Journal of Agricultural Science; Vol. 16, No. 4; 2024
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Lethality of Simulated UV and Solar Diffuse Radiations to DetachedUrediniopores of *Phakopsora pachyrhizi*

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Received: January 25, 2024 Accepted: February 23, 2024 Online Published: March 15, 2024

doi:10.5539/jas.v16n4p22 URL: https://doi.org/10.5539/jas.v16n4p22

Abstract

Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, is the most destructive disease on the crop. This work aimed to generate data to understand the detached uredospores survival during the winter fallow in Mato Grosso state, Brazil. Experiment 'A'—the detached urediniospores maintained and multiplied on Ativa soybean cultivar, were deposited on soybean estract-agar and kept at 25 °C temperature in closed plastic petri dishes and exposed to UV-C (100-280 nm) radiation, distant 30 cm from the plates surface for ' 0'; 60; 120; 180; 240; 300; 360, 420, 480 and 560 minutes. Experiment 'B'—was similar to previous experiment but spores were exposed to UV-B (280-320 nm) radiation. In Experiment 'C'—the urediniospores were deposited into empty plastic petri dishes without lids and exposed to direct diffuse sunlight for 0; 60; 120; 180; 240; 300; 360; 420; and 300 minutes. Solar radiation was measured with a pyranometer (Spectrum Technologies, Inc.). After the exposition time, urediniospores were plated on soybean-extract agar, incubated at 25 °C under dark for 8 h and germination assessed under an optical microscope (400×). Data were submitted to analyses of variance and regression. All experiments were repeated twice. The detached spores exposed to UV-C were killed with 496 minutes, and under UV-B radiation killed with 962.7 minutes and those submitted to direct diffusion solar radiation killed by 15.6 MJ m^{-2} dose, or 240 minutes exposition to 1,250 w/m^2. It can be inferred that the incident solar diffused radiation in Mato Grosso, during the winter fallow, has the daily potential to kill the detached *P. pachyrhizi* urediniospores.

Keywords: *Glycine max*, spore survival, viability, soybean rust, winter fallow

1. Introduction

Soybean Asian rust (SAR) the disease that causes the most crop damage is incited by the obligate basidiomycete fungus, *Phakopsora pachyrhizi* (*Pp*) (H. Sydow & P. Sydow, 1914). The average damage (Nutter et al., 1993) calculated with data (from the 2006-2021 season) from the Antirust Consortium was 64% (unpublished data).

To minimize the damage, control measures such as the soybean free period, the restriction of the sowing date and chemical control have been indicated (Godoy et al., 2020). Related to the soybean free period, it is likely that the host plant does not survive the water deficit of 43.9 mm during the 90 days winter fallow (June to September) in Mato Grosso (MT) (Marcuzzo et al., 2012; Reis et al., 2021a).

Phytopathogenic fungi depend on the host (s) for survival and multiplication. However, hosts are not always available such happen during the MT winter fallow. Therefore, it has been questioned whether urediniospores. can maintain viability when detached from the host in this adverse weather period (Reis et al., 2021a, 2021b).

The main inoculum of *Pp*, produced in soybean under Brazilian climatic conditions, are the asexual, diploid spores, the urediniospores. The fungus survival throughout the year depends on the continuous production of this inoculum in a host plant. Besides soybean no other host plant has been reported in MT considering that only the urediniospores are functional. The period of the year that the fungus needs to survive is in the off-season, or in the winter fallow, which in MT extends from June to September Savana climate.

Since long-distance dispersal of SAR urediniospores has been confirmed (Krupa et al., 2006), there has been an increase in new epidemiological studies to identify the inoculum source for MT state specially during the natural winter fallow.

When spores of different fungi move in the environment during transport in the air, detached from the host, their viability is affected by temperature, solar radiation and relative humidity (Isard et al., 2006; Twizeyimana & Hartman, 2010; Young et al., 2012). A classic work on solar radiation on spores viability was published by Rotem et al. (1985) working with *Peronospora tabacina*, *Uromyces phaseoli* and *Alternaria solani*.

To better understand the detached urediniospores viability during winter fallow (June to September) in MT has been a challenge and priority for researchers. In this sense, the lack of data showing the presence of green bridges in October in MT due to the environment during the winter fallow has been questioned (Reis et al., 2021a).

The identification of primary inoculum sources and its viability at the end the off-season and the mechanism for the fungus survival can improve ASR management. Therefore, the elimination of irrigated soybean was fundamental in reducing the inoculum source during the off-season in MT (Seixas & Godoy, 2007). But, what is the role of detached urediniospores on the fungal survival or can they survive detached from the host? Can they be inoculum for the next season?

In this sense, studies have been carried out, not on detached spores, but on detached infected leaves. For example, Patil et al. (1998) reported that urediniospores.maintained their viability for 55 days on stacked soybean leaves and under the shade of a tree, not directly exposed to solar radiation as occurs in MT during winter fallow. References to data obtained with detached spores were also found, but in a laboratory environment (Godoy & Flausino, 2004; Beledelli et al., 2012) and again, not subjected to direct diffusion solar radiation.

Therefore, in the absence of the soybean plant during the winter fallow, detached urediniospores can maintain viability exposed directly to diffuse solar radiation, whether on senescent leaves, or on any other surface or present in the air? Like the soybean plant, the hypothesis formulated is that the urediniospores also do not remain viable during the winter fallow in MT.

The objective of this work was to determine the damaging effects UV (UV-B and UV-C) of direct diffusion solar radiations, provided by natural or simulates sunlight on detached urediniospores germination.

2. Materials and Methods

2.1 Exposure of Detached Spores to UV-C Radiation

The indoor experiments were conducted in BOD at Agris Institute, Passo Fundo, RS, Brazil. In this experiment spores viability was studied exposing them to short UV-C radiation wavelengths which are absent at the earth surface but are instrumental in elucidating the mechanisms of detached urediniospores survival. Radiation source were two Osram 30 W emitting UV-C radiation (100-280 nm) lamps distant 30 cm from the plates surface and turned on 30 minutes before spores exposure to stabilize radiation. One mL spores suspended in distilled sterile water were poured in plastic petry dishes containing soybean leaf extract agar medium and exposed for 0; 60; 120; 180; 240; 300; 360; 420; 480; and 560 minutes. At the end of each exposure time, the plates were removed from the radiation source and kept at 22-23 ^{o}C until completing 8 h time for maximum germination and 1.0 mL of the acetone + Amann’s blue dye mixture/plate was added to stop growth. The material was kept in closed plastic bags in a refrigerator at 5 ^{o}C until evaluated.

2.2 Exposure of Detached Spores to Simulated UV-B Radiation

The difference between the previous experiment was the radiation source, two UV-B light (280-320 nm, peak 311 nm) lamps.

2.3 Direct Exposure of Detached Spores to Solar Diffusion Radiation

This field experiment was conducted also in Passo Fundo, RS, Brazil. Fresh urediniospores produced on Fundacep 55 soybean cultivar, grown in green house, with seven days old were deposited in open, empty plastic petri dishes (9 cm in diameter) and exposed to direct sunlight at different times: 0, 15, 30, 60, 120, 180, 240, 300 minutes, corresponding to 10:00, 10:15, 10:30, 11:0, 12:0, 13:0, 14:0 and 15:0 h in May sunny days. Finishing each exposure time, the urediniospores were suspended in distilled-sterilized water containing Tween 20 (1.0 drop/L water), plated (1 mL/dish) on the soybean leaf extract agar medium (Blum et al., 2015) and maintained for 8 hours in the dark at 22-23°C in an incubator. The spores were inactivated adding 1.0 ml/plate acetone + Amann’s lactophenol blue dye and stored in a refrigerator in a sealed plastic bags until evaluated. Uredospore germination was determined under a microscope (400×) considering viable the spore with a germ tube longer than its longest diameter.

2.4 Experimental Design

Four treatment repetitions were observed, in completely randomized design. The experimental unit was represented by a petri dish. Data were subjected to analysis of variance and regression. The three experiments were repeated twice.

3. Results

3.1 Exposition to UV-C Radiation

The percent survival of urediniospores decreased linearly as the simulated solar irradiance flux increased. A linear regression model was fit to the data; y = -0.1458x + 72.379, R^2 = 0.87 (Figure 1). The spore germination was reduced as the exposure time increased and completely killed with 496 minutes exposure (Figure 1).

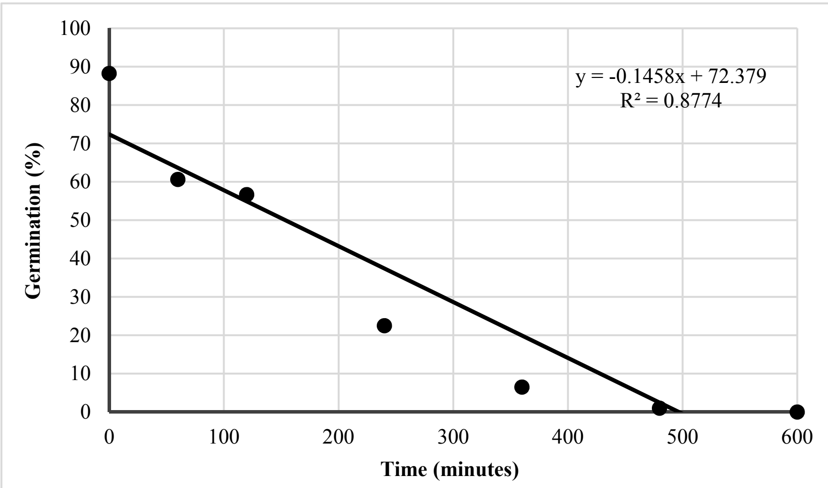

Figure 1. Negative relationship between *Phakopsora pachyrhizi* urediniospores germination and exposure to UV-C radiation time. Means of two experiments

3.2 UV-B Radiation

The percent survival of urediniospores decreased linearly as the simulated solar irradiance flux increased. A linear regression model was fit to the data; Y = 0.0906x + 87.227 (R^2 = 0.8099) (Figure 2). The spore germination was reduced as the exposure time increased, but total lethality would need 962.7 minutes exposure.

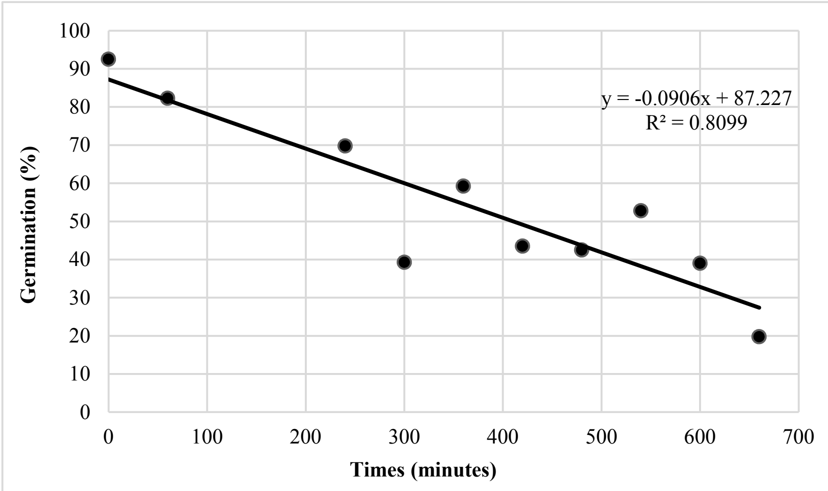

Figure 2. Negative relationship between *Phakopsora pachyrhizi* urediniospores germination and exposure to UV-B radiation time. Means of two experiments

3.3 Exposure to Direct Solar Diffusion Radiation

In these experiments carried out in Passo Fundo (RS) (latitude 28°13′50.62″S, longitude 52°22′59.13″W, altitude 683 m a.s.l.), in May, 2022, under solar diffuse radiation of 1,250 W m^{-2} and with four-hour (240 minutes) exposure corresponding to 15.18 MJ m^{-2} dose resulted in total death of *P. pachyrhizi* urediniospores (Figure 3).

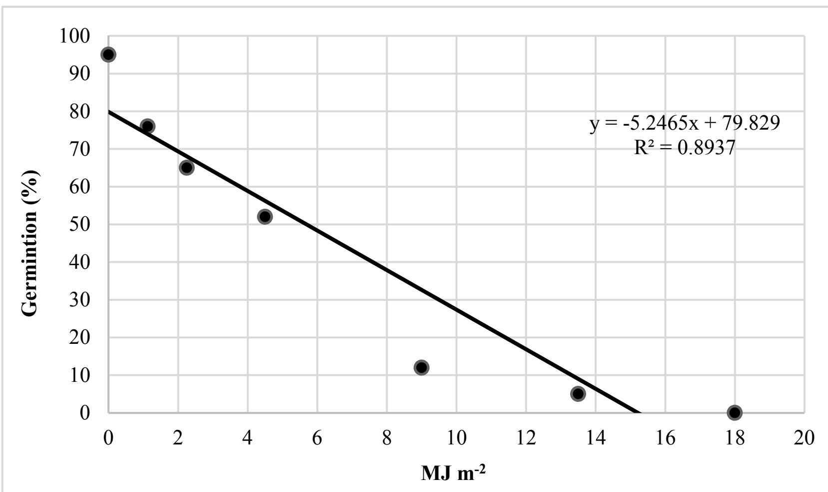

Figure 3. Negative relationship between *Phakopsora pachyrhizi* urediniospores germination and exposure to diffuse solar radiation doses (MJ m^{-2}). Means of two experiments

The viability of detached urediniospores can be predicted as a function of incident solar radiation. In our work each 1.0 MJ m^{-2} reduces 5.2465% germination, considering 79.82 initial germination (R = 0.89).

4. Discussion

Ultraviolet (UV) radiation is a natural component of sunlight and a part of the environment in which life evolved and adapted. The UV component of terrestrial radiation from the midday sun comprises about 95% UV-A and 5% UV-B; UV-C and most of UV-B are removed from extraterrestrial radiation by stratospheric ozone.

According to Kerr and Fioletov (2008) UV radiation is classified as UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm).

Regarding UV-C, Molina and Rowland (1974) proposed that stratospheric ozone might be destroyed by industrially produced chlorine- and bromine-containing stable substances, such as chlorofluorocarbons commonly used in spray cans, refrigerators and air conditioners.

4.1 Solar Diffusion Radiation

But the lethal solar diffuse radiation dose found in our experiment does occur in MT? According to Souza et al. (2016) and Zamadei et al. (218) during the winter fallow, from June to August, the daily insolation in MT lasts 300 h month and with 16.4 to 18.3 MJ m^{-2} day^{-1} radiation dose (Souza et al., 2016), is enough to kill the *Pp* urediniospores.

It is important to discuss the fact that the implementation of 90-day duration of the soybean free period in MT was based on the work of Patil et al. (1998) where the viability of non-detached spores in soybean leaves was reported as 55 days (Godoy et al., 2020). The time reported by Patil et al. (1998) is longer than ours and Young et al. (2012) data with detached urediniospores exposed to UV radiation.

It is likely that spores present in the air, or on any surface, including senescent and decaying leaves (June/July/August) under one day can be killed by diffuse solar radiation which confirm our hypothesis.

Considering ours and Young et al. (2012) data, the detached spores exposure submitted to solar radiation for one day in MT, as occurs in the days of June, July, August (Souza et al., 2016), can be killed. However, in MT there are 90 continuous days with more than 10 hours of daily sunshine. For example, in Sorriso (MT) the diffuse irradiation is 1,601 in May, 1,515 m in June and 1,634 in July an average of 1,533 Wh m^2 day more than enough to kill de *Pp* detached inoculum (Souza et al., 2016).

Regarding to soybean free period its great contribution was to avoid the cultivation of irrigated soybean crop in MT.

As shown by our and other works, solar radiation has potential to eliminate the detached urediniospores from the host, nonetheless, some questions remain yet: what was the importance of soybean free period and sowing time restrictions to fight *Pp* resistance to site-specific fungicides and where is the *Pp* inoculum source for soybean in MT?

However, it must be remembered that the frequency and duration of the liquid water resulting in daily leaf wetness is fundamental to the *Pp* infectious process to soybean (Marcheti et al., 1976). But, as reported by Lubin and Jensen (1995), and Isard et al. (2006), the removal and transport of urediniospores by the wind in cloudy days are protected from lethal solar radiation. Based on Souza et al. (2026), and Zamadei et al. (2018) this condition, that favors the development of rust, frequently occurs annually in MT from October to April.

In this 90 days period (June-August) there should be no present live host multiplying and renewing the inoculum (Reis et al., 2021a). The last moment for this should occur in the last green crops in December/January or in green bridges until May. According to our data, the detached asexual spores are unlikely to survive for 4-5 months, until the presence of new soybean plants.

Another evidence that should be stressed is that ASR occurs in MT with high frequency in December/January (Godoy et al., 2020). This reinforces the fact that urediniospores in MT are not present or if so inviable. If they were present and viable, both in voluntary soybeans or as detached spores, the disease should be observed on the first emerged plants and not later.

Finally, it can be concluded that both the soybean plant, due to water deficit, and the detached urediospores, due to the dose of diffuse solar radiation, do not survive during the winter fallow in MT.

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Acknowledgments

We would like to thank Aprosja MT for the financial support.

Authors Contributions

Not applicable.

Funding

This work was supported by Aprosja MT.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and Peer Review

Not commissioned; externally double-blind peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.