeacris pseudopunctulatus*, *Dichroplus maculipennis*, *Dichroplus vittatus*, *Neopedies brunneri*, *Scotussa lemniscata*), and one gomphocerine (Gomphocerinae) (*Staurorhectus longicornis*). Average spore load among heavily infected grasshoppers ranged from $8.7 \pm 0.5 \times 10^7$ to $1.1 \pm 0.7 \times 10^9$. Only females of *B. pseudopunctulatus* and *S. longicornis* showed significantly higher spore loads than the males.

Paranosema locustae, an entomopathogen of grasshoppers and locusts that primarily infects the adipocytes of fat body tissue, remains the only microsporidium registered and commercially available for long-term pest control (Solter et al., 2012). Several factors allowed for the successful development of *P. locustae* as a grasshopper control agent. A broad host range among grasshoppers and locusts (more than 100 species), efficient horizontal transmission complemented with vertical transmission, moderate virulence that permits a high level of spore production, and the ability of spores to withstand frozen storage for prolonged periods are among the pathogen's traits considered to be favourable (Bardi et al., 2012; Lange, 1997; Lange and Cigliano, 2005; Lockwood et al., 1999). Spore production in a variety of hosts may be of relevance for persistence and dispersal of *P. locustae* in grasshopper communities.

After introductions from North America, *P. locustae* became established in grasshopper communities in areas of north-western Patagonia and the western Pampas of Argentina (Bardi et al., 2012; Lange and Azzaro, 2008). Field infections in establishment areas have been detected in 22 species of grasshoppers, and prevalence was often higher than reported for North American grasshopper populations (Bardi et al., 2012; Lange, 2010). Some estimates of field spore loads of *P. locustae* in Argentine grasshoppers have been reported, particularly for three of the most commonly affected grasshoppers, the melanoplines (Acrididae: Melanoplinae) *Baeacris punctulatus*, *Dichroplus elongatus*, and *Dichroplus pratensis* (Lange, 2010, 2003). Further field monitoring enabled us to measure the infection intensity by evaluating spore loads in more individuals of a variety of grasshopper species and the results are presented here. Although in North America and China the intensity of field infections have been categorized according to five-level scales based on number of spores observed in microscope fields (Bomar et al., 1993; Henry, 1972; Johnson, 1989; Shi et al., 2009), to our knowledge natural intensities estimated as total spore counts per individual have not been reported elsewhere.

Collection of live grasshoppers by means of net sweeping and detection of microsporidiosis by compound microscopy were performed as recently reported (Bardi et al., 2012; Lange and Azzaro, 2008). After detection of an infection in a grasshopper by tissue examination or whole-body homogenization in double distilled water, a 5 ml spore suspension was produced by filtering homogenate through cheesecloth, washing twice in double distilled water and centrifuging (90 g, 15 min) as generally described by Undeen (1998). Spore counts were performed using an improved Neubauer haemocytometer after the procedure by Undeen and Vávra (1997). Since the aim was to quantify the maximum spore production capability of *P. locustae* in different field-infected grasshopper species, only heavy infections as categorized by earlier workers for the same volume of homogenate (Ewen and Mukerji, 1980; Johnson, 1989) were counted. Differences between spore loads of males and f-

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Table 1
Mean (±SE), minimum, and maximum spore loads of *Paranosema locustae* in six field-infected species of grasshoppers in Argentina. Statistical differences between males and females are represented by letters after mean values.

Grasshopper species	Sex	*n*	Spore loads		
			Mean ± SE	Minimum	Maximum
Baeacris pseudopunctulatus	♀	17	$3.4 \pm 0.9 \times 10^8$ a	5.8×10^6	1.5×10^9
	♂	30	$1.4 \pm 0.3 \times 10^8$ b	9.0×10^5	4.8×10^8
Dichroplus maculipennis	♀	3	$1.1 \pm 0.7 \times 10^9$ a	1.9×10^8	2.4×10^9
	♂	5	$4.1 \pm 2.2 \times 10^8$ a	9.0×10^6	1.1×10^9
Dichroplus vittatus	♀	8	$2.9 \pm 1.0 \times 10^8$ a	1.8×10^7	9.0×10^8
	♂	8	$1.3 \pm 0.5 \times 10^8$ a	3.9×10^6	3.0×10^8
Neopedies brunneri	♀	5	$2.3 \pm 1.2 \times 10^8$ a	3.9×10^7	6.9×10^8
	♂	3	$4.1 \pm 2.0 \times 10^8$ a	1.7×10^7	6.6×10^8
Scotussa lemniscata	♀	5	$3.0 \pm 2.3 \times 10^8$ a	1.5×10^6	1.2×10^9
	♂	7	$3.7 \pm 1.0 \times 10^8$ a	7.0×10^7	6.9×10^8
Staurorhectus longicornis	♀	4	$1.0 \pm 0.3 \times 10^9$ a	4.8×10^8	1.9×10^9
	♂	3	$8.7 \pm 0.5 \times 10^7$ b	3.2×10^7	1.9×10^8

emales of each species were statistically evaluated with the Mann–Whitney U test, using XLSTAT 7.5.3 software (Addinsoft, 2005).

Spore loads of *P. locustae* were obtained for adult individuals of six species of grasshoppers, five melanoplines (*Baeacris pseudopunctulatus*, *Dichroplus maculipennis*, *Dichroplus vittatus*, *Neopedies brunneri*, *Scotussa lemniscata*), and one gomphocerine (Acrididae: Gomphocerinae, *Staurorhectus longicornis*) (Table 1). Average spore loads of heavily infected grasshoppers (±Standard Error) ranged from a minimum of $8.7 \pm 0.5 \times 10^7$ in male *S. longicornis* to a maximum of $1.1 \pm 0.7 \times 10^9$ in female *D. maculipennis*. The lowest individual spore load counted was 9×10^5 in a male *B. pseudopunctulatus*, and the highest was 2.4×10^9 in a female *D. maculipennis*. Fig. 1 shows an area of the haemocytometer corresponding to a *P. locustae* infection of approximate 5.5×10^7 in a male *B. pseudopunctulatus* individual. *B. pseudopunctulatus* and *S. longicornis* females produced significantly higher spore loads than males (MW-U $p < 0.05$) whereas there were no significant differences between sexes in the other four species (MW-U $p > 0.05$).

Average spore loads among heavily infected grasshoppers obtained in this study are congruent with values previously recorded for the melanoplines *B. punctulatus*, *D. elongatus*, and *D. pratensis* (Lange, 2003). As such, they revalidate the ability of *P. locustae*, regardless of host sex, to produce large quantities of spores under field conditions in several Argentine species of grasshoppers. Although *P. locustae* is orally infective, the alimentary canal and associated structures are not the primary target tissues for reproduction and spores are presumably not often passed out with feces, a possibility once noted as preliminary laboratory observations (Henry, 1972). Therefore, unless spores are disseminated in some way during the life of the host, they are probably contained in the carcass until the host decomposes following death. Consumption of infected grasshoppers by other grasshoppers, either still alive or recently dead, has been suggested to be a pathway for horizontal transmission of *P. locustae* in North American species of grasshoppers (Ewen and Mukerji, 1980; Henry, 1972; Henry and Oma, 1981; Lockwood, 1988). Unfortunately, cannibalism, necrophagy, and scavenging in general have not been studied in grasshoppers of Argentina. Given the close phylogenetic relationship and similar ecological roles between many of the components of the North and South American grasshopper fauna (Amédégnato et al., 2003; Chintauan-Marquier et al., 2011; Litzenberger and

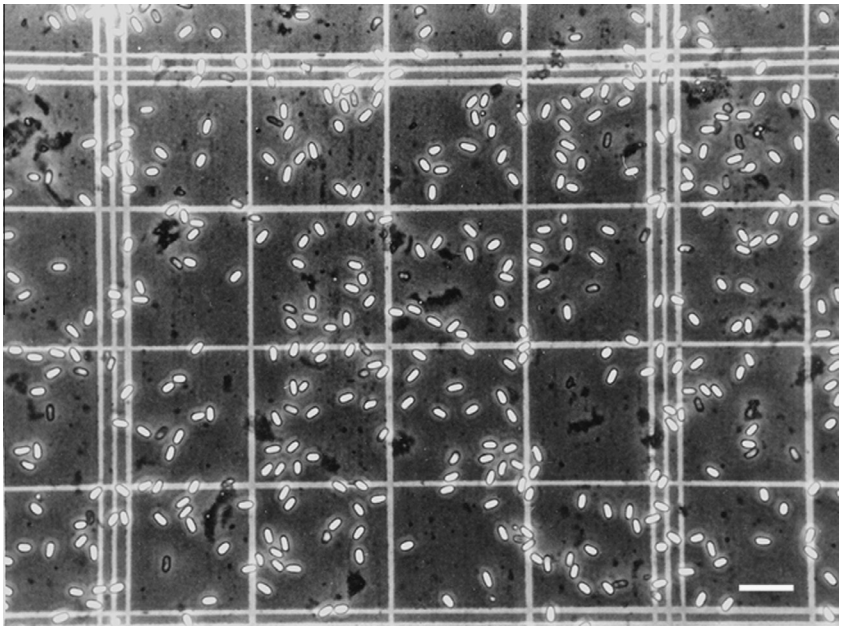

Fig. 1. Visual field of an improved Neubauer haemocytometer with spores of *Paranosema locustae* from one male of *Baeacris pseudopunctulatus* homogenized in 5 ml of water. Infection intensity near 5.5×10^7 spores. Bar = 20 μm.

Chapco, 2003), such feeding habits are likely to occur in Argentine species. They should be studied for an understanding of the transmission and host range of *P. locustae* in Argentina.

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