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Short Communication

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Preservation of *Phakopsora pachyrhizi* Uredospores

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Abstract

This study compared different temperatures and dormancy-reversion procedures for preservation of *Phakopsora pachyrhizi* uredospores. The storage temperatures tested were room temperature, 5°C, −20°C and −80°C. Dehydrated and non-dehydrated uredospores were used, and evaluations for germination (%) and infectivity (no. of lesions/cm^2) were made with fresh harvested spores and after 15, 29, 76, 154 and 231 days of storage. The dormancy-reversion procedures evaluated were thermal shock (40°C/5 min) followed or not by hydration (moist chamber/24 h). Uredospores stored at room temperature were viable only up to a month of storage, regardless of their hydration condition. Survival of uredospores increased with storage at lower temperatures. Dehydration of uredospores prior to storage increased their viability, mainly for uredospores stored at 5°C, −20°C and −80°C. At 5°C and −20°C, dehydrated uredospores showed increases in viability of at least 47 and 127 days, respectively, compared to non-dehydrated spores. Uredospore germination and infectivity after storage for 231 days (7.7 months), could only be observed at −80°C, for both hydration conditions. At this storage temperature, dehydrated and non-dehydrated uredospores exhibited 56 and 28% of germination at the end of the experiment, respectively. Storage at −80°C also maintained uredospore infectivity, based upon levels of infection frequency, for both hydration conditions. Among the dormancy-reversion treatments applied to spores stored at −80°C, those involving hydration allowed recoveries of 85 to 92% of the initial germination.

Introduction

Soybean rust is caused by the basidiomycete *Phakopsora pachyrhizi* Syd. & P. Syd. In addition to soybean (*Glycine max* (L.) Merrill), this fungus can infect more than 60 plant species in 26 genera (Ono et al., 1992). In the New World, the disease was reported to occur in epidemic conditions only in 2001, then spreading across South America (Yorinori et al., 2005). In 2004 it was reported in the United States (Schneider et al., 2005).

The disease is highly destructive to above-ground parts of the soybean plant, causing considerable losses in several countries (Sinclair and Hartman, 1999). *Phakopsora pachyrhizi* is a biotrophic fungus, a trait that prevents it from being grown in culture medium. For research purposes, uredospores are produced by means of inoculations in soybean plants. This requires the availability of labour, time and greenhouse space. In addition, genetic characteristics of the pathogen population or isolates are difficult to maintain. Therefore, an effective uredospore preservation method would facilitate plant pathology assays. Several methodologies for the preservation of uredospores are described in the literature for other rusts, including wheat (Bromfield, 1964), sugarcane (Garcia et al., 2007) and bean rust (Faleiro et al., 2000).

Thus, this study aimed to develop a methodology to preserve *P. pachyrhizi* uredospores for extended periods, while maintaining their viability and infectivity.

Materials and Methods

Uredospores were collected from naturally rusted soybean plants in the Piracicaba area, state of Sao Paulo, Brazil. Part of these uredospores was dehydrated (in silica gel for 24 h) prior to storage, while another part was not dehydrated.

About 0.05 g of spores were placed inside 0.5 ml plastic microtubes and tightly sealed. These microtubes of both dehydrated and non-dehydrated spores were kept at the following temperatures: 25 ± 3°C (room temperature), 5 ± 2°C (refrigerator), −20°C (freezer) and −80°C (deep freezer).

Evaluation of uredospore viability was carried out by means of *in vitro* germination tests, whereas infectivity was verified by inoculations in soybean plants, performed immediately prior to storage and after 15, 29, 76, 154 and 231 days. Reversion of dormancy induced by storage was accomplished by subjecting the

spores to a thermal shock at 40°C for 5 min in a thermocycler, followed by hydration for 24 h inside a moist chamber (Bromfield, 1964).

Suspensions containing 10^5 uredospores/ml in distilled water + Tween 20 (0.05%) were used in the *in vitro* germination tests. Aliquots (0.5 ml) were spread onto Petri dishes containing water–agar culture medium (2%) and maintained at 20°C in the dark. The germination process was stopped after 6 h of incubation by adding lactoglycerol to the dishes. The percentage of germinated uredospores was determined by examination of 100 randomly selected spores/dish under an optical microscope at 100× magnification. Each treatment contained six dishes. Uredospores exhibiting a germ tube at least as long as its largest dimension were considered to be germinated.

In the infectivity tests, the same spore suspension used in the germination tests was sprayed onto the adaxial surface of soybean leaves (cv. BRS 154, phenological stage V_1), using six potted plants per treatment. After inoculation, the plants were placed at 23°C in the dark, remaining under moist chamber conditions during the first 24 h. Evaluations were performed 14 days after inoculation, and to accomplish this, the pustules present in a 1 cm^2 area in the centre of each inoculated leaflet were counted, using a stereoscopic microscope.

A new assay using dehydrated and non-dehydrated spores was implemented in order to evaluate in detail uredospore loss of viability at room temperature. These spores were placed inside microtubes and maintained at 25 ± 3°C. Viability was evaluated using the same methodology previously mentioned, at the onset of the experiment and after 4, 6, 9, 14, 16, 20 and 22 days.

Dehydrated uredospores, stored for 30 days at −80°C, were used in the dormancy-reversion studies. After that period, the uredospores were submitted to the following treatments: (i) without thermal shock and without hydration, (ii) thermal shock (40°C/5 min) only, (iii) hydration (moist chamber/24 h) only and (iv) thermal shock followed by hydration. As a control treatment, spore germination was evaluated on the day spores were collected. Viability of these spores was evaluated by following the same procedures as used previously.

Results and Discussion

Initial germination was 50.6 and 62.2%, and initial infectivity was 62 and 106 lesions/cm^2 for dehydrated and non-dehydrated uredospores, respectively (Fig. 1). Therefore, dehydration decreased initial spore germination and infectivity.

In general, it was observed that the dehydration process provided higher spore viability at all storage temperatures. However, this was more evident for spores stored at room, refrigerator and freezer temperatures.

Uredospore germination and infectivity after storage for 231 days (7.7 months) could only be observed for those kept at −80°C, regardless of the hydration condi-

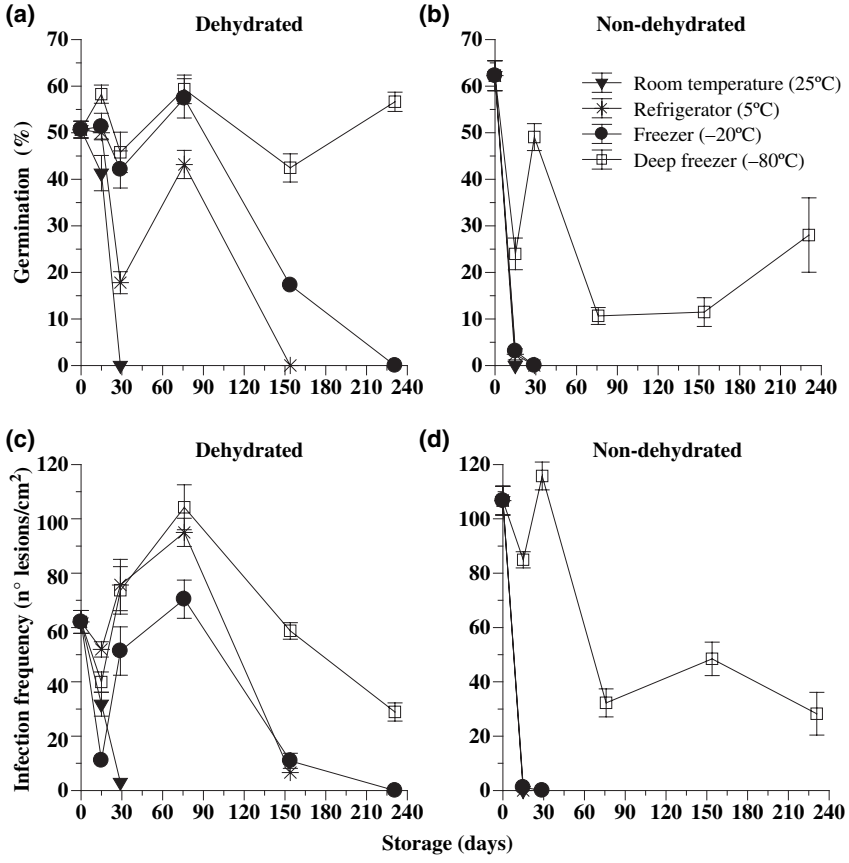

Fig. 1 *In vitro* germination (a and b) and infectivity on soybean leaves (c and d) of dehydrated and non-dehydrated *P. pachyrhizi* uredospores. Data are means of six replicates. Bars represent standard error

tion. However, dehydrated uredospores exhibited a higher germination rate.

Spores that germinated *in vitro*, irrespective of the germination rate, also maintained infectivity on soybean plants, although we could not establish a correlation between these two variables in most cases (Fig. 1). However, this lack of correlation apparently is common in most experiments comparing spore germination on artificial and natural surfaces (Tessman and Dianese, 2002; Garcia et al., 2007). This pattern can be explained by the differences observed between these two conditions. Germination on natural surfaces, besides spore viability, also depends on environmental conditions and host characteristics, like stimulatory substances (Lucas and Knights, 1987; French, 1992). Regardless the lack of correlation, results showed that *P. pachyrhizi* uredospores remained viable and infective at the end of the experiment.

In *Puccina melanocephala* (sugarcane rust), the best spore preservation treatments were dehydration in silica gel, followed by storage at −20°C and −80°C. Under these conditions, the spores remained viable and infective even after 1 year of storage (Garcia et al., 2007).

In the assay where viability was only evaluated at room temperature, the results showed that *P. pachyrhizi* uredospores did not have a good survival rate under that condition. However, spore dehydration, although causing a reduction in initial viability, allowed the preservation period to be extended for 6 days (Fig. 2).

Among the dormancy-reversion treatments, those involving hydration provided the highest germination recovery (Table 1). Hydration/thermal shock and hydration/no thermal shock treatments were similar. Initial germination observed before storage was 43%.

Rust spores apparently become dormant when stored at low temperatures, and show reduced germination rates (Bromfield, 1964). Loegering et al. (1961) froze *Puccinia graminis* f. sp. *tritici* spores in liquid nitrogen and observed germination levels similar to non-frozen spores after submitting them to thermal shock for a few minutes at temperatures between 36°C and 60°C. Bromfield (1964), also working with *P. graminis* f. sp. *tritici*, observed higher dormancy-reversion rates when spores were submitted to thermal shock followed by hydration. Using dormancy reversion, Schein (1962) was successful in recovering *Uromyces appendiculatus* spores after storage for almost 2 years at −60°C.

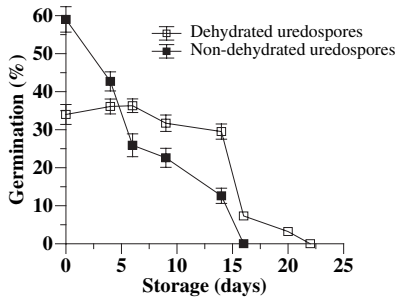

Fig. 2 Survival of *P. pachyrhizi* uredospores under room temperature (25 ± 3°C). Data are means of six replicates. Bars represent standard error

Table 1
Dormancy reversion of *Phakopsora pachyrhizi* uredospores stored for 30 days at −80ºC

Treatment	Germination (%)
With thermal shock and hydration	39.9 A
Without thermal shock, with hydration	36.8 A
With thermal shock, without hydration	22.5 B
Without thermal shock and without hydration	2.9 C
Control (before storage)	43.0

Values in the column with different letters are significantly different at $P < 0.05$ in accordance with Tukey test.

Finally, the preservation period obtained in the present study (dehydrated uredospores, −80°C) is sufficient to meet soybean off-season experimental requirements. In addition, it was demonstrated that the hydration process of *P. pachyrhizi* uredospores is more important in reverting dormancy than thermal treatment.

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