Susceptibility of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) to the fungal entomopathogen *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin s.l. (Hypocreales: Clavicipitaceae)

Susceptibilidad de *Rhyzopertha dominica* (Coleoptera: Bostrichidae) y *Sitophilus oryzae* (Coleoptera: Curculionidae) al hongo entomopatógeno *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin s.l. (Hypocreales: Clavicipitaceae)

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Abstract

Control measures of stored grain pests include the excessive utilization of chemical insecticides that generate negative environmental impact. Current trends in integrated pest management are oriented towards the preservation of the environment using natural biopesticides, among these products arise entomopathogenic fungi. This study aimed to test the efficacy of a native strain of *Beauveria bassiana* to control two main stored grain pests such as *Sitophilus oryzae* and *Rhyzopertha dominica* and also evaluate the persistence of the fungus on wheat grains. The *B. bassiana* strain controlled 89%±0.07 of *R. dominica* adults and 80% ±0.14 of *S. oryzae.* The survival analysis showed that MST was 4.27 ±0.19 days for *R. dominica* adults and 4.27 ±0.20 days for *S. oryzae*. Furthermore, results of long rank test for the comparison of the Kaplan-Meier curves did not present significant differences between the survival of both stored grain pests. Dual choice tests demonstrated that *B. bassiana* LPSc1227 presented a repellent action against both stored grain pests. The seed persistence of conidia was 100% in treated seeds after 45 days. Further research will contribute to elucidate more insecticidal features of the *B. bassiana* LPSc 1227 strain against *S. oryzae* and *R. dominica*, two main stored grain insect pests.

Keywords
stored grain pests • fungal entomopathogens • biopesticides

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Resumen

Las medidas de control de plagas de granos almacenados incluyen la utilización excesiva de insecticidas químicos generando un impacto negativo al ambiente. Las tendencias actuales en el manejo integrado de plagas están orientadas a la preservación del ambiente utilizando biopesticidas naturales, entre estos surgen los hongos entomopatógenos. Este estudio tuvo como objetivo probar la eficacia de una cepa nativa de *Beauveria bassiana* para controlar dos de las principales plagas de granos almacenados, *Sitophilus oryzae* y *Rhyzopertha dominica*, y también evaluar la persistencia del hongo en granos de trigo. La cepa *B. bassiana* controló el 89% de los adultos de *R. dominica* y el 80% de *S. oryzae*. El análisis de supervivencia mostró que el TMS fue de 4,27 días para *R. dominica* y de 4,27 días para *S. oryzae*. Además, los resultados del "long rank" test no presentaron diferencias significativas en la supervivencia de ambas plagas. La prueba de elección demostró que *B. bassiana* presentó una acción repelente frente a ambas especies de insectos. La persistencia de los conidios en las semillas fue del 100% en las semillas tratadas. Futuros estudios permitirán dilucidar la capacidad de *B. bassiana* LPSc 1227 para controlar los principales insectos plaga de granos almacenados, *S. oryzae* y *R. dominica*.

Palabras clave

plagas de granos almacenados • entomopatógenos fúngicos • biopesticidas

Introduction

Food production faces the challenge of keeping up high levels of quality, considering aspects of food safety and production systems with fair remuneration for producers (8).

Grain storage arises as a consequence of the randomness and seasonality of agricultural production. Inside stored commodities, the temperature and humidity conditions favor the appearance of insect pests that find the food and protection to display their multiplication potential. It has been estimated that 5-30% of post-harvested losses worldwide are due to insect damage (25). The damages are qualitative and quantitative, including reduction of hectoliter weight, increased commercial rejection levels, alterations in the nutritional value, deterioration of industrial features and decreased seed germination power (13). Some species in the Order Coleoptera, due to their ubiquity and high destructive potential, constitute one of the greatest entomological problems of stored grains. The "weevil" (*Sithophilus oryzae* L.) and the "cereal borer" (*Rhyzopertha dominica* F.) are primary infestation beetles that initiate the deterioration of healthy grains, the larvae feed on the endosperm, leaving holes that facilitate the entry of secondary infestation species (13).

Despite the negative consequences associated with the utilization of synthetic insecticides, these substances are still the main solution utilized in preventive and curative treatments of stored grains. The use of chemical-synthetic insecticides involves a series of disadvantages: such as the presence of toxic residues in the grain, intoxication of users and consumers, contamination of the environment, and development of insect resistance (1, 4). The urgent need for a control method that ensures the elimination of insect pests, leads in many cases to incorrect and excessive applications of these harmful products, risking food safety and therefore the health of the consumer, generating a negative environmental impact and the rejection of grains in the market (13).

The current demand for healthier food and the change in production paradigms require the total or partial replacement of synthetic pesticides by non-polluting methods. In this sense, a biological alternative, not detrimental to the environment that is also safe for the producers and the consumers is urgently needed to control stored grain pests. Current trends in integrated pest management are oriented towards preserving the environment together with the use of natural biopesticides with less toxicity. Among these products are the entomopathogenic fungi (14, 20).

The species within the genera *Beauveria* and *Metarhizium* are widely used due to their specificity and effectiveness as biological insecticides (11, 22, 26, 27). Several studies have demonstrated the capacity of entomopathogenic fungi to protect stored seeds and have demonstrated their insecticidal capacity to control different beetles (3, 9, 10, 13, 14) highlighting the importance of conducting bioassays for the selection of highly virulent isolates given the high genetic variability presented by these microorganisms.

The hypotheses tested were that native strains are able to control several species of stored grain pests and that the fungus is able to persist on the surface of wheat grains. Thus, this study was conducted to test the efficacy of a native strain of *B. bassiana* to control two main stored grain pests such as *S. oryzae* and *R. dominica* and also evaluate the persistence of the fungus on wheat grains in laboratory conditions.

Material and methods

Insect rearing

Two main stored grain pests *R. dominica* and *S. oryzae,* were selected to perform bioassays. The insects were acquired from the Department of Agricultural Zoology (Faculty of Agronomy, National University of Buenos Aires, Argentina) and a laboratory colony was established at the Spegazzini Institute, La Plata National University, Argentina. The insects were kept inside glass containers (400 mL) and provided with wheat grains (cultivar Klein Capricornio, Cauda Semillas, Chacabuco, Argentina) as food source. The colonies were maintained in a climatic chamber under controlled conditions of temperature and humidity (26 ±2°C and 70±5% RH).

All trials were carried out with adults of 7-10 days of age.

Fungal strain

The fungal strain used to carry out the laboratory tests was provided by the mycological collection of the Spegazzini Institute, La Plata National University, Argentina. The *Beauveria bassiana* LPSc 1227 strain (GeneBank Accession number MG012792) was isolated from *Schistocerca cancellata* (Orthoptera: Acrididae) in Santiago del Estero Province, Argentina during 2016. This strain was selected based on its entomocidal capacity (15). The inocula was obtained from cultures maintained in potato dextrose agar (PDA) for a week at 25°C in the dark. Conidia were harvested with a sterile loop, placed in test tubes and stirred for 2 minutes using a vortex. The conidial concentration was determined as in Goettel & Inglis (1997). The conidia were counted using a Neubauer chamber under a light microscope and the concentration was adjusted to 1×10^8 conidia/ml. The viability of the conidia was determined according to Goettel & Inglis (1997). The fungal suspension (400 µl) was inoculated into slides containing a thin layer of PDA culture media. Slides were kept for 24 h in Petri dishes containing a moistened filter paper to allow conidia germination. Conidia were considered germinated when the germ tube exceeded half its length. Three repetitions were made at different times and 300 conidia were counted on each case. The conidia exhibited a 99% germination rate.

Mortality test

Rhyzopertha dominica and *S. oryzae* adults were inoculated with the fungal solution (1×10^8 conidia/ml) using a hand glass sprayer (20 mL). Control insects were sprayed with a conidia free solution of Tween 80 ® (Merck) 0.01% (v/v). Afterwards, adults were individualized in Petri dishes containing wheat grain as food source and placed inside a climatic chamber under controlled conditions (24±2°C, 75%). Mortality was recorded daily for 14 days. Humid chambers were set up according to Goettel & Inglis (1997) to confirm death by mycosis.

As data did not meet normality insect mortality was analyzed using the Wilcoxon test.

Survival curves and mean survival time (MST) were estimated using the Kaplan-Meyer analysis. Pairwise comparisons between survival curves were made by Logrank test (6, 15). Infostat software was used to performe the statistical analyses (5).

Choice test

A dual-choice olfactometer with static air (supplementary material) was utilized to evaluate the insect preference according to Mitina *et al.* (2020) methods. One pot (20 cm^3) containing 20 g of treated wheat grains was placed at one end of the tube and a container with untreated grains (control) was placed at the other end. Treated group grains were sprayed with the conidial suspension (1× 10^8conidia/ml). In the control group, grains were sprayed with a Tween 80 solution. Insects were placed individually at the center of the olfactometer, and the chosen direction was recorded. The olfactometer was rotated to ensure that the behavior of the insects depended only on the repellent action. Individuals who did not show a response after 10 minutes were not considered in the analysis. A total of 100 insects of each species were employed in this experiment.

To characterize the olfactory response of weevils, the "index of aggregation" (19, 29) was calculated using the following formula:

$$\mathbf{IA= (O\text{-}K)/ (O+K) \times 100\%}$$

where:
O = number of insects in the tube with the treated sample
K = number of insects in the control tube

If differences are obtained between the mean number of insects in the treatment and control, and the value of the index is positive indicates an attractive effect of the sample. On the other hand, a repellent action of the sample is recorded when the value of the index is a negative number. If no differences are observed a neutral action of the sample is inferred (19, 29).

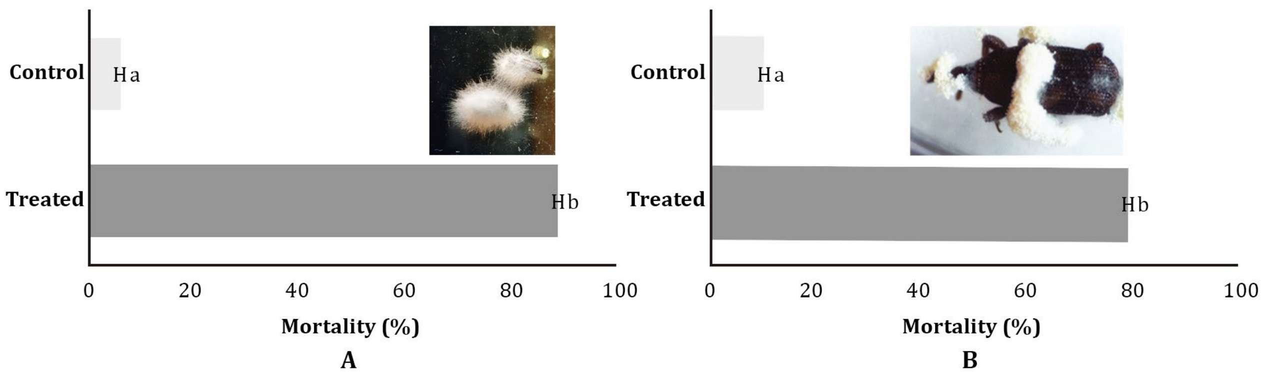
Seed persistence

To evaluate the conidial persistence in wheat over time, seeds (100 g) were autoclaved for 20 minutes, and then were sprayed with 30 ml of the fungal suspension (1×10^8conidia/ml). Inoculated seeds were placed in Erlenmeyer flasks (250 ml) and were maintained at 24°C in the darkness. Three seeds were randomly selected every week for 45 days and were placed in Petri dishes containing PDA. Control seeds were not mixed with the fungal inocula. The Petri dishes were kept under controlled conditions of temperature (25°C) and in darkness for seven days. The persistence was recorded when fungal colonies grew around seeds.

Results

Mortality tests showed significant differences between treatments for both insect species, *R. dominica* (W=135, p < 0.0001) and *S. oryzae* (W=55, p=0.0001). The *B. bassiana* strain controlled 89%±0.073 of *R. dominica* adults and 80% ±0.14 of *S. oryzae* (figure 1).

The Kaplan-Meier analysis exhibited a mean survival time (MST) of 4.27 ±0.19 days for



Different letters show significant differences according to Wilcoxon test (<0.05).
Letras diferentes indican diferencias significativas de acuerdo con el test de Wilcoxon (<0,05).

Figure 1. Percentage of adult mortality after the treatment with *Beauveria bassiana* LPSc 1227 strain. **A**: *Rhyzopertha dominica* and **B**: *Sitophilus oryzae.*

Figura 1. Mortalidad porcentual de adultos de **A**: *Rhyzopertha dominica* y **B**: *Sitophilus oryzae* inoculados con la cepa de *Beauveria bassiana* LPSc 1227.

R. dominica adults and 4.27 ±0.20 days for *S. oryzae*. Results of long rank test (X^2) for the comparison of the Kaplan-Meier curves did not present significant differences between the survival of both stored grain pests (log-rank test p>0.05) (figure 2).

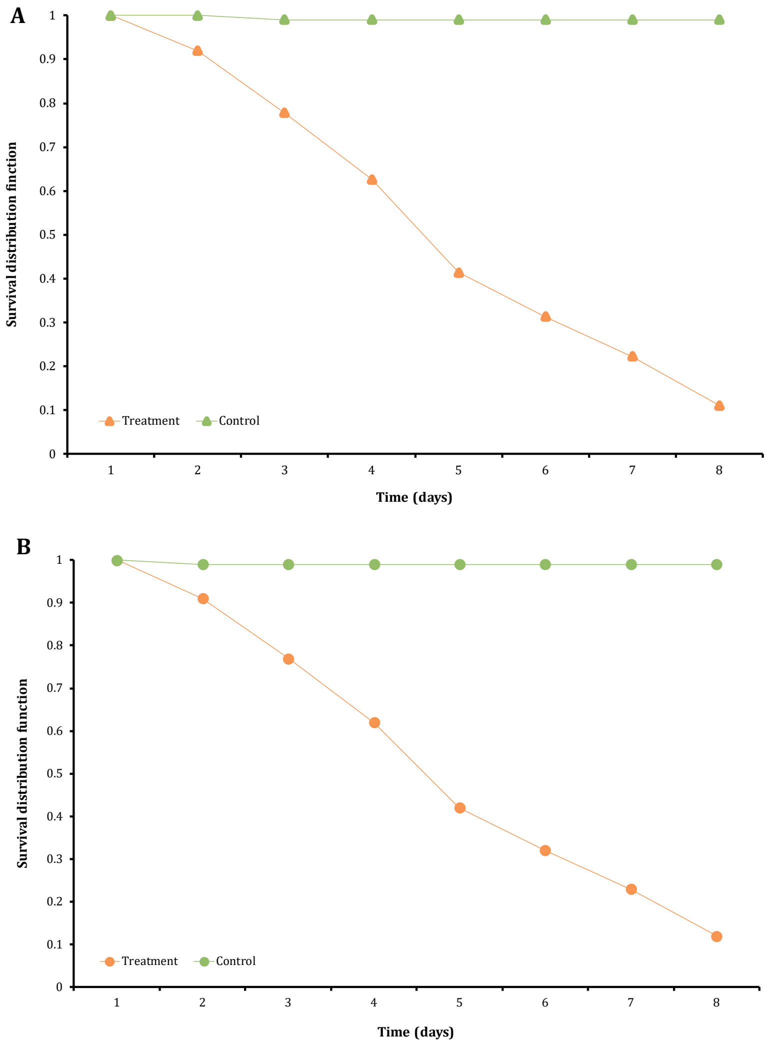


Figure 2. Kaplan-Meier survival curves of *Rhyzopertha dominica* **A:** and *Sitophilus oryzae* **B:** after the treatment with *Beauveria bassiana* LPSc 1227 strain.

Figura 2. Curvas de supervivencia de Kaplan-Meier **A**: *Rhyzopertha dominica* y **B**: *Sitophilus oryzae* luego del tratamiento con la cepa de *Beauveria bassiana* LPSc 1227.

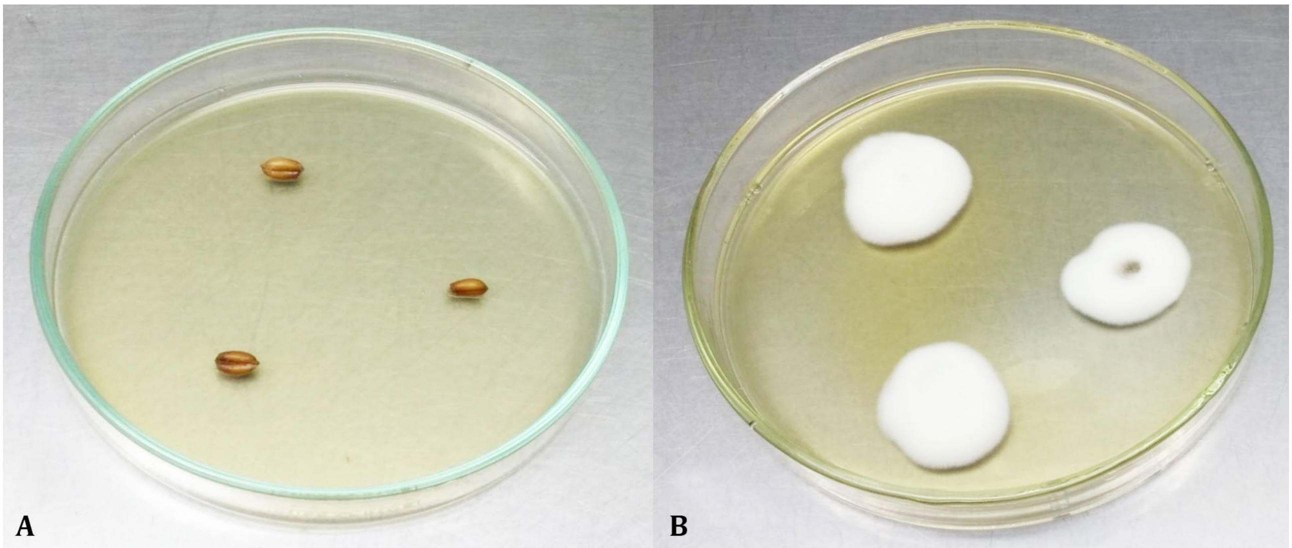
Dual choice tests demonstrated that *B. bassiana* LPSc1227 presented a repellent action against both stored grain pests (table 1).

Table 1. Effect of *Beauveria bassiana* on treated wheat grains on the preference of *Sitophilus oryzae* and *Rhizopertha dominica.*

Tabla 1. Efecto de *Beauveria bassiana* sobre la preferencia por granos tratados de *Sitophilus oryzae* y *Rhizopertha dominica* para granos de trigo con y sin tratamiento de *Beauveria bassiana*.

Species	N° of insects, mean ± SE, %		IA%	Effect
	Treated grain	Control grains		
S. oryzae	14±0.66	86±0.69	-72	Repellent
R. dominica	0	100	-100	Repellent

After 45 days, 100% of wheat seeds studied preserved viable conidia on the surface (figure 3).



A: Control (not inoculated) and **B:** Treated with *Beauveria bassiana* LPSc 1227.
A: Control (no inoculadas) y **B:** Tratadas con *Beauveria bassiana* LPSc 1227.

Figure 3: Persistence of *Beauveria bassiana* conidia 45 days after the wheat grain treatment.
Figura 3: Persistencia de conidios de *Beauveria bassiana* en semillas de trigo luego de 45 días.

Discussion

The results provided by this investigation constitute a starting point in the utilization of the *B. bassiana* strain LPSc 1227 as an effective entomopathogen to control two main stored grain pests.

In this study, *B. bassiana* LPSc 1227 exhibited mortality levels of 80-89% against *R. dominica* and *S. oryzae* respectively, showing a good performance to control both coleopteran species *in vitro*. Many studies presented similar results where the effectiveness of the entomopathogens relied on the fungal strain and the pest species tested. Wakil *et al.* (2021 b) found that *R. dominica* was the most susceptible species to *B. bassiana* and *M. anisopliae*. Also, Reza Pourian & Alizadeh (2021) reported that an isolate of *B. bassiana* was effective in killing 60-73% of *Callosobruchus maculatus* (F.) (Chrysomelidae) and *Oryzaephilus surinamensis* (L.) (Silvanidae). Kordali *et al.* (2021) registered high mortalities (from 62.6% to 100%) of *C. maculatus* adults using different species of entomopathogenic fungi. Furthermore, Yanar *et al.* (2019) also found that the *S. granarius* mortality varied depending on the fungal isolate utilized registering up to 70% mortalities. When analyzing fungal entomopathogens against *R. dominica*, Musso *et al.* (2020) reported that *B. bassiana* strains were the most effective in controlling adults causing up to 65% mortality.

Mean survival time (MST) constitutes an important parameter to describe when characterizing entomopathogens that gives an approximation of the pathogenicity rate of the fungus. In this study, median lethal times were similar for both insects (4.27±0.19 for *R. dominica* and 4.27±0.20 for *S. oryzae*) confirming the good performance of the fungus.

In the Same fashion, El Khourry *et al.* (2022) estimated for four different stored grain pests MST of 3.5±0.3 for *Cathartus quadricollis* (Guerin-Meneville) (Coleoptera: Silvanidae), of 3.8±0.6 days for *Callobrosuchus maculatus* F. (Coleoptera: Chrysomelidae), of 3.8±0.2 for *Sitophilus granarius* L. (Coleoptera: Curculionidae) and of 4.1±0.2 for *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) using a *B. bassiana* strain. Similarly, Kassa *et al.* (2002) when studying several strains of *B. bassiana* to control *Sitophilus zeamais* Motschulsky

(Coleoptera: Curculionidae) and *Protephonus truncates* Horn (Coleoptera: Bostrichidae) registered MST that ranged between 2.85±0.05 to 6.28±0.41 days. On the contrary, higher MST have been found by other authors indicating a poor entomodicidal capacity of the strains employed, for instance Al-Zunti *et al.* (2023) when studying the effect of *B. bassiana* on larval stages of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) found MST of 5-6 days and in the case of *R. dominica*, Musso *et al.* (2020) obtained MST values for *B. bassiana* strains of 8 to 9 days.

The differential susceptibility of different species of stored grain insect pests towards fungal entomopathogens has been attributed to differences in the composition of the insect cuticle, to the conidial concentration or to fungal specificity (3, 4, 27). The entomocidal capacity of the *B. bassiana* LPSc 1227 strain towards two of the main primary stored grain pests represents an important finding in the search for the formulation of an effective bioinsecticide.

Fungal-insect interactions are of great interest to understand the fundamental behavioral processes that occur between insects and pathogens. This issue remains crucial when trying to exploit fungal entomopathogens as biological control agents. Results provided by this study show that the *B. bassiana* strain inflicted a repellent effect on both insect species. Many studies revealed that insects tend to avoid the presence of fungal entomopathogens (16, 17, 23). This behavior has been attributed to volatile compounds released by fungi and to the capacity of insects to detect these specific signals. In this regard, Selitskaya *et al.* (2016) confirmed that *B. bassiana* strain Yuk-4 had a strong repellent effect towards the granary weevil. Similar results were also obtained by Mitina *et al.* (2020) using *B. bassiana*. The authors obtained negative values of the index of aggregation, showing repellence towards the fungus. Also, Selitskaya *et al.* (2014) investigated the behavior response of *S. oryzae* to several *Fusarium* strains and found differential responses of the insects according to the strains.

Seed persistence of conidia on wheat grains over prolonged periods may provide extra protection against stored primary pests since it may contribute to suppressing progeny production (27). Results in this study demonstrated that the strain *B. bassiana* LPSc 1227 remains viable on the seed surface for at least 45 days.

When developing a microorganism-based product the study of multitrophic interactions should be considered and included as part of the basic research of an entomopathogen, future research will contribute to elucidating further properties of the promising strain studied.

Conclusion

This study demonstrates that the native strain of *B. bassiana* LPSc 1227 can effectively control stored grain pests and also persist on the surface of wheat grains.

Further research will contribute to elucidating additional insecticidal features of the *B. bassiana* LPSc 1227 strain against *S. oryzae* and *R. dominica*, two primary pests of stored grain. Additionally, it aims to evaluate whether the conidia present on the seed surface after 45 days retain their germination and insecticidal capacity.

Supplemmentary material

Squematic figure of the dual-choice olfatometer utilized in choice test with stored grain pests:
https://drive.google.com/file/d/1dRT3o9FaT9fXJbjrz71VPs3ybds2wukv/view?usp=sharing

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