

Susceptibility of *Spodoptera frugiperda* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) to infections caused by *Metarhizium rileyi*¹

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ABSTRACT

Due to the reduced host spectrum of the entomopathogenic fungus *Metarhizium rileyi*, its pathogenicity against different target insects must be assessed to develop biopesticides capable of controlling more than one pest species. This study aimed to evaluate the susceptibility of the pest species *Chrysodeixis includens* and *Spodoptera frugiperda* to different isolates of *M. rileyi* and, thus, determine a possible influence of the host of origin on the pathogenic activity of these isolates. Three isolates [CG1312 (*C. includens* as original host), CG381 (*S. frugiperda* as original host) and a new wild isolate (*C. includens* as original host)] were tested against larvae of *C. includens* and *S. frugiperda*, in third instar age, by treatment of surfaces with conidial suspension, under laboratory conditions. Both species were susceptible to the isolates of *M. rileyi*, with mortality rates of 53-58 % for *C. includens* and 74-84 % for *S. frugiperda*. The results suggest that the host of origin may not be determinant in the selection of pathogenic isolates of *M. rileyi* against these two pest insects.

KEYWORDS: Biopesticide, soybean looper, fall armyworm, green muscardine, entomopathogenic fungus.

INTRODUCTION

Chrysodeixis includens (Walker) and *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) are pest species of great economic importance in grain and fiber crops such as soybean, corn, bean and cotton (Pitre & Hogg 1983, Bueno et al. 2011, Baldin et al. 2014, Specht et al. 2015).

RESUMO

Suscetibilidade de *Spodoptera frugiperda* e *Chrysodeixis includens* (Lepidoptera: Noctuidae) a infecções causadas por *Metarhizium rileyi*

Em razão do reduzido espectro hospedeiro do fungo entomopatogênico *Metarhizium rileyi*, sua patogenicidade contra diferentes insetos alvos deve ser avaliada para o desenvolvimento de bioinseticidas capazes de controlar mais de uma espécie-praga. Objetivou-se avaliar a suscetibilidade das espécies-pragas *Chrysodeixis includens* e *Spodoptera frugiperda* a diferentes isolados de *M. rileyi* e, assim, determinar uma possível influência do hospedeiro de origem na atividade patogênica desses isolados. Três isolados [CG1312 (*C. includens* como hospedeiro original), CG381 (*S. frugiperda* como hospedeiro original) e um novo isolado selvagem (*C. includens* como hospedeiro original)] foram testados contra lagartas de *C. includens* e *S. frugiperda*, em idade de terceiro instar, por tratamento de superfícies com suspensão de conídios, em laboratório. Ambas as espécies foram suscetíveis aos isolados de *M. rileyi*, com índices de mortalidade de 53-58 % para *C. includens* e 74-84 % para *S. frugiperda*. Os resultados sugerem que o hospedeiro de origem pode não ser determinante na seleção de isolados patogênicos de *M. rileyi* contra essas duas espécies de praga.

PALAVRAS-CHAVE: Biopesticida, lagarta falsa-medideira, lagarta-do-cartucho, muscardine verde, fungo entomopatogênico.

Traditionally, synthetic insecticides and genetically modified plants (Bt-crops) are among the main methods for controlling larval infestations. However, the overuse of chemicals may result in damage to human and environmental health and cause the death of pollinators and natural enemies, in addition to selection of resistant pest populations (Bernardi et al. 2012, Sosa-Gómez & Omoto 2012, Martins &

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Tomquelski 2015, Sorgatto et al. 2015, Bernardi et al. 2017, Fernandes et al. 2019). Studies around the world have been carried out to develop alternatives for managing pests in different crops, evidencing the need to advance in the adoption of control methods other than chemical, such as the use of biopesticides based on entomopathogenic fungi (Lacey et al. 2015).

The fungus *Metarhizium rileyi* (Farlow) Kepler, S. A. Rehner & Humber (Hypocreales: Clavicipitaceae) (formerly *Nomuraea rileyi*) has been studied as an alternative in managing lepidopteran-pests (Vimala Devi & Prasad 2000). There are records of 60 lepidopteran species susceptible to this pathogen, of which half are Noctuidae (Fronza et al. 2017). Therefore, *M. rileyi* is an entomopathogenic fungus with a great potential to control noctuid pests in several agricultural systems. For instance, *C. includens* and *S. frugiperda* larvae are susceptible to this entomopathogen, and the occurrence of natural epizootics caused by *M. rileyi* on these pest species is often observed in soybean and corn crops, respectively (Pavone et al. 2009, Sosa-Gómez 2017, Souza et al. 2019, Lopes et al. 2020). Furthermore, the risks posed by this fungus on natural enemies or pollinators are minimal or inexistent, making its use safer, when compared to chemical pesticides (Matter & Sabbour 2013).

The control of lepidopteran larvae by *M. rileyi* in the field is directly related to its ability to increase in density by dispersing the inoculum from primary foci established in the crop, inducing an epizootic cycle on the host population and becoming a medium to long-term control strategy of caterpillars (Hajek & St. Leger 1994). On the other hand, natural outbreaks of this fungus are highly dependent on the host species, as well as their density, occurring normally when the pest population has already caused significant damages to the crops (Allen et al. 1971). Thus, the inoculum increment by field applications of the fungus may be determinant for effectively controlling lepidopteran pests.

An important step to develop new biopesticides based on *M. rileyi* is to select virulent strains to different caterpillar species (Souza et al. 2019). Fungal strains capable of infecting a certain species may or may not cause high mortality rates in other species, since there is a host specificity of naturally occurring strains (Tigano-Milani et al. 1995, Srisukchayakul et al. 2005). Genetic variations inherent to each fungal strain provide them with

varied characteristics related to pathogenicity and virulence, directly influencing host-pathogen interactions (Bianco & Perrota 2015). Therefore, in the present study, laboratory experiments were carried out to evaluate the susceptibility of *C. includens* and *S. frugiperda* to *M. rileyi* isolates and, thus, to determine some influence of the original host on the biological activity of these isolates.

MATERIAL AND METHODS

The experiments were carried out at the Embrapa Agrossilvipastoril (Sinop, Mato Grosso state, Brazil), in 2019. *S. frugiperda* and *C. includens* colonies were kept in the laboratory for successive generations at 26 ± 1 °C, relative humidity (RH) of 60 ± 10 % and photophase of 14 hours. The adult insects were kept inside cages made with polyvinyl chloride (PVC) tubes, coated with A4 paper for oviposition and covered with voile fabric. For adult feeding, 10 % sucrose aqueous solutions were provided and, every two days, the papers presenting eggs were removed from the cages and placed in a Gerbox containing an artificial diet (adapted from Greene et al. 1976). Next, the caterpillars were individualized in test tubes containing an artificial diet for a new breeding cycle or were used in bioassays.

Three *M. rileyi* isolates were selected for this study: CG381 (originally obtained from infected *S. frugiperda* caterpillars, in Planaltina, DF, Brazil) and CG1312 (originally obtained from infected *C. includens* caterpillars, in Buritis, Minas Gerais state, Brazil), both from the invertebrate-associated fungal collection of the Embrapa Recursos Genéticos e Biotecnologia; and a wild isolate named Native, collected from *C. includens* cadavers in Sinop (Mato Grosso state, Brazil). The request for access to the genetic heritage for the isolates above mentioned was registered with the SisGen certificate code AF5D9B3.

For both the caterpillar species, bioassays were conducted in a completely randomized design, with three isolates + control treatment (water), in 10 replicates, with each isolate being composed of 100 third-instar larvae, divided into repetitions of 10 individuals each. The isolates were initially inoculated with conidial suspension (c.a. 2×10^7 conidia mL⁻¹) on third-instar caterpillars from their original hosts and reisolated on sabouraud maltose agar + yeast extract (SMAY) culture medium, from the mummified cadavers, before the bioassays.

Next, the isolates were incubated for 9 to 12 days at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, $70\% \pm 10\%$ RH and 12-h photophase. After this period, the conidia on the culture media were collected using a stainless steel spatula and stored at $-20\text{ }^{\circ}\text{C}$, until the bioassays were carried out.

Before performing the bioassays, the viability of the conidia of each isolate was verified (adapted from Neves et al. 2001). Briefly, *M. rileyi* conidia were suspended in water + Tween 80™ (0.1 %) and then 0.1 mL of the conidial suspension was spread using a Drigalski loop in four Petri dishes containing SMAY culture medium. The plates were incubated at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, $70\% \pm 10\%$ RH and 12-h photophase and, after 48 hours, the percentage of germinated conidia in five fields per Petri dish was quantified with an optical microscope (400x of magnification). Only samples with conidial viability above 80 % were used in the bioassays. The conidial concentrations for the bioassays were determined using a Neubauer™ chamber.

The contaminated surface method was used to perform the bioassays, in which suspensions of each isolate were prepared in sterile water + Tween 80™ (0.1 %), at the concentration of 2×10^9 viable conidia mL^{-1} . The suspensions (800 μl) of each isolate were sprayed on Petri dishes (15 cm in diameter) using 2-mL glass flasks with a spray valve, making sure that the entire surface of the plates was covered with the cell suspension. Only sterile distilled water + Tween 80™ (0.1 %) were sprayed on the plates in the control treatment. The insects were transferred to the treated plate after the water evaporation and, after one hour in the plates, the larvae were individualized in glass tubes containing 5 mL of an artificial diet and kept in an acclimatized chamber

at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, $70\% \pm 10\%$ RH and 12-h photophase. The larval mortality was evaluated daily up to the pupae formation. Individuals were considered killed by the fungus when typical signs of infection, such as mummified body, followed by mycelium growth and formation of greenish conidia, were seen.

The normality and homoscedasticity of mortality data were assessed by the Shapiro-Wilk and Bartlett tests. As the data set was well-modeled by normality and homoscedasticity precepts, the analysis of variance was applied and the means were compared by the Tukey test at 5 % of probability. The Kaplan-Meier survival analysis was performed in pairwise comparisons by the log-rank test (Mantel-Cox) to verify the differences between survival curves. The R 3.6.1 free software was chosen for these analyzes, and the “survival” package of this software was used to carry out the survival analyzes (R Development Core Team 2019). LT_{50} (mean lethal time) was calculated using the PoloPlus 0.03 software (LeOra Software 2003).

RESULTS AND DISCUSSION

The larval mortality rates caused by the isolates at the end of the experimental period did not differ significantly for the *C. includens* ($F_{2,27} = 0.23$; $p = 0.79$) and *S. frugiperda* ($F_{2,27} = 2.3367$; $p = 0.11$) caterpillars. For *C. includens*, the larval mortality was $55.0\% \pm 2.68$ for the CG1312 isolate, $53.0\% \pm 5.78$ for CG381 and $58.0\% \pm 6.46$ for Native. For *S. frugiperda*, these same isolates caused larval mortality rates of $77.5\% \pm 2.80$, $84.0\% \pm 3.39$ and $74.5\% \pm 3.20$, respectively. No mortality was observed in the control treatments (Figure 1).

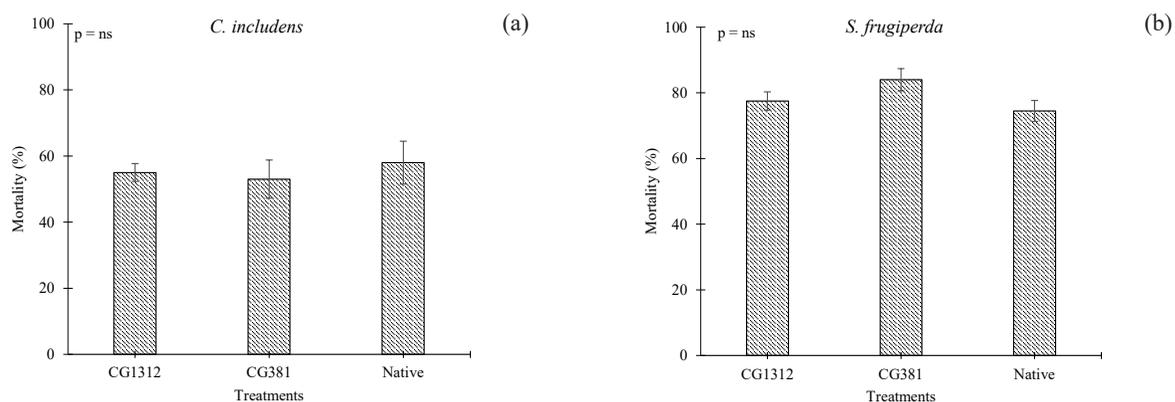


Figure 1. Mortality rates (mean \pm standard error) of *Chrysodeixis includens* and *Spodoptera frugiperda* caterpillars caused by *Metarhizium rileyi* isolates after surface treatment at a concentration of 2×10^9 viable conidia mL^{-1} . ns: non-significant mean differences at 5 % of probability by the Tukey test.

It was observed, therefore, that the original hosts of the isolates did not influence the fungal activity on the different target insects, when they were exposed to high concentrations of conidia. The ability of the *M. rileyi* fungus to cause epizootics in caterpillar populations in the field (Ignoffo 1981, Sujii et al. 2002) and the increased incidence of the disease in commercial crops after releasing propagules of the fungus (Ignoffo et al. 1976a) indicate its great potential to control lepidopterans. The use of isolates with a larger host spectrum opens a new perspective for the future development of biopesticides against infestations of multiple species of caterpillars in different crops. In the present study, it was possible to identify *M. rileyi* isolates with a high activity on the two important caterpillar pests in grain crops.

The survival analyzes also indicated that there was no difference among the treatments for caterpillars of *C. includens* ($\chi^2 = 1.9$; $df = 2$; $p = 0.4$) and *S. frugiperda* ($\chi^2 = 5$; $df = 2$; $p = 0.08$) (Figure 2). The larval survival started to decrease on the fifth day after the larval exposure, and this reduction was more prominent between the sixth and eighth day of evaluation.

The estimated average lethal times (LT_{50}) for the *C. includens* caterpillars were 7.5, 8.2 and 7.3 days, when respectively exposed to the isolates CG1312, CG381 and Native (Table 1). In the bioassays performed with *S. frugiperda* larvae, the estimated LT_{50} were 5.6, 5.9 and 5.7 days, respectively for CG1312, CG381 and Native (Table 1).

Previous studies have shown a variation in the susceptibility of lepidopterans to infections caused by

Table 1. Lethal time (LT_{50}) for *Chrysodeixis includens* and *Spodoptera frugiperda* larvae treated with CG1312, CG381 and Native *Metarhizium rileyi* isolates.

Treatments	LT_{50} (days)	95 % confidence interval (days)	Equation ^(a)	Df ^(b)	χ^2
<i>C. includens</i>					
CG1312	7.5	7.13-8.19	$Y = -2.425 + 8.456 \log X$	6	7.7*
CG381	8.2	7.73-9.04	$Y = -2.383 + 8.056 \log X$	7	14.2*
Native	7.3	7.02-7.61	$Y = -2.814 + 9.059 \log X$	6	4.8*
<i>S. frugiperda</i>					
CG1312	5.6	5.05-6.03	$Y = 0.521 + 5.987 \log X$	4	4.4*
CG381	5.9	5.30-6.49	$Y = 0.070 + 6.341 \log X$	4	6.7*
Native	5.7	5.08-6.19	$Y = -1.469 + 8.524 \log X$	4	8.8*

^(a) Equations obtained by probit analysis, where Y is the probit value and X the conidia concentration. ^(b) Df: degree of freedom. * Significant values by the Chi-square (χ^2) test at 5 % of probability.

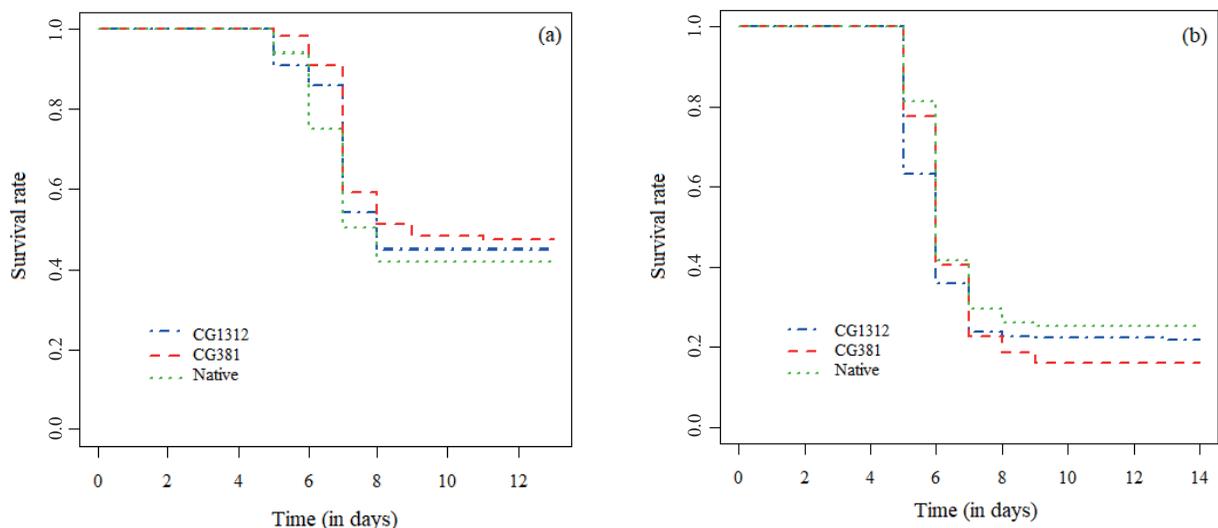


Figure 2. Survival rates of *Chrysodeixis includens* (a) and *Spodoptera frugiperda* (b) larvae after exposure to different isolates of *Metarhizium rileyi* (CG1312, CG381 and Native).

the *M. rileyi* fungus (Ignoffo et al. 1976b, Puttler et al. 1976, Ignoffo & Garcia 1987, Kulkarni & Lingappa 2002). The successful infection and evolution of the disease caused by *M. rileyi* in larvae have a direct relation with the specificity of the different fungal isolates, as well as the tolerance of the host species (Fronza et al. 2017). The absence of certain attributes of the isolate related to the penetration mechanisms and/or characteristics of the host integument may influence the germination success and host invasion by the pathogen (Ignoffo & Garcia 1985). Although *M. rileyi* isolates may show high specificity to some caterpillars, causing high mortality rates in less tolerant hosts, such variation in susceptibility can be considered to improve the screening studies of strains with a broader host spectrum (Boucias et al. 1982). For example, candidates for the control of defoliating caterpillars in soybean could be selected based on their activity only against *C. includens*, since *S. frugiperda* seemed to be more susceptible to the fungus, according to our bioassays.

The host and geographic origin of *M. rileyi* isolates to a specific target have also been the objective of specificity studies. Ignoffo & Boucias (1992) suggest that the homogeneity of the fungus isolates in their ability to infect larvae of some lepidopterans is more dictated by the host than the geographic origin. According to Boucias et al. (2000), *M. rileyi* isolates show a greater virulence to the host species from which they were isolated. However, this effect was not evidenced in our study, since the final mortality of the individuals was similar for the three isolates evaluated in the two hosts, although the LT_{50} values of *C. includens* exposed to the CG1312 and Native isolates (*C. includens* as original host) were lower than for the CG381 isolate (*S. frugiperda* as original host). This trend was not repeated for the LT_{50} of *S. frugiperda*, as the three isolates did not differ.

Other factors, such as the amount of inoculum and the larvae age, may influence the virulence of the tested isolate. A study by Patil et al. (2014) demonstrated that the LT_{50} of *M. rileyi* isolates for some Lepidoptera species, when tested in the 4th and 5th instars, were superior to those observed in the present study for earlier developmental instar, indicating the importance of standardizing studies on host susceptibility and isolate screening. The germination period of conidia and the complete development cycle of *M. rileyi* in the host generally

occur up to 6-8 days, varying according to the isolates, inoculum concentration and host species involved (Srisukchayakul et al. 2005).

The interest of growers in the use of biopesticides has increased worldwide in recent decades, due to the increased cost of developing new safer chemical compounds (Glare et al. 2012) and a concern with the evolution of resistance to chemical insecticides and pest resurgence (Dutcher 2007). The soybean and corn production has been limited, among other factors, by the attack of lepidopteran pests, and the use of *M. rileyi* may contribute to the management of these insects, in order to reduce the environmental and economic impacts provoked by the intensive use of synthetic insecticides. In general, the isolates evaluated in the present study have the potential to be used as biopesticides to simultaneously control *C. includens* and *S. frugiperda* larvae, in soybean and corn crops. Fronza et al. (2017) point out the difficulty of mass production and formulation as technological bottlenecks to use some entomopathogenic fungi. Given this, complementary studies on the formulation, large-scale production and efficiency of these isolates against these pest species under field conditions are necessary to develop a future biopesticide based on *M. rileyi*.

CONCLUSIONS

The pest insects *Chrysodeixis includens* and *Spodoptera frugiperda* showed to be susceptible to isolates of *Metarhizium rileyi* (e.g., CG1312 and CG381, respectively isolated from *C. includens* and *S. frugiperda* caterpillars; and another native wild from *C. includens* cadavers). Our results suggest that the host of origin may not be determinant in the selection of pathogenic isolates of *M. rileyi* against these pest insects.

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