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Activity of oil-formulated conidia of the fungal entomopathogens *Nomuraea rileyi* and *Isaria tenuipes* against lepidopterous larvae

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ABSTRACT

The fungi *Nomuraea rileyi* and *Isaria tenuipes* (=*Paecilomyces tenuipes*) are ecologically obligate, widespread pathogens of lepidopterans. Bioassays were carried out to evaluate the activity of oil-suspended conidia of *N. rileyi* and *I. tenuipes* against larvae of *Spodoptera frugiperda, Spodoptera exigua, Helicoverpa zea,* and *Heliothis virescens*. The tests consisted of two bioassay sets. In the first set, conidia of *N. rileyi* and *I. tenuipes* were suspended in water + Tween 80, and in vegetable (canola, soybean) and mineral (proprietary mixture of alkanes and cyclic paraffins) oils, and tested against *S. frugiperda*. Both fungi were highly compatible with oils and caused mortalities near 100% in all oil treatments; the lowest LT_{50} values were 4.7 days for *N. rileyi* in mineral oil and 6.0 days for *I. tenuipes* in soybean oil. The second set included additional fungal strains and oil formulations (mineral, canola, sunflower, olive and peanut oils) tested against larvae of *S. exigua, S. frugiperda, H. zea* and *H. virescens*. The highest activity was that of *N. rileyi* in mineral oil against *Spodoptera* spp., with LT_{50} values of 2.5 days (strain ARSEF 135) and 3 days (strain ARSEF 762) respectively. For two different isolates of *I. tenuipes* the lowest LT_{50} values (5.1–5.6 days respectively) were obtained with mineral oil formulations against *Spodoptera* spp. and *H. zea* respectively. Additionally, we tested both fungi against prepupae of all four lepidopteran species. Mortalities with *I. tenuipes* against *S. exigua* ranged from 90% to 100% (strains ARSEF 2488 and 4096); *N. rileyi* caused 95% mortality on *S. frugiperda*. The activity of formulations depended on host species and oil used; *Spodoptera* spp. was more susceptible to these fungi than *Heliothis* and *Helicoverpa*. The results indicate that a comprehensive evaluation of these entomopathogens in agriculture using oil application technologies is advisable, particularly, in organic and sustainable settings.

1. Introduction

Entomopathogenic fungi are frequent natural enemies of arthropods worldwide (Hajek and Leger, 1994; Shah and Pell, 2003). Research on these fungi has focused predominantly on aspects related to population regulation and control of arthropods (Roy et al., 2006). *Nomuraea rileyi* (Farlow) Samson and *Isaria tenuipes* Peck (=*Paecilomyces tenuipes* (Peck) Samson) (Hypocreales: Clavicipitaceae) are anamorphic (i.e. asexual phases of) fungi. *N. rileyi* infects larvae of Lepidoptera in the family Noctuidae, including agricultural pests like the cabbage looper (*Trichoplusia ni* [Hübner]), velvetbean caterpillar (*Anticarsia gemmatalis* [Hübner]), soybean looper (*Pseudoplusia includens* [Walker]), bollworm (*Helicoverpa armigera* [Hübner]), corn earworm or bollworm (*Helicoverpa zea* [Boddie]), tobacco budworm (*Heliothis virescens* F.), and many species of *Spodoptera* armyworms, including the African cotton leafworm, *S. littoralis* (Boisduval), beet armyworm (*S. exigua* [Hübner]), and fall armyworm (*S. frugiperda* [J.E. Smith]) (Ignoffo, 1981; Ignoffo and Boucias, 1992; Sanchez-Peña, 2000; Martins et al., 2005). *I. tenuipes* is parasitic on pupae and larvae of various lepidopterans, usually in forested habitats, forming yellowish asexual fruit bodies (synnemata) (Sanchez-Peña, 1990; Kana-uchi and Fukatsu, 1999). Both fungi (particularly *N. rileyi*) are essentially ecologically obligate pathogens of Lepidoptera (Samson, 1974; Ignoffo, 1981; Humber, 1992).

Some of the main issues in the successful use of fungi for pest control include application, infectivity, and persistence of their inoculum in the environment (Moore and Prior, 1993). Application of oils in agriculture, either as active ingredients *per se* or as carriers, has clear advantages over aqueous and other formulations in specific situations (e.g. when water is scarce and low volume applications are required). Suspending entomopathogenic fungal conidia in oil frequently improves their environmental persistence and virulence against insects, compared to using water suspen-

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sions (Prior et al., 1988; Bateman et al., 1993). The conidia of other entomopathogenic fungi, such as *Beauveria* and *Metarhizium* spp. suspend easily in oils, and, in field applications, oil prevents small droplets from evaporating before reaching the target insect (Prior et al., 1988; Bateman et al., 1993; Lomer et al., 1993; McClatchie et al., 1994; Vimala-Devi and Prasad, 1996). To our knowledge, there are very few or no reports (e. g. Vimala-Devi and Prasad, 1996) on the suitability of oils as carriers for *N. rileyi* and *I. tenuipes*. Besides their proven virulence against important pests (Ignoffo, 1981; Tang and Cheng, 1999), the ecological specificity of these two fungi makes them promising biological control agents. In this study we compared the virulence of oil-suspended and water-suspended conidia of several isolates of *N. rileyi* and *I. tenuipes* against *S. frugiperda, S. exigua, H. zea,* and *H. virescens*.

2. Materials and methods

The experiments were conducted at two locations: (1) Saltillo, Coahuila, Mexico; strains used there were: freshly-collected and isolated *N. rileyi* strains (NrDN1, NrL1 and NrSS2) and one *I. tenuipes* strain collected in Tamaulipas, Mexico and accessed in the USDA., Agricultural Research Service (USDA-ARSEF) collection (ARSEF strain 2489). These were tested against *S. frugiperda*. (2) Stoneville, Mississippi, USA; there, American, Brazilian and Mexican strains of *N. rileyi* and *I. tenuipes* from the USDA-ARSEF collection were tested against *H. virescens*, *H. zea*, *S. frugiperda*, and *S. exigua*. All strains tested and their origins are listed in Table 1. For all tests and fungi, conidial viability was verified by conidia germination, spreading conidial suspensions on a thin layer of potato dextrose agar (PDA) in petri dishes, followed by incubation at room temperature and examining these dishes under the microscope at 24-h intervals; conidial viability was never under 80% and usually 90–96% in all tests.

2.1. First set of bioassays at Saltillo

Strains of *N. rileyi* were obtained from dead larvae of *S. frugiperda* collected on corn (*Zea mays* L.) in Saltillo on 15 September 2006, then isolated in PDA with egg yolk (Sanchez-Peña, 2000); the *I. tenuipes* strain ARSEF 2489 (Table 1) was also used. All fungi were transferred to PDA plus 2% yeast extract (PDAY) and incubated under diffuse fluorescent light. After two subcultures from larvae, *N. rileyi* produced abundant aerial mycelium but low amounts of conidia on agar plus egg yolk. Subsequently we used PDAY for culture of both fungi, where production of infective conidia was stable and sufficient. For inoculation, 3rd instar larvae from a *S. frugiperda* colony obtained from corn in Saltillo were used; this colony was maintained in the laboratory for six generations on wheat-germ based diet (Blanco et al., 2009).

2.1.1. Preliminary tests

These were conducted to confirm the virulence of the local *N. rileyi* strains from Saltillo against *S. frugiperda* larvae. Insects were immersed in aqueous suspensions of *N. rileyi* conidia (strains NrDN1, NrL1 and NrSS2). First, we dipped 2nd, 3rd and 4th instar larvae (termed L2, L3 and L4 respectively) (eighty of by treatment) each one for five seconds in a fungal suspension of 2.5×10^7 conidia/ml in water plus 0.05% Tween 80 (Merck, Hohenbrunn, Germany). Larvae were kept individually in 37-ml plastic containers (Solo Co., Urbana, IL) with bermuda grass (*Cynodon* spp.) leaves as food and a ball of moist cotton at 25 °C. For the second test, eighty third-instar larvae by treatment, each one were immersed in aqueous fungal suspensions of the three strains as above. In both tests control larvae were dipped in 0.05% Tween 80 solution in water with no conidia. Mortality was evaluated two, four and seven days after inoculation.

2.1.2. Oil and water-suspended conidia of N. rileyi and I. tenuipes against 3rd instar larvae of S. frugiperda

Conidia of *N. rileyi* (strain NrDN1) were taken from agar cultures and suspended at different formulations by stirring in water plus Tween 80 and the following oils: (1) soybean oil (Soraya, Industrial Aceitera, Naucalpan, Mexico), (2) mineral oil (Mennen, Colgate-Palmolive, Mexico, DF) and (3) canola oil (Maravilla, Industrial Aceitera, Naucalpan, Mexico). *I. tenuipes* conidia (ARSEF 2489) were suspended in soybean oil only. Oil suspensions (2 μl of a suspension having 2.5×10^7 conidia/ml, equivalent to 5×10^4 conidia / larva) were applied to the abdominal dorsum of larvae utilizing a micropipette; 2 μl of soybean oil were applied to control larvae, for the aqueous suspensions larvae were immersed by 2 s, also for this bioassay was used eighty larvae by treatment. Inoculated larvae were incubated as described; larval mortality was determined daily for nine days.

2.2. Second set of bioassays at Stoneville

N. rileyi (ARSEF 762 and 135) and *I. tenuipes* (ARSEF 4096, 2488 and 2489) (Table 1) were grown on PDAY at 25 °C under diffuse fluorescent light. Conidia were harvested from agar after 7–10 days and suspended in either canola, peanut (LouAna, Ventura Foods, Brea, CA), sunflower, olive (Kroger, Cincinnati, OH), and mineral oil (Johnson and Johnson, Langhorne, PA) (all commercial-grade oils). We used one hundred larvae for each treatment and bioassay, and applied 2 μl of suspensions (2.5×10^7 conidia/ml, equivalent to 5×10^4 conidia/larva) on the abdominal dorsum of *S. frugiperda, S. exigua, H. zea,* and *H. virescens* larvae. The first three insect species were obtained from Benzon, Inc. (Benzon Research, Carlisle, PA), while *H. virescens* were obtained from the USDA-ARS colony at Stoneville, MS. After inoculation larvae were kept at 25 °C in 37-ml plastic cups with artificial diet. Mortality was registered daily for eight days.

2.2.1. Mortality of 2–4th instar larvae of four species of lepidopterans exposed to ARSEF strain 762 of N. rileyi in a mineral oil formulation

To compare the effect of larval instar on the activity of a mineral oil formulation of strain ARSEF 762 of *N. rileyi*, 2nd, 3rd and 4th instars of *S. frugiperda, S. exigua, H. zea* and *H. virescens* were inoculated with 2 μl of oil suspensions, as described above. Mortality was recorded daily for eight days.

Table 1
N. rileyi and *I. tenuipes* strains used in bioassays. Three *N. rileyi* strains from Saltillo were used; these were not accessed in the ARSEF collection.

Fungus	ARSEF strain number	Locality of origin and (habitat)	Host
N. rileyi	Not accessed	Saltillo, Coahuila, Mexico (corn)	Spodoptera frugiperda
N. rileyi	135	Stoneville, Mississippi, USA	Lepidoptera: Noctuidae
N. rileyi	762	Columbia, Missouri, USA (alfalfa)	Plathypena scabra
I. tenuipes	2488 and 2489	Gomez Farias, Tamaulipas, Mexico (rainforest)	Unidentified lepidopterous host, passed through Spodoptera frugiperda
I. tenuipes	4096	Brazil (no locality listed)	Lepidoptera: Noctuidae