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SHORT COMMUNICATION

Prevalence and infection intensity of the biocontrol agent *Paranosema locustae* (Microsporidia) in field-collected, newly-associated hosts (Orthoptera: Acrididae: Melanoplinae)

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Host range, prevalence, and infection intensity of *Paranosema locustae* in grasshoppers at an establishment site in Patagonia, Argentina, were recorded. Results agreed with earlier observations at other introduction-establishment areas. Affected grasshoppers were melanoplines (*Baeacris punctulatus*, *Dichroplus elongatus*, *Dichroplus maculipennis*). Sporulation was not observed in instars I, II, and III.

Keywords: biocontrol agent; *Dichroplus*; grasshopper; melanoplines; microsporidia; *Paranosema (Nosema) locustae*

Recently, a new case of long-term persistence of the biocontrol agent *Paranosema locustae* (Canning) in melanopline grasshoppers of Argentina following its introduction from North America was documented (Lange and Azzaro 2008). Coupled with two earlier introduction-establishment events in the country (Lange and Cigliano 2005) and a similar outcome in China (Shi, Wang, Lv, Guo, and Cheng 2009), our findings corroborated the ability of the pathogen to recycle through the years in grasshopper communities having susceptible species other than natural hosts (as defined by Onstad et al. 2006). Data supporting a connection between *P. locustae* and long-term attenuation of grasshopper outbreaks are not available. However, according to ranchers, grasshoppers seem to no longer show the outbreaks that they had prior to the introductions or that still occur in similar areas where *P. locustae* was either not introduced or established. The fate of *P. locustae* in other countries where it was introduced (Australia, Cape Verde, Mali, Mauritania, Niger) is unknown. Within this context, it seems that the value of *P. locustae* as a long-term (i.e., through the years) biocontrol agent, as it was originally conceived (Henry 1990), might have not been fully appreciated, at least when target species are not natural hosts (i.e., new associations). Even when used against natural hosts, Onsager (1988) noted that the effects of *P. locustae* are subtle and accumulate slowly over time during seasons after the year of treatment, a characteristic that turn traditional insecticide-based criteria not adequate for its evaluation. Unfortunately, aside from a few cases (Bomar,

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Lockwood, Pomerinke, and French 1993; Johnson and Dolinski 1997), the agent was evaluated under the 'chemical control paradigm' which is not appropriate for slow-acting entomopathogens (Inglis, Goettel, Erlandson, and Weaver 2007).

Although *P. locustae* may increase mortality, what actually counts, as with most microsporidia (Becnel and Andreadis 1999), are the sublethal-type effects. These include reduction of fertility and longevity, delayed development, decreased activity, reduced feeding, molting abnormalities, flying difficulties, and disruption of aggregation (Lange and Cigliano 2005). In this sense, it is relevant to record the persistence, prevalence, host range, and infection intensity of *P. locustae* in areas where it was not originally present.

During the 2008–2009 season, and using sweep nets (Larson, O'Neill, and Kemp 1999), grasshopper samplings were conducted at the new establishment site (Loncopué, Neuquén province, northwestern Patagonia). Samples were frozen (-32°C) for later examination by homogenization or dissection (Lange and Henry 1996; Undeen and Vávra 1997). *Paranosema locustae* is readily recognized under the microscope (400×, 1000×) from other microsporidia associated to Argentine grasshoppers (Lange and Azzaro 2008; Sokolova, Lange, Mariottini, and Fuxa 2009). When sporulation was reached, infection intensity was estimated according to Henry's category (1972), employed by several authors (Johnson 1989; Bomar et al. 1993; Habtewold, Landin, Wennergen, and Bergman 1995). Infections were rated as trace, light, moderate, and heavy, based on the number of spores per microscopic field (400×). In the absence of sporulation, early-stage infections were diagnosed by presence of proliferative stages (meronts).

As shown in Table 1, infections were found in older nymphs (instars IV, V, VI) and adults of three of the four species of melanoplines collected, *Baeacris punctulatus*, *Dichroplus elongatus*, and *Dichroplus maculipennis*. No infections were detected in younger nymphs (instars I, II, III) of these species, nymphs and adults of the melanopline *Scotussa lemniscata*, and species of other subfamilies, the Gomphocerinae *Borellia bruneri* ($n=997$) and *Borellia pallida* ($n=434$), and the Copiocerinae *Aleuas lineatus* ($n=33$). Infections ranged from some meronts in IV instar nymphs of *D. elongatus* and *D. maculipennis* (Figure 1) to heavy infections in adults of the three affected melanoplines. *Paranosema locustae* ranged in prevalence from 2.5% in IV instars of *D. maculipennis* to 50% in adults of *B. punctulatus*. However, the highest prevalence in a larger sample was 21.5% in adults of *D. elongatus* ($n=65$).

Results for older nymphs and adults agree in terms of affected hosts, not affected hosts, and prevalence with the accumulated knowledge of the pathogen in Argentina (Lange and Cigliano 2005; Lange and Azzaro 2008). *Dichroplus elongatus, D. maculipennis* and *B. punctulatus*, along with another melanopline, *Dichroplus pratensis*, not recorded in Loncopué, had been previously recognized as the species with highest susceptibility among Argentine grasshoppers. The opposite (absence of susceptibility) held true for *B. bruneri*, *B. pallida*, and *A. lineatus* which were never found infected (Lange 2005). Lack of detection of *P. locustae* in *S. lemniscata* in 2008–2009, albeit present during the previous season (Lange and Azzaro 2008), could be related to the small sample size for the species. Prevalence values were within the range of those recorded for melanoplines in the western Pampas (Lange and Cigliano 2005).

Table 1. Prevalence and infection intensity of *Paranosema locustae* in nymphs (instars I to V–VI), and adults of four melanopline grasshoppers at Loncopué, northwestern Patagonia, Argentina.

Species and development stage	Collection date			
	11-18-08	12-09-08	01-09-09	02-18-09
Dichroplus elongatus				
I	0 (41)	–	–	–
II	0 (52)	–	–	–
III	0 (19)	0 (2)	–	–
IV	–	**13.3** (30) **(2T, 2m)**	–	–
V	–	**4.8** (42) (**2T**)	0 (5)	–
Adult	–	0 (1)	**14.7** (34) **(2L, 2M,1H)**	**21.5** (65) **(2L, 7M, 5H)**
Total	0 (112)	**8.0** (75)	**12.8** (39)	**21.5** (65)
Dichroplus maculipennis				
I	0 (73)	–	–	–
II	0 (184)	–	–	–
III	0 (102)	–	–	–
IV	–	**2.5** (39) (**1m**)	–	–
V, VI	–	**5.5** (73) (**4L**)	0 (8)	–
Adult	–	0 (2)	**6.8** (133) **(6L, 3H)**	**4.9** (123) **(4L, 2H)**
Total	0 (359)	**4.4** (114)	**6.4** (141)	**4.9** (123)
Baeacris punctulatus				
I	0 (15)	–	–	–
II	0 (16)	–	–	–
III	0 (43)	0 (3)	–	–
IV	–	0 (10)	–	–
V	–	0 (32)	–	–
Adult	–	**50** (4) (**2L**)	**17.8** (28) **(2L, 2M, 1H)**	**28.6** (7) (**2L**)
Total	0 (74)	**4.1** (49)	**17.8** (28)	**28.6** (7)
Scotussa lemniscata				
I	0 (14)	–	–	–
II	0 (33)	–	–	–
III	0 (8)	–	–	–
IV	–	0 (4)	–	–
V	–	0 (10)	0 (1)	–
Adult	–	–	0 (5)	0 (7)
Total	0 (55)	0 (14)	0 (6)	0 (7)

In each column, the first number indicates prevalence (percent infection), followed by the number of grasshoppers collected-examined, and the number and intensity of infections (**T**, trace infection; **L**, light infection; **M**, moderate infection; **H**, heavy infection; **m**, meronts only infection).

One aspect of interest is the apparent absence of sporulation in younger nymphs. Spores, sporoblasts or sporonts were not detected in the three initial instars of the melanoplines (Table 1). It might be argued that meronts were not observed either, but

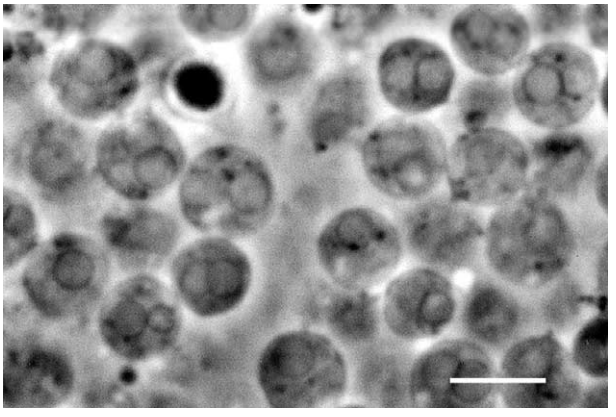

Figure 1. Proliferative stages (meronts) of *Paranosema locustae* isolated from fat tissue of a fourth instar nymph of *Dichroplus elongatus* from Loncopué, Neuquén province. Scale bar = 5 μm.

since they were observed in instars IV of *D. elongatus* and *D. maculipennis*, it is highly likely that meronts must have been present in some younger nymphs, albeit in a low number and too scattered for detection. The possible absence of sporulation in younger nymphs does not agree with observations in natural hosts of *P. locustae* in the field and laboratory. Following applications of *P. locustae* in New Mexico, Bomar et al. (1993) observed spores in progeny younger nymphs of two *Melanoplus* species, and suggested that they were capable of causing mortality in first and second instar nymphs. Raina, Das, Rai, and Khurad (1995) reported conspicuous sporulation (mean spore loads: $4.1 \times 10^3 - 7.0 \times 10^5$) and associated mortality (43.2–83.3%) in progeny younger nymphs of the Oedipodinae *Locusta migratoria* in the laboratory. Unfortunately, at present we do not have explanations for the lack of sporulation in younger nymphs of melanoplines from Loncopué, but it might well be related to the new association condition of pathogen and hosts. For instance, vertically transmitted infections, the source of disease at the beginning of each new season (Lange and Cigliano 2005), might take longer to develop in the newly-associated hosts than in natural ones. Anyway, regardless of the reason why sporulation seems not to occur in younger nymphs in Loncopué, its absence would suggest a lack of *P. locustae*-associated mortality early in the grasshopper life cycles, because in most microsporidia, disease pathology and mortality is normally proportional to the number spores generated (Dunn, Terry, and Smith 2001). If such is the case, the eventual attenuation of outbreaks reported by ranchers at establishment areas of *P. locustae* should be mostly due to sublethal effects, as originally envisaged during its development as a biocontrol agent.

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