Effect of low temperatures on the survival of *Phakopsora pachyrhizi* urediniospores in overwinter volunteer soybean plants in Entre Ríos Province, Argentina

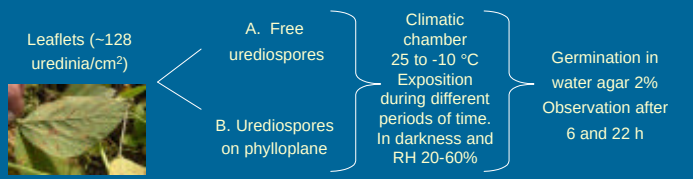
de Souza1, J.; N. Formento1; T. Boca2; C. Godoy3 & A. del C. Ridao4
1 Fact. Bióticos y Prot. Veg. INTA EEA Paraná (Entre Ríos, Argentina), 2 Inst. de Clima y Agua. CIRN INTA Castelar (Buenos Aires, Argentina),3 Embrapa Soja (Londrina, Brasil) & 4 Fac. de Cs. Agrarias. Univ. Nac. de Mar del Plata (Buenos Aires, Argentina).
jdesouza@parana.inta.gov.ar; nformento@parana.inta.gov.ar; tereboca@yahoo.com; godoy@cnpso.embrapa.br; ridaoaz@balcarce.inta.gov.ar

INTRODUCTION

The Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Syd. & P Syd., originated in tropical and subtropical zones, occurs in all soybean producing regions in the world. The survival of biotrophic pathogen depends on the continuous production of urediospores (Pivonia and Yang, 2004; Agrios, 1997). In temperate regions, temperature is probably the environmental factor of major significance in the survival of *P. pachyrhizi* and it has been reported that under 7°C there is no spore germination (Marchetti *et al.*, 1976). Moschini *et al.* (2005) consider 11°C as the threshold of coldness stress and found that in Paraná, Entre Ríos (ER), half the years present favorable winters for the survival of fungus. However, in ER Province during the growing seasons 2004-05 and 2005-06 active uredinia have been observed in volunteer soybean plants surviving to successive agroclimatic frosts (Formento *et al.* 2006; Formento y de Souza, 2006) out of the growing season. This could constitute an epidemiological risk situation for Argentina. The aim of the present study was to analyze the effect of low temperatures on the viability of urediospores under controlled conditions.

MATERIALS AND METHODS

An analysis of low extreme temperatures at 5 cm above ground was carried out (period 1966-2005; Meteorological Station at INTA EEA Paraná) that could affect the survival of urediospores present in volunteer soybean plants.
The spore germination was evaluated as an indicator of the resistance to coldness stress (frost) during winter. Two strategies of pathogen survival were analyzed: A) free spores in the atmosphere and B) spores on the phylloplane of soybean plants. The laboratory trial was carried out twice each with 4 repetitions. Every 2 days during 45 days central soybean leaflets were collected with abundant recent sporulation and the germination was determined at twelve different temperatures (-10 to 25°C) during five exposure periods (1 to 24 h). Controls were considered to determine the levels of initial germination.



After the thermal treatment urediospores were suspended in 1 ml of a solution of sterile distilled water with 0.01% of Tween 20 and 100 ml/l of chloramphenicol and were placed in water agar at 2%. They were incubated at 22 ± 1° C in the darkness and the percentage of germination was registered in 200 urediospores after 6 and 22 h.
The percentage of germination was normalized relative to the mean initial germination of each respective trial (controls). The differences between the distribution observed of the germination control percentages and the normal distribution were analyzed graphically with the PROC UNIVARIATE and with the Kolmogorov–Smirnov test.
The progress of the germination was drawn according to the thermal treatments and the hours of exposure and the models of lineal regression were adjusted with the PROC REG.
To analyze the effect of temperature on germination an ANOVA was carried out considering three polynomial models (Fig. 2). The selection of the cubical model was carried out on the basis of the analysis of residual graphics, the coefficients of determination (R2) and the level of significance of the parameters estimated for the model in t test.
The relative rate of germination was estimated (Zadoks & Schein, 1979) with the mean germination values (v) for each temperature evaluated after 6 and 22 h of observation.
For the statistical analysis of the data the SAS statistical package (SAS, 1999) was used.

RESULTS AND DISCUSSION

During the period 1966-2005 every year 11°C at 5 cm from the ground was recorded at least once; 0°C was observed in 23 years (29%) and -4°C in 15 years (38%) (Table 1). The lowest record was -10°C and it occurred only once in 39 years. These records were used in the trial design to simulate conditions of low temperatures recorded in the region.

Urediospores presented variable levels of initial germination: 22 to 36% (mean: 29) after 6 h and 48 to 73% (mean: 60.5) after 22 h of evaluation.

Exposure to temperatures between 11 and -10°C indicates that frosts and winter temperatures in Paraná may affect germination of the inoculum that remains locally in volunteer plants (Fig. 1). Survival of urediospores, evaluated through the percentage of normalized germination, $\hat{a}_0$ and the relative rate (Fig. 3 and 4) diminished significantly with decrease of the temperature and prolonged exposure to constant temperatures between 6 and -10°C (Fig. 2).

Table 1. Frequency of annual extreme low air temperatures (5 cm height) in Paraná, Entre Ríos,1966 to 2005*

Lowest daily temperature (°C)	Frequency of ocurrente (years)	
	Number	%
11	39	100
10	38	97
9	36	92
8	35	90
7	34	87
6	33	85
5	32	82
4	31	79
3	30	77
2	28	72
1	26	67
0	23	59
-1	20	51
-2	18	46
-3	17	44
-4	15	38
-5	11	28
-6	4	10
-7	3	8
-8	1	3
-9	1	3
-10	1	3

* Data collected at the INTA EEA Paraná weather station.

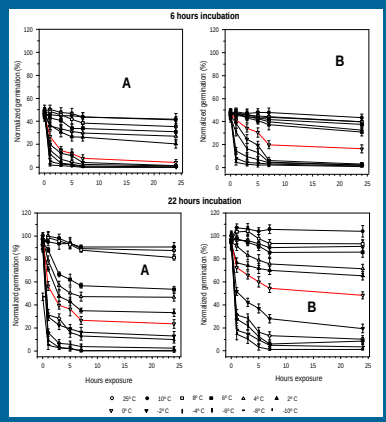


Fig. 1. Normalized germination (%) of *P. pachyrhizi* exposed to constant temperatures of 25, 10, 8, 6, 4, 2, 0, -2, -4, -6, -8 and -10º C for 1, 3, 5, 7 y 24 h. Free spores (A) and Spores on phylloplane (B). Data points for each temperature represent means of experiments replicated four times y repeated twice. Vertical line drawn through data point indicates ± one standard error of the mean.

Germination of urediospores decreased significantly at less than -4°C, even for brief periods. At -10°C very low values were obtained, almost zero germination.

After 22 h of incubation under optimum conditions (22°C), after exposure to coldness, germination was increased approximately 50% with respect to the evaluation after 6 h, independently of the treatment applied (Fig. 1).

Brief periods of exposure to temperatures of less than 6°C negatively affected the survival of urediospores. However at higher temperatures a significant reduction of germination was not observed, especially for the spores kept in the phylloplane (Fig. 1, B).

The phylloplane of volunteer plants could provide some protection to the urediospores. Even with periods of up to 24 hours, germination remained above the values determined for the free urediospores, especially at temperatures below 6°C (Fig. 1 and 2).

Urediospores of *P. pachyrhizi* may remain viable after being exposed to environmental conditions of cold stress (frost), however, this viability might be affected by other factors not considered in this study.

For the winter conditions of Paraná (ER), low temperatures would not constitute a limiting factor for the survival of *P. pachyrhizi* and urediospores could remain active on volunteer plants until the sowing of new soybean crops.

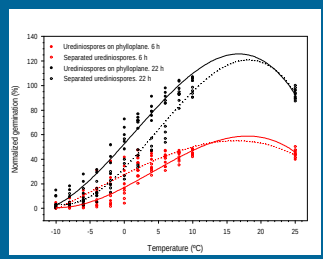


Fig. 2. Relationship between normalized germination (%) of *P. pachyrhizi* to constant temperatures for different periods of times. Polynomial model (p<0.0001) lines are shown. : y = 27.612 + 2.757x -0.029x2 – 0.023 x3, adjusted R2 = 0.84; : y = 17.040 + 2.872x + 0.068 x2 – 0.005 x3, adjusted R2 = 0.92; : y = 52.586 + 6.256x +0.036 x2 – 0.009 x3, adjusted R2 = 0.95; : y = 32.919 + 5.775x +0.166 x2 – 0.012 x3, adjusted R2 = 0.94.

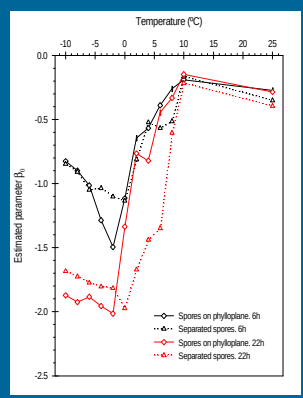


Fig. 3. Effect of temperature on the estimated parameter â0 for the lineal models (y = â0 + â1x + â2x² + â3x3) fitted to normalized germination (%) and constant temperature data in a multiple regression analysis.

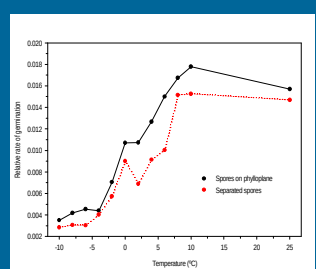


Fig. 4. Progress of relative rate of germination (v) over temperature, estimated with the germination mean in percentage for each temperature according to v = ((x2 – x1)/ X(t2-t1)). x1 and x2 are the number of germinated spores at time t1 and t2, X is the number of total spores.