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The Fate of *Nosema locustae* (Microsporida: Nosematidae) in Argentine Grasshoppers (Orthoptera: Acrididae)

CARLOS E. LANGE[1] AND MARÍA L. DE WYSIECKI[1]

Centro de Estudios Parasitológicos y de Vectores (CEPAVE), Universidad Nacional de La Plata (UNLP)/CONICET, Calle 2 No. 584, 1900 La Plata, Argentina

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Surveys to detect *Nosema locustae*, a microsporidian pathogen of orthopterans introduced in Argentina several times from 1978 to 1982 to control pest grasshoppers, were conducted during the 1994 and 1995 seasons at locations in Buenos Aires and La Pampa provinces. A total of 7535 grasshoppers collected from 13 different sites were examined. Infected grasshoppers were found at 4 of the sites, 2 of them 75 km from the closest introduction site. Infections were diagnosed in 10 species: 7 Melanoplinae (*Baeacris punctulatus, Dichroplus elongatus, D. pratensis, D. vittatus, Neopedies brunneri, Scotussa daguerrei,* and *S. lemniscata*), 2 Gomphocerinae (*Staurorhectus longicornis* and *Rhammatocerus pictus*), and 1 Romaleidae (*Diponthus argentinus*). Prevalence of infection ranged from 0.7% in *D. pratensis* to 33.3% in *R. pictus* and averaged 7.9%. *B. punctulatus* appeared as the most susceptible species to infection. *N. locustae* is well established in grasshopper communities of central Argentina. Some comments are included about concerns that were raised on the use of exotic agents for the control of native pests. © 1996 Academic Press, Inc.

KEY WORDS: Microsporidian; Protozoan; Argentina; biological control.

INTRODUCTION

Nosema locustae Canning is a microsporidian pathogen of orthopterans that is used as an agent for grasshopper control (Henry and Oma, 1981). It infects the fat tissue, thereby disrupting the host's intermediary metabolism and competing with it for energy reserves. Pathogenicity is expressed in many ways, among which are increased mortality rates (Lockwood and Debrey, 1990), reduced fecundity (Ewen and Mukerji, 1980), delayed development (Henry, 1969a), decreased activity (Bomar *et al.*, 1993), and reduced food consumption (Johnson and Pavlicova, 1986). *N. locustae* was registered by the United States Environmental Protection Agency (EPA) in 1980 and is the only protozoan commercially produced as a microbial insecticide.

Following the standard procedure of broadcasting wheat bran bait treated with spores (Henry and Oma, 1981), *N. locustae* was experimentally introduced between 1978 and 1982 in natural and improved pastures in the surroundings of eight localities in Argentina, five in Buenos Aires province (Gorchs, 1978, 1980; Casbas, 1979; General Lamadrid, 1981; Coronel Suárez, 1981; Coronel Pringles, 1981), two in La Pampa province (Macachín, 1982; Santa Rosa, 1982), and one in Chubut province (Gualjaina, 1981, 1982) (Ronderos and Rossi, personal communication) (Fig. 1). The spores used were produced either by the USDA-ARS Rangeland Insect Laboratory (Bozeman, MT) or by Bio-Ecologists, Inc. (Denver, CO). Samples of grasshoppers (100–300 net beats along transects in the plots) were taken 24–48 h before each treatment. The grasshoppers in the samples were examined by the homogenization method (Henry *et al.*, 1973) to determine the presence/absence of microsporidian infections. *N. locustae* was never found in the thousands of grasshoppers that constituted the pretreatment samples (Ronderos and Rossi, personal communication).

Regretably, the short-term effects of these introductions will remain unknown because no reports or publications were produced, and density and infectivity data are not available. Similarly, no efforts were expended to evaluate the long-term outcome of the introductions, and the fate of *N. locustae* in grasshopper communities of Argentina also remained unknown. Almost 10 years after the last introduction, *N. locustae* was found parasitizing three species of grasshoppers in areas of two provinces (Lange, 1992). The purpose of this paper is to provide further information regarding the establishment of *N. locustae* as an additional control factor in grasshopper communities of central

[1] Researchers from CIC and CONICET, respectively.

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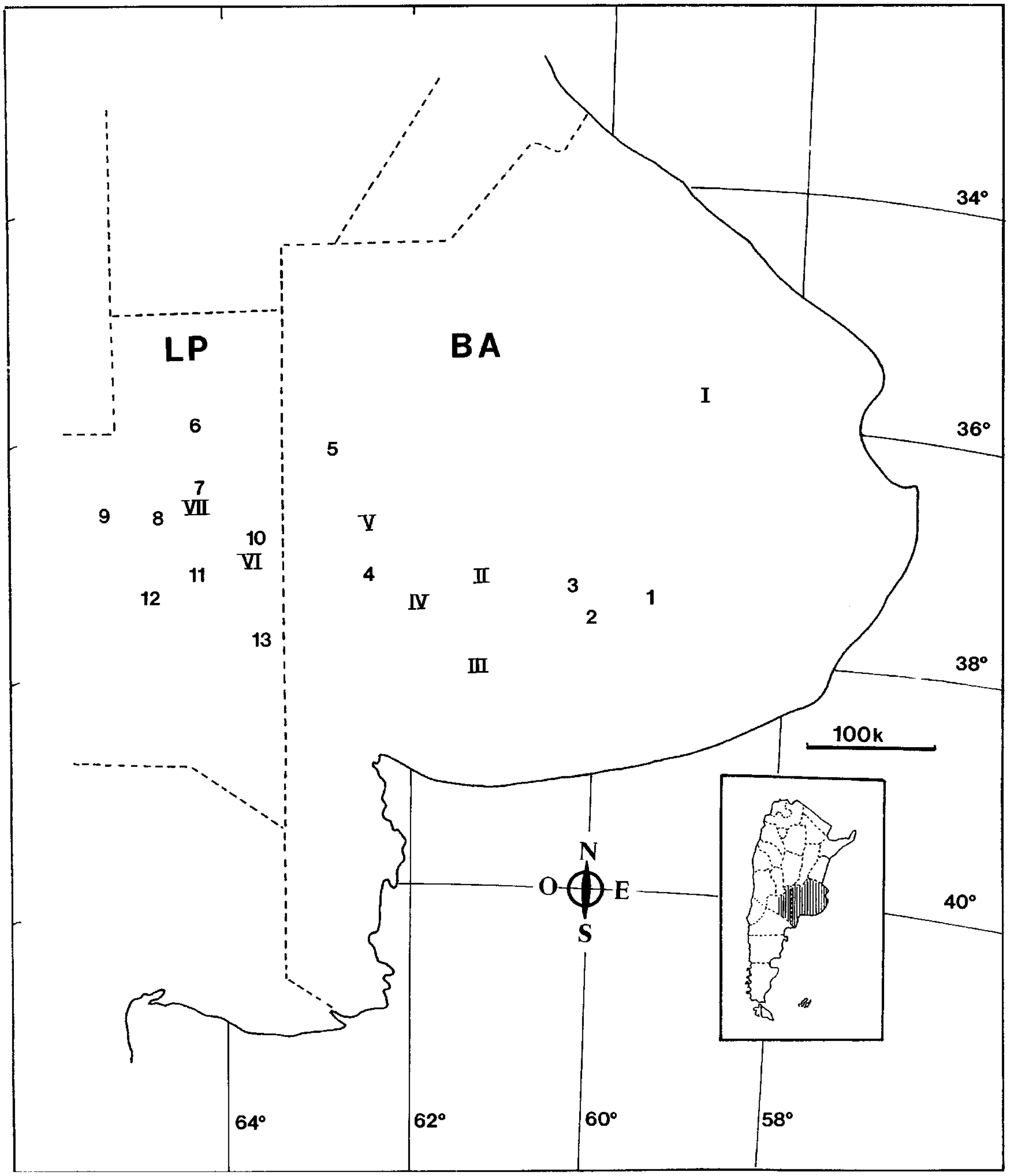

FIG. 1. Sites of introduction of *Nosema locustae* (Roman numerals) and sites of sampling of grasshoppers (Arabic numerals) in Buenos Aires and La Pampa provinces, Argentina. Sites of introduction: I, Gorchs; II, General Lamadrid; III, Coronel Pringles; IV, Coronel Suárez; V, Casbas; VI, Macachín; VII, Santa Rosa. Sites of sampling: 1, María Ignacia; 2, Coronel Bunge; 3, El Luchador; 4, 20 km east of Carhué; 5, 30 km southwest of Trenque Lauquen; 6, Eduardo Castex; 7, 15 km north of Santa Rosa; 8, 30 km west of Toay; 9, El Durazno; 10, 20 km north of Macachín; 11, 10 km north of Padre Buodo; 12, 14 km west of General Acha; 13, 20 km south of Guatraché.

Argentina by presenting data on host range, prevalence, and geographical dispersion.

MATERIALS AND METHODS

A resurrection of the grasshopper problem in some areas of the pampas grasslands during the 1994–1995 seasons caused increased concern among authorities and ranchers, which in turn prompted the opportunity to conduct surveys for pathogen detection. Samples of grasshoppers (nymphs and adults) were taken from 13 different sites, 5 in Buenos Aires province (20 km east of Carhué, Coronel Bunge, El Luchador, María Ignacia, 30 km southwest of Trenque Lauquen) and 8 in La Pampa province (Eduardo Castex, El Durazno, 14 km west of General Acha, 20 km south of Guatraché, 15 km north of Santa Rosa, 10 km north of Padre Buodo, 20 km north of Macachín, 30 km west of Toay) (Fig. 1). No

TABLE 1

Occurrence of *Nosema locustae* in Grasshopper Species of Buenos Aires and La Pampa Provinces, Argentina

Site/date	*B. punctulatus*	*D. argentinus*	*D. elongatus*	*D. pratensis*	*D. vittatus*	*N. brunneri*	*R. pictus*	*S. daguerrei*	*S. lemniscata*	*S. longicornis*
20 km north of Macachin										
1/26/94				6.9 (72)		9.4 (32)[a]				4.8 (21)
12/21/94	0.9 (111)									
2/07/95	8.5 (234)		4.1 (49)	12.5 (96)						
15 km north of Santa Rosa										
2/24/94	10.3 (29)									
Eduardo Castex										
12/21/94	1.6 (185)[b]			0.7 (147)[b]	2.3 (43)[b]					
2/08/95	8.7 (92)	25.0 (4)	1.4 (69)	2.8 (106)	4.8 (45)					1.4 (71)
Trenque Lauquen										
2/24/94	19.1 (47)						33.3 (3)			
2/09/95	6.9 (72)		1.2 (85)					7.7 (13)	9.1 (11)	7.3 (41)

Note. The first number indicates prevalence (% of infection), and the second indicates number of insects examined.
[a] Two nymphs were infected.
[b] One nymph was infected.

samples were collected in Chubut province. One to four samples from each of these sites were obtained along transects with beating nets during the two-season period of the study (January–February 1994 and December 1994–February 1995).

Once in the laboratory, the samples were frozen, and the species, stage of development (as adults or nymphs), and sex of each grasshopper were determined. For disease diagnosis, the homogenization method described by Henry *et al.* (1973) was employed, but trace infections were not considered. Frequently, when a grasshopper was suspected of being infected before freezing, it was dissected, and pieces of tissues and organs were removed for microscopic examination. Developmental stages and spores were observed as fresh mounts with Ringer's solution (Poinar and Thomas, 1984) or distilled water and as Giemsa-stained smears prepared according to the procedure by Wang *et al.* (1991).

RESULTS

The grasshoppers collected ($n = 7535$) were *Aleuas lineatus* Stal (1.0% of the total), *Allotruxalis strigata* (Bruner) (0.66%), *Amblytropidia australis* Bruner (0.01%), *Atrachelacris unicolor* Giglio-Tos (0.01%), *Baeacris punctulatus* (Thunberg) (12.23%), *Chromacris* sp. (0.05%), *Dichroplus bergii* (Stal) (0.15%), *D. elongatus* Giglio-Tos (30.0%), *D. maculipennis* (Blanchard) (0.01%), *D. pratensis* Bruner (20.0%), *D. vittatus* Bruner (4.83%), *Dichroplus* sp. (0.23%), *Diponthus argentinus* Pictet & Saussure (0.06%), *Euplectotettrix* sp. (0.65%), *Leiotettix pulcher* Rehn (1.9%), *Neopedies brunneri* (Rehn) (4.0%), *Parorphula graminea* Bruner (4.4%), *Rhammatocerus pictus* (Bruner) (3.0%), *Scotussa lemniscata* (Stal) (1.34%), *S. daguerrei* Liebermann (1.18%), *Staurorhectus longicornis* Giglio-Tos (14.0%), *Scotussa* sp. (0.18%), *Xyleus laevipes* (Stal) (0.01%), and *Zoniopoda tarsata* (Serville) (0.10%).

Table 1 summarizes the results of the surveys conducted for this study. The pathogen was encountered in 10 species of grasshoppers collected at 4 of the 13 sampling sites: 20 km north of Macachín, 15 km north of Santa Rosa, Eduardo Castex, and 30 km southwest of Trenque Lauquen. Grasshopper species that showed infection were *B. punctulatus*, *D. argentinus*, *D. elongatus*, *D. pratensis*, *D. vittatus*, *N. brunneri*, *R. pictus*, *S. daguerrei*, *S. lemniscata*, and *S. longicornis*. At all four sites where *N. locustae* occurred, individuals of *B. punctulatus* were found to be infected. Infected *S. longicornis* and *D. elongatus* were present at 3 sites, while infected *D. pratensis* was present at 2. Infections in the remaining species were restricted to individuals belonging to just 1 of the sites. Prevalence of infection ranged from 0.7% in *D. pratensis* of Eduardo Castex (December 21, 1994) to 33.3% in *R. pictus* from Trenque Lauquen (February 24, 1994) and averaged 7.9%. Table 2, which was compiled using data from this study and from Lange (1992), lists the species of grasshoppers examined that were not found to be infected with *N. locustae*.

DISCUSSION

Identification. Since ultrastructural or molecular studies of isolates from the different grasshopper species were not conducted, it might be argued that the pathogen cannot be unequivocally identified as *N. locustae*. However, all available evidence makes it difficult to think that a species other than the intro-

TABLE 2

Species of Argentine Grasshoppers in Which Infections by *Nosema locustae* Have Not Been Found

Acrididea: Acridinae
Allotruxalis strigata (Bruner)
Metaleptea brevicornis (L.)
Parorphula graminea (Bruner)
Acrididae: Melanoplinae
Atrachelacris unicolor Giglio-Tos
Baeacris pseudopunctulatus Ronderos
Dichroplus bergii (Stal)
D. maculipennis (Blanchard)
D. vittiger Bruner
Leiotettix pulcher Rehn
Scotussa cliens (Stal)
Acrididae: Gomphocerinae
Amblytropidia australis (Bruner)
Laplatacris dispar Rehn
Scyllinops brunneri (Rehn)
Sinipta dalmani Stal
Acrididae: Copiocerinae
Aleuas lineatus Stal
Romaleidae: Romaleinae
Chromacris speciosa (Thumberg)
Elaeochlora viridicata (Serville)
Xyleus leavipes (Stal)
Zoniopoda tarsata (Serville)

Note. Compiled from surveys in Lange (1992) and in the present study.

duced *N. locustae* is responsible for the infections for the following reasons: (1) Although spore appearance alone does not warrant an accurate identity, the size and shape of spores, including megaspores, isolated from argentine grasshopper species did not differ from what is known for *N. locustae* (Henry and Oma, 1981). (2) All developmental stages observed were diplokaryotic, sporogony appeared disporoblastic, and sporophorous vesicles were never seen. (3) The adipose tissue was the site of infection and the histopathology was typical of *N. locustae* (Henry and Oma, 1981). (4) When inoculated into susceptible grasshopper species (*Dichroplus* spp.), the spores caused the typical patterns of mortality and infectivity (Henry, 1990) known for *N. locustae* (Lange, unpublished observation). (5) As mentioned earlier, the pathogen was never found in pretreatment samples (Ronderos and Rossi, personal communication). (6) The pathogen was never encountered in samples from areas relatively far from application sites but with grasshopper communities having several susceptible species (southeast and far north Buenos Aires province). The only other known microsporidium from Argentine grasshoppers, *Perezia dichroplusae* Lange, is easily distinguished from *N. locustae* by its much smaller spores, the presence of moniliform haplokaryotic sporonts, polysporoblastic sporogony, and development in the Malpighian tubules (Lange, 1987a).

Host range. The host range of *N. locustae* in grasshoppers of Argentina was greatly expanded. Until 1992 only three species of grasshoppers (*D. pratensis, D. vittatus, N. brunneri*) were known to be infected by *N. locustae* (Lange, 1992). Our study demonstrates that at least seven more species (*B. punctulatus, D. argentinus, D. elongatus, R. pictus, S. daguerrei, S. lemniscata,* and *S. longicornis*) are naturally susceptible. This widened host range, observed many years after the last introductions, confirms its establishment in grasshopper communities of central Argentina.

Seven of the ten species that had infections were Acrididae in the subfamily Melanoplinae (*B. punctulatus, D. elongatus, D. pratensis, D. vittatus, N. brunneri, S. daguerrei,* and *S. lemniscata*). Surveys and studies carried out in North America (Henry, 1969b; Henry *et al.*, 1973), where *N. locustae* is native, also indicated that the melanoplines show a relatively high degree of susceptibility to infection. The infections in *S. longicornis* and *R. pictus* represent the first natural records for grasshoppers in the subfamily Gomphocerinae. Aside from the infected specimens of *D. argentinus* reported here, only one other species of Romaleidae was reported to be infected (Henry, 1969b).

B. punctulatus was the only species with individuals showing infections in all localities where *N. locustae* occurred. This observation suggests that it is the most naturally susceptible species in the sampled grasshopper communities. While most species are univoltine, *B. punctulatus* has two generations per season when conditions are favorable (COPR, 1982) and this might be the explanation for *N. locustae* widespread occurrence. Infected *B. punctulatus* have developed from eggs from field-collected grasshoppers where *N. locustae* was present, showing that vertical transmission, either transovarial or transovum, does occur (Lange, personal observation).

We list the species of grasshoppers in which no *N. locustae* was found. When dealing with the fate undergone by a recently introduced organism, particularly if introduction was deliberately oriented to a preconceived goal, establishing the absence of the introduced organism in a given potential host might be as informative as determining its presence. If caution is exerted, this information might prove useful in the future.

Prevalence. So much time elapsed between introductions and the observed prevalences that it is best if we compare them with natural occurrence elsewhere in the world. Such information seems to be available for only North America and India. Extensive surveys conducted throughout the western United States and Canada demonstrated that natural infections were normally less than 1%, with rare peaks of 2 to 5% (Henry and Oma, 1981; Ewen, 1983). Prevalences monitored in India were 1 to 2%, sporadically reaching

5% (Raina *et al.*, 1987). Disregarding those instances where insect samples were low (*D. argentinus* in Eduardo Castex; *R. pictus, S. lemniscata,* and *S. daguerrei* in Trenque Lauquen), the prevalences registered in central Argentina still fluctuated around relatively high values when compared with *N. locustae* in grasshoppers of North America and India.

Aside from the presumably "inflated" prevalences in *R. pictus, D. argentinus, S. lemniscata,* and *S. daguerrei,* the species that showed higher prevalences were *B. punctulatus* (19.1% at Trenque Lauquen; 10.3% at 15 km north of Santa Rosa) and *D. pratensis* (12.5% at 20 km north of Macachín).

Dispersion. Since microsporidia are intracellular obligate parasites, and their spores are normally short-lived in the outside environment (Brooks, 1988), they greatly depend on the movements of their hosts for spread. A gross approximation of the magnitude of the dispersal attained by *N. locustae* in grasshoppers of Buenos Aires and La Pampa provinces can be inferred by comparing the locations of sites of application with those of sites of findings. The maximum distance between a site with infected individuals and the nearest application site was approximately 75 km (Casbas—30 km southwest of Trenque Lauquen; Santa Rosa—Eduardo Castex). Although most grasshoppers exhibit sedentary habits, some species that showed infections are known to sporadically perform migratory or dispersal flights (Carbonell, 1957; Liebermann, 1961; COPR, 1982). If *N. locustae* exhibits an effective means of horizontal and vertical transmission, a few lightly infected grasshoppers still capable of flying would provide enough inoculum for the initiation of an enzootic or even an epizootic in a site where it previously did not occur.

Impact. Although the purpose of this study was to show evidence that *N. locustae* is well established in Argentina, with no intention of measuring its eventual impact in grasshopper communities, a few words are necessary regarding a recent controversy about the use of parasites in the control of pests. Following the introduction of a pathogenic fungus, a pathotype of *Entomophaga grylli* (Fresenius) (Zygomycetes: Entomophthorales), and the proposed introduction of a parasitic wasp, *Scelio parvicornis* Dodd (Hymenoptera: Scelionidae), from Australia into the western United States for the control of rangeland grasshoppers, Lockwood (1993) raised concerns about the use of an exotic agent to control a native pest. The major concerns were the eventual negative impacts on nontarget grasshopper species and native control agents and the irreversibility of the process of introduction. Carruthers and Onsager (1993) disagreed with most of Lockwood's points of view and warned that increased mandatory regulation resulting from his approach on the matter would restrict implementation of biological control programs.

The long-term impact produced by the introduction of *N. locustae* will probably remain unknown and be a matter of considerable speculation. The lack of quantitative studies on the abundance and diversity of grasshoppers, particularly before the introductions, is the main drawback that does not permit us to compare pre- and postintroduction scenarios. However, we need to address Lockwood's concerns.

One of the possible negative impacts cited by Lockwood (1993) was the extinction of nontarget, rare species of grasshoppers. With only one possible exception (*D. argentinus*), none of the infected species can be considered rare. On the contrary, all are very common and seven of them are regarded as harmful to agriculture (COPR, 1982).

Another possible negative impact pointed out by Lockwood (1993) was the eventual competitive displacement of native pathogens by the introduced agent. Despite extensive surveys, only one native microsporidian pathogen, *P. dichroplusae* (Lange, 1987a,b), is currently known in Argentine grasshoppers. Host range and prevalence studies have shown no evidence that *P. dichroplusae* occurred or occurs in areas where *N. locustae* is established, whereas its presence was common in *D. elongatus* (13.5%, $n = 296$) collected in Coronel Suárez (Lange, 1992), a site where introduction of *N. locustae* apparently did not result in establishment. The opposite picture of *P. dichroplusae* precluding the establishment of *N. locustae* in certain areas by competitive interactions cannot be ruled out.

We need to continue surveying for pathogens in the grasshopper communities of central Argentina for as many seasons as possible. These surveys could increase our knowledge regarding the fate undergone by *N. locustae* and provide much needed insight into the use of exotic agents to control native pests.

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