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Effect of the pathogen *Nosema locustae* (Protozoa: Microspora) on mortality and development of nymphs of the South American locust, *Schistocerca cancellata* (Orthoptera: Acrididae)

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Abstract

Laboratory procedures previously used by other authors were employed to conduct experimental bioassays with *Nosema locustae* on cultured *Schistocerca cancellata*. Third-instar nymphs were individually, *per os* inoculated with 10^5 spores and maintained under controlled conditions until their death or termination of the experiment after 30 days. Mortality and development of *S. cancellata* were affected by *N. locustae*. Mortality did not differ among treated and control nymphs at 20 days post-inoculation, but was significantly higher for infected nymphs at 30 days post-inoculation. Infections developed in 52.5 % of the treated nymphs. While 92.5 % of control nymphs reached adulthood, no infected individuals reached the adult stage, most succumbing as fifth or sixth instar nymphs. Control insects reached the sixth nymphal instar 6 days before infected nymphs and 4 days before non-infected (but treated) nymphs. Given the current preventive strategy employed in Argentina for locust control, the results obtained in this study suggest that *N. locustae* could be of value as a long-term biocontrol agent of *S. cancellata* in the outbreak areas of Northwest Argentina.

Introduction

The South American locust, *Schistocerca cancellata* (Serville), has been considered the most serious agricultural pest in Argentina (Gastón 1969). Since the mid-fifties however, invasion of agricultural lands has been prevented by application of chemical insecticides against nymphal bands in outbreak areas of the northwestern provinces of Catamarca and La Rioja (Hunter & Cosenzo 1990). Although this strategy has so far proven effective, it would be desirable to switch to non-toxic alternatives.

Nosema locustae Canning is a pathogen of the fat body of orthopterans that is used as a microbial control agent (Henry & Oma 1981). It has been extensively tested and employed, with varying results, against different species of grasshoppers in several regions of the world (for a detailed review see Johnson 1997). Following its introduction into eight localities in Argentina from 1978 to 1982, *N. locustae* became established at relatively high prevalences in at least 14 grasshopper species in the center (eastern La Pampa and western Buenos Aires provinces) and the southwest (northwestern Chubut province) of the country (Lange & de Wysiecki 1996, Lange 2000). Although Luna *et al.* (1981) mentioned in passing that *S. cancellata* was susceptible to *N. locustae*, its potential as a control agent against *S. cancellata* has not been explored. Because it is generally a slow acting pathogen that causes sublethal effects and relatively low mortality rates, its value for locust control has been considered to be minimal (Streett & Henry 1990).

The aim of this study was to investigate the effect of *N. locustae* on mortality and development of nymphs of *S. cancellata*. Results obtained from laboratory bioassays, and some speculations about its eventual usefulness in field situations are presented.

Materials and methods

Insects. Locusts used in this study were obtained from a colony that was developed at "Centro de Estudios Parasitológicos y de Vectores" following general procedures described by Henry (1985) for the rearing of grasshoppers and locusts. The colony was initiated with older nymphs collected near Telaritos, Catamarca province, and in the vicinity of Las Lomitas, Formosa province, both places located well in the center of the known range of *S. cancellata* (COPR 1982).

Pathogen. In eastern La Pampa and western Buenos Aires provinces, *N. locustae* is readily available from infected insects in the field, particularly the melanoplines (Melanoplinae) *Dichroplus pratensis* Bruner, *Dichroplus elongatus* Giglio-Tos and *Baeacris punctulatus* (Thunberg) (Lange & de Wysiecki 1999). Spore suspensions were prepared by homogenizing infected insects in distilled water as described by Henry & Oma (1974). A hemocytometer was used for counting the spores, and desired dilutions were made.

Inoculations. The *per os* experimental inoculations were conducted on third instar nymphs as described by Habtewold *et al.* (1995). Twenty-four hours before the beginning of the experiment, nymphs were individually placed in 20 ml foam-plugged glass vials and starved. A lettuce disk (5 mm

diameter) with 10^5 spores was then administered to each insect. Both the dose of pathogen and the age of experimental insects were selected in order to comply with previously used protocols (Henry 1990, Habtewold *et al.* 1995).

Treatment procedure. Forty insects ingested the spore-loaded bait (treated), and forty others ingested untreated disks (control). Immediately after consumption, the control and treated insects were transferred to individual acetate tubes (Henry 1985) and carefully observed for development and mortality several times a day until death or termination of the experiment after 30 days. Since insects were individually treated and maintained thereafter, each grasshopper represented a replicate. *Nosema locustae*-treated and control nymphs were placed in separate equal rooms under the same conditions (30°C, 14L-10D, around 40 % RH) and fed a daily diet of lettuce, wheat seedlings, and wheat bran. Infection was diagnosed after the death of each insect during the experiment or when killing survivors at the end. Diagnosis was by observation of spores and developmental stages of *N. locustae* in small drops of homogenates of each cadaver as fresh slide mounts with distilled water or Ringer's solution (Poinar and Thomas 1984) under phase contrast microscopy (X400; X1000). Giemsa-stained smears of small pieces of different tissues and organs (mainly fat body), as described by Wang *et al.* (1991), were also prepared prior to homogenization of cadavers. Giemsa-stained smears were used in order to confirm those cases in which infection did not develop.

Data analysis. Chi-square tests were used for comparing mortalities of treated and control insects at days 20 and 30 after inoculation. These times were selected in order to be able to compare the results with available information on other hosts (Henry, 1990). ANOVA was employed to determine if differences existed between treatments in development time of nymphs from third to sixth instar and to adult. Means were compared by the least significant difference (LSD) test ($P < 0.01$).

Results

Both survival and development time of *S. cancellata* were affected by *N. locustae*. Mortality of treated nymphs was higher than that of control nymphs. The number of treated nymphs that died during the experiment was 7 and 14 at 20 and 30 days post inoculation (dpi), respectively, while only 3 control nymphs died at both 20 and 30 dpi. Percentage of mortality was 17.5% and 35 % at 20 and 30 dpi, respectively, in treated nymphs, and 7.5 % at both 20 and 30 dpi, in controls. Mortality did not differ among treated and control nymphs at 20 dpi ($\chi^2 = 1.83$, df = 1, P = 0.17), but it was significantly different at 30 dpi ($\chi^2 = 8.72$, df = 1, P = 0.003).

Infection developed in 52.5 % of the treated nymphs. Most infected individuals died as fifth or sixth instars or still remained as moribund sixth instars at the end of the experiment; no infected individuals reached the adult stage. Indeed, none of the 19 individuals (47.5 %) that reached adulthood were infected. Percentage of control nymphs reaching adulthood was 92.5 %.

Mean development times from third to sixth nymphal instar and to adult in treated and control are shown in Fig. 1. Treated nymphs developed more slowly than control nymphs to reach both the sixth instar ($F=56.07$, df=2, 60, $P=0.001$) and the adult stage ($F=251.8$, df=1, 52, $P=0.001$).

Control insects reached the sixth nymphal instar 6 days before infected insects and 4 days before nymphs that were treated but did not develop infections. Non-infected but treated nymphs molted to the adult stage approximately 5.5 days later than controls (Fig.1).

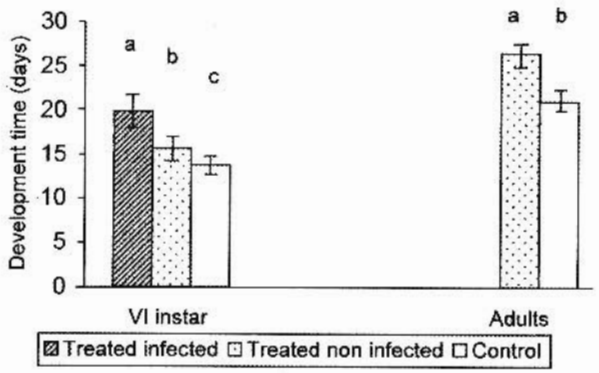

Fig. 1. Mean development times of *S.cancellata* from III to VI nymphal instar and to adult stage in *Nosema*-treated and control nymphs. Vertical, capped lines are S.D. Mean times topped by the same letter are not significantly different ($P < 0.01$).

Discussion

Mortality. Most effects associated with the pathogenicity of *N. locustae* in a given host are of a sublethal nature, such as reductions in fecundity, activity, and food consumption, as well as alterations in development. However, increased mortality rates may also play an important role, depending primarily on host susceptibility and dose (Johnson 1997).

Laboratory and field studies on the host range of *N. locustae* carried out in North and South America (Henry 1969a, Henry *et al.* 1973, Lange & De Wysiecki 1996) indicated that grasshopper species in the subfamily Melanoplinae are more likely to contract infection than other grasshopper taxa. Bioassays that can be used as a reference for comparisons, have established an LD^{50} of about 10^5 spores at 20–25 dpi for third instar nymphs of the North American melanoplines *Melanoplus sanguinipes* (Fabricius) and *Melanoplus bivittatus* (Say) (Henry 1978, 1990). Similar values have been obtained for the South American melanoplines *Dichroplus schulzi* Bruner and *D. elongatus* (Lange, unpublished results). Although *N. locustae* increased the mortality of *S. cancellata*, values obtained in this study at 20 dpi (17.3 %) and 30 dpi (35 %) are much lower than

those known for melanopline hosts. Direct mortality then would not have a major impact on the South American locust.

Development. Since infections by *N. locustae* occur primarily in the fat body, which performs a dynamic role in both intermediate metabolism and energy storage, energy reserves for growth of the host are greatly compromised by *N. locustae* infection. As a result, abnormal host development is commonly manifested in infected grasshoppers (Henry 1969b). Alterations in host development were clearly observed in our study as a delay in reaching the sixth instar of those individuals that contracted infection. In addition, infected nymphs did not molt to the adult stage. Although to a lesser extent, treated nymphs that did not become infected also showed delays in reaching the adult stage, possibly because some energy reserves were used for initial, albeit successful, immune response.

Field situation scenario. Successful control of the South American locust began in 1954 when the strategy followed by the officially conducted control campaigns was changed from reactive action (large-scale actions against far-reaching swarms) to preventive action (Gastón 1969). The original concept underlying the change was to prevent the formation of swarms by tracking and individually treating bands of nymphs in outbreak areas with chemicals. Although laborious, the approach, which is still in practice, proved to be effective. This is probably because the outbreak area of *S. cancellata* is relatively small, well-defined, and contained within a single country, when compared to other locusts, particularly the closely related desert locust, *S. gregaria* (Forskål), in Africa and Asia (Hunter & Cosenzo 1990; Steedman 1990).

The results obtained in this study suggest that *N. locustae* might have some value as a long-term biocontrol agent of *S. cancellata* in Northwestern Argentina. Although *N. locustae* does not cause conspicuous mortality as do the chemicals currently used, the increased mortality and development time, and the fact that infected nymphs are apparently unable to reach adulthood, should have an impact on the intrinsic rate of population increase, leading to lower population density levels in the long run. According to Habtewold *et al.* (1995), reduction of survival and development time in late instars were the most effective demographic traits in reducing population growth rate in the grasshopper *Aiolopus longicornis* Sjöstedt. For several reasons, inducing infections in the field should be feasible. It is well known that *S. cancellata* readily consumes wheat-bran baits (Gastón, 1946) the way in which *N. locustae* is delivered (Henry & Oma 1981). Nymphs normally occur in well-defined bands that are routinely tracked by experienced personnel. In large sectors of the outbreak area ground cover is relatively poor (Köhler 1982), meaning that baits would be easily located by nymphs. Since infected nymphs did not reach adulthood, it is unknown if vertical transmission, as demonstrated by Raina *et al.* (1995) in *Locusta migratoria migratorioides* (Reiche & Fairmaire), would play a role in the maintenance of the pathogen in field populations. Further research efforts should probably be directed at assessing the levels of spore production per infected locust and whether *S. cancellata* exhibits cannibalistic habits, both central factors for the occurrence of significant horizontal transfer of the pathogen in nature.

Acknowledgements

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