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ORIGINAL ARTICLE

A substitute host for *Paranosema locustae* (Microsporidia)

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Summary

The microsporidium *Paranosema locustae* is one of the only two species of entomopathogens developed and registered worldwide as biological control agents for grasshopper and locust management. It is a pathogen of fat body cells that normally causes a chronic type of disease rather than an acute one, debilitating and altering hosts in many ways and reducing feeding, longevity, and fecundity. Spores of *P. locustae* for field applications are produced *in vivo* in natural hosts, the Migratory locust *Locusta migratoria* in China, and the grasshoppers *Melanoplus bivittatus* and *M. differentialis* in USA. We conducted laboratory en masse inoculations on fourth instar nymphs of the melanopline grasshopper *Ronderosia bergii*, one of many grasshopper species newly associated with *P. locustae* in areas of Argentina following introductions of this biocontrol agent decades ago. All *R. bergii* individuals in the assays (n= 812 in four cages) became infected (100 % prevalence) and spore production levels were high in all assays (in low values of the 10^{11} order of magnitude). Although natural hosts used for production of *P. locustae* spores are comparatively more productive, results obtained with the newly associated host *R. bergii* are considered also appropriate. Such results coupled with some owned biological traits of *R. bergii*, such as absence of obligate embryonic diapause that allows continuous rearing of colonies, polyphagia (Dicotyledoneous and gramineous plants), and a meek behaviour that simplifies colony handling make this grasshopper a suitable substitute host for *P. locustae* spore production.

Key words: *Antonospora*, Biocontrol agent, *Nosema, Ronderosia bergii*

Introduction

The microsporidium *Paranosema locustae* is one of only two species of entomopathogens developed and registered worldwide as biological control agents for grasshopper and locust management, the other being the fungus *Metarhizium acridum* (Solter et al., 2012a; Vega et al., 2012). Sokolova et al. (2005) provided the reasons on why the combination *P. locustae* should be used instead of *Nosema locustae* or *Antonospora locustae*, other names employed for the same species. Since attempts aimed at *in vitro*

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propagation have not become operational (Raina and Ewen, 1979; Kurtti and Munderloch, 1987; Kurtti et al., 1990; Solter et al., 2012b), *P. locustae* is produced in vivo by infecting and rearing diseased hosts until massive spore loads are reached and then harvested (Zhang and Lecoq, 2021). This is possible because *P. locustae* normally causes a chronic type of disease in the adipose tissue of the host rather than an acute one, although heavy doses may cause faster mortality (Lange and Cigliano, 2005; Chen et al., 2020). Infected hosts are debilitated and altered in many ways including reduction in feeding, longevity, and fecundity. *Paranosema locustae* is commercially produced at facilities only in two countries: USA, where it was originally developed (Henry and Oma, 1981; Lange and Sokolova, 2017; Henry, 2017) and it is mostly used by organic farmers against a variety of grasshopper species (Solter et al., 2012a), and China, where it is extensively applied against both grasshoppers and locusts (Zhang and Hunter, 2017). In addition, Gerus et al. (2016) succeeded in propagating *P. locustae in vivo* in Russia. In all these cases, natural hosts as defined by Onstad et al. (2006), are used, the melanopline grasshoppers *Melanoplus bivittatus* and *M. differentialis* (Acrididae: Melanoplinae) in the USA, and the Migratory locust, *Locusta migratoria* (Acrididae: Oedipodinae), in China and Russia (Zhang and Lecoq, 2021).

In Argentina, where *P. locustae* became naturalized in grasshopper communities in areas of the western Pampas and north-western Patagonia following introductions from North America (Lange and Azzaro, 2008; Lange at al., 2020), natural hosts do not occur (Carbonell et al., 2022). However, twenty-nine grasshopper species have been found to be susceptible to *P. locustae*, either in the field and/or the laboratory, constituting new pathogen-host associations (Lange et al., 2020). It is conceivable that one or more of these newly associated hosts could be useful for the local production of *P. locustae*. Given that importation of non-indigenous biological agents became an elusive option for pest control over the years due to the environmental issues involved (Howarth, 2001), the eventual availability of a locally produced biocontrol agent would contribute significantly to a more environmental-friendly management of pest grasshoppers in the country, reducing the exclusive dependence on chemical insecticides. Previous laboratory and field studies (Lange, 2003; Plischuk et al., 2013; Lange et al., 2020) indicated that some of the melanopline grasshopper species inhabiting Argentina could be useful for *P. locustae* production. We conducted some en masse inoculations using the Berg's grasshopper, *Ronderosia bergii*, a melanopline widely distributed in Argentina and most neighbouring countries, and here we report on the feasibility of using such species as a substitute host (as defined by Onstad et al. 2006: A host, other than the natural host, chosen for laboratory propagation of a pathogenic microorganism or parasite) for producing large quantities of spores of *P. locustae* for practical implications.

Fig. 1. Adult female of *Ronderosia bergii*.

Material and methods

Ronderosia bergii is one of the 24 species of grasshoppers in Argentina in which field infections with *P. locustae* have been found (Lange et al. 2020). The Berg's grasshopper is a medium (Female: 18–28 mm; Fig. 1) to small (Male: 14–22 mm) size winged melanopline that inhabits much of central and North of the country and areas of bordering Bolivia, Paraguay, Brazil, and Uruguay (Cigliano et al. 2014; Carbonell et al. 2022). It is a polyphagous (Dicotyledoneous and gramineous plants) species having no obligatory embryonic diapause and developing through five nymphal instars prior to adulthood (Mariottini et al. 2010; Carbonell et al. 2022). *R. bergii* has been often reared in our laboratory for several generations and resulted to be relatively easy to establish and maintain compared to other grasshopper species due in part to the lack of obligatory embryonic diapause, polyphagia, and meek behaviour. For assays performed in this study we employed laboratory-reared, second-generation individuals of *R. bergii*, the original stock of which was collected as adults at fields in the vicinity of Gobernador Roca (27°11′25″S, 55°28′09″W), Misiones province, in northeast

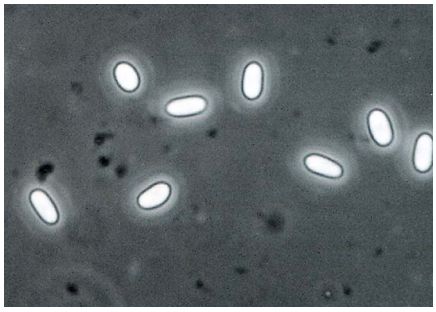

Fig. 2. Mature spores of *Paranosema locustae*, (mean size: 4.95×2.65 microns).

Fig. 3. Spraying aqueous suspension of spores of *Paranosema locustae* on lettuce leaves.

Argentina. The source of *P. locustae* utilized were spores (Fig. 2) isolated, purified, and counted by the homogenization method described by Plischuk et al. (2013) from field-infected grasshoppers captured in the western Pampas, one of the general areas were *P. locustae* became naturalized following introductions from North America (Bardi et al. 2012; Lange et al. 2020). We followed Henry's (1985) procedure of en masse inoculation. Four large screened aluminium cages (0.4×0.4×0.4 m) were seeded with 220 fourth-instar nymphs of *R. bergii* each. A 20 ml aqueous suspension containing 10^9 spores of *P. locustae* was evenly sprayed to each of four batches of 450 gr of thoroughly washed lettuce (*Lactuca sativa*) leaves (Fig. 3). When the surface of the leaves were dried 150 gr were introduced in each of the four cages (day 1 of inoculation), an offering that was repeated on days 3 and 4 of the 31-day long experience (Fig. 4). According to Henry (1985) such a long inoculation protocol (skipping day 2) allows for most if not all the nymphs to consume the lettuce baits, including those that might be molting or simply not hungry at times. After inoculation, grasshoppers were maintained under the routine conditions in our rearing rooms (30 ºC, 14L:10D, and 40% RH) until the end of the experiment when all survivors were frozen (-32 ºC). Our interests were the infectivity and the spore production; hence we did not measure mortality (or other effects). However, we recovered those cadavers or moribund individuals that were not cannibalized or scavenged (common grasshopper behavior under crowded conditions; Henry and Oma, 1981) for later examination. Infection diagnosis and spore counts in cadavers and survivors were performed through the homogenization and dissection methods, and hemocytometer counts as previously described (Plischuk et al., 2013; Pocco et al., 2020).

Results and discussion

Results were remarkably consistent in all four assays in terms of both prevalence of infection and overall spore production (Table 1). All grasshoppers in the assays became infected (100 % prevalence) and each of the repetitions (cages) produced spores within the same order of magnitude (10^{11}) regardless of small differences in the number of insects reaching the final stages of infection at the end of the assays. The 10^{11} order of magnitude obtained in all four assays ended up being coherent with previous work (Lange, 2003) where individually inoculated males and females averaged spore loads of 7.9×10^8 and 1.8×10^9, respectively. Infections in grasshoppers dying during the first three weeks comprised mostly of stages prior to sporogenesis (prespore developmental stages) with almost no spores (Fig. 5). As such, these grasshoppers were discarded and hence did not contribute to the production effort. Grand total production for the four cages was 10^{12} (n = 812) which at the standard rate of field application of 2.5×10^9 spores/ha (Henry and Oma, 1981) allows for treatment of 400 hectares.

The consistency of results in our assays is a desirable outcome when a procedure is intended to become a reliable starting point for a standardized protocol. Although *R. bergii* is less productive of *P. locustae* spores than the natural hosts, *M. bivittatus, M. differentials*, and *L. migratoria*, which can reach a spore yield per individual within the low values of the 10^{10} order of magnitude (2×10^{10} in *Melanoplus* spp. and 3.4×10^{10} in *L. migratoria*; Henry, 1981, 1985; Zhang and Lecoq, 2021), it is still a conceivable prospect as a species for production where natural hosts are not available. As seen in our assays, susceptibility of *R. bergii* to *P. locustae* is very high (100

Fig. 4. Cage housing *Ronderosia bergii* nymphs after inoculation with *Paranosema locustae* spore-sprayed lettuce leaves.

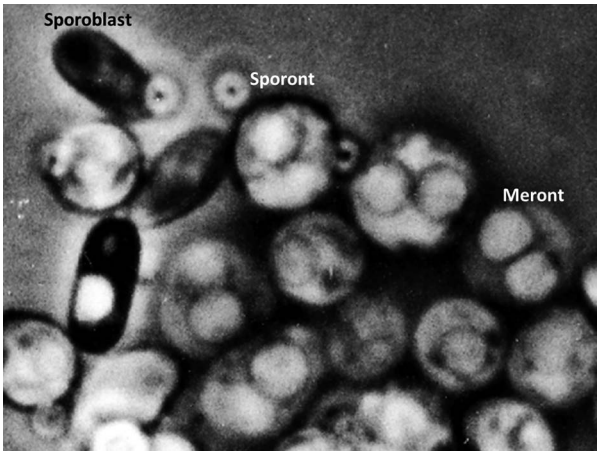

Fig. 5. Meronts, sporonts, sporoblasts, but not mature spores of *Paranosema locustae* in a typical infection within the first three weeks of the assays.

% prevalence) and spore yield is reasonable (all four assays at 10^{11} order of magnitude). These attributes coupled with a lack of obligatory embryonic diapause that permits continuous rearing, polyphagous diet that simplifies feeding, and easy handling make maintaining *R. bergii* for the microsporidium propagation a relatively simple and inexpensive task. Overall, it seems that *R. bergii* is a suitable substitute host for production of *P. locustae*.

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Table 1. Prevalence of infection and spore production of *Paranosema locustae* in the melanopline grasshopper *Ronderosia bergii* (Acrididae: Melanoplinae) in four 31 day-long assays under controlled conditions (30 ºC, 14L: 10D, and 40% RH) with an initial stock of 220 IV-instar nymphs per cage treated with 10^9 spores/cage.

Assay (Cage)	Grasshoppers not contributing spores		Grasshoppers contributing spores	Prevalence of infection (%)	Number of harvested spores
	Lost to cannibalism and necrophagy	Not reaching sporogenesis prior to death (first 3 weeks)			
1	17	5	198	100	1.3×10^{11}
2	10	2	208	100	2.9×10^{11}
3	3	–	217	100	4.6×10^{11}
4	27	4	189	100	1.2×10^{11}

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