THE INFLUENCE OF CULTURE MEDIA ON THE SPORULATION OF *SEPTORIA GLYCINES* HEMMI, CAUSAL AGENT OF SOYBEAN BROWN SPOT[1]

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ABSTRACT - This work was undertaken to obtain a culture medium for fast and abundant spore production of *Septoria glycines*. Iniatially, oat meal agar, PDA, Czapek agar, tomato extract agar, soybean leaf extract agar, corn meal agar, corn meal agar, Fries agar, Sabouraud dextrose agar, and wheat flour agar media were compared. In a second trial the effect of adding a filter paper disc on the top of solidified media was tested. In both experiments the greatest number of pycnidiospores were produced on Fries medium. With the exception of Fries agar medium, filter paper did not increase spore production.

Index terms: fungus, pycnidiospores, spores, agar.

INFLUÊNCIA DE MEIOS DE CULTURA NA ESPORULAÇÃO DE *SEPTORIA GLYCINES* HEMMI, AGENTE CAUSAL DA MANCHA-PARDA DA FOLHA DA SOJA

RESUMO - O presente trabalho teve como objetivo determinar um meio de cultura que proporcione produção rápida e abundante de picnidiosporos de *Septoria glycines*. Para isto, foram realizados dois experimentos: no primeiro, compararam-se os meios de cultura: aveia ágar, BDA, Czapek ágar, extrato de tomate ágar, extrato de folha de soja ágar, farinha de milho ágar, Fries ágar, Sabouraud dextrose ágar e semolina ágar; no segundo, testou-se o efeito da sobreposição de um disco de papel-filtro aos meios de cultura. Destacou-se dos demais, nos dois experimentos, o tratamento Fries ágar, sendo que a sobreposição de um disco de papel-filtro não proporcionou aumento na produção de picnidiosporos para a maioria dos meios testados.

Termos para indexação: fungos, picnidiosporos, esporos, ágar.

INTRODUCTION

The fungus *Septoria glycines* Hemmi, causal agent of soybean (*Glycine max* (L.) Merrill) brown spot is distributed worldwide and it has been reported in the United States, Brazil, Canada, China, Germany, Italy, Japan, Corea, Manchuria, Taiwan, Russia and Yugoslavia (The American Phytopathological Society 1975). In a survey carried out by Lehman et al. (1976), in the two most southern states of Brazil, of Rio Grande do Sul and Santa Catarina, the fungus was found on 65% of the observed farms. According to Araújo et al. (1973), the disease was present in 1973 on soybeans in the state of Paraná (another southern state of Brazil), causing lesions both on seedlings and adult plants.

The attack of the fungus on cotyledons, leaves, stems, and legumes results in yellowing and leaf fall and a reduction in plant height, number of pods per plant, and seed weight. Under natural epidemics, crop losses have been estimated at 10% according to Pataky & Lim (1981a) and with artificial inoculation under field conditions up to 33% (Lim 1980).

Resistant cultivars to *S. glycines* have not been found, but differences in susceptibility of some genotypes have been observed by Lim (1979) and Reis (1973). Screening tests to find resistant cultivars require artificial inoculation under controlled environmental conditions and a large quantity of inoculum at the desired time.

Wolf & Lehman (1926) used potato dextrose agar (PDA), corn meal agar, cassava, and soybean legumes media for sporulation. However, these authors did not quantify spore production on the cited substrata. Benedict (1964) tested Sabouraud dextrose agar obteining good fungus growth. Nevertheless he did not quantify spore production. Picinini (1978) found that culturing on PDA under

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continuous light, with temperatures between 23°C and 30°C and pH 6.0, obtained the highest sporulation.

The effect of the addition of filter paper to media has been studied by several workers. Lukens (1960) and McDonald & Martens (1963) obtained increased sporulation of *Helminthosporium* sp. and *Alternaria* sp. when they added a filter paper disc to the top of solidified media. Bean & Wilcoxson (1964) used filter paper as a source of carbon to increase sporulation of *Helminthosporium* sp. This technique was also used with *Colletotrichum dematium* var. *truncata* by Reis (1973), *C. falcatum* by Kimati (1975), and with *Fusarium oxysporum* by Berton (1981) but it did not increase sporulation. This work was undertaken to obtain a suitable culture medium for fast and abundant pycnidiospores production by *S. glycines*.

MATERIAL AND METHODS

Isolates of *S. glycines* used throughout this work were obtained from soybean leaves of several cultivars showing symptoms of brown spot in the county of Passo Fundo, state of Rio Grande do Sul. Isolates were identified morphologically and by pathogenicity tests. They were maintained throughout this work in PDA slants.

Experiment 1

In this trial the following media were compared for pycnidiospores production: oat meal agar (oat meal 60 g, agar 12 g, water 1,000 ml); PDA (sliced potatoes 140 g, dextrose 10 g, agar 15 g, water 1,000 ml); Czapek agar ($NaNo_3$ 3 g, $K_2HPO_4.7H_2O$ 1 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4.7H_2O$ 0.01 g, sucrose 30 g, agar 15 g, water 1,000 ml); soybean leaf extract agar (soybean leaves 200 g, sucrose 10 g, agar 15 g, water 1,000 ml); tomato extract agar (tomato extract 380 g, $CaCO_3$ 6 g, agar 30 g, water 1,000 ml); corn meal agar (corn meal 40 g, agar 15 g, water 1,000 ml); Fries agar [$(NH_4)_2$ $C_4H_4O_6$ 5 g, NH_4NO_3 1 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, $CaCl_2$ 0.13 g, NaCl 0.1 g, yeast extract 1 g, sucrose 30 g, agar 15 g, water 1,000 ml]; Sabouraud dextrose agar (neopeptone 10 g, dextrose 40 g, agar 15 g, water 1,000 ml); and wheat flour agar (100 g of wheat flour, agar 12 g, water 1,000 ml). The pH was adjusted to 6,0 prior autoclaving. To avoid the growth of undesirable bacteria, 200 μg/ml of streptomycin sulfate was used in each medium.

To grow the fungus, a 1.5 ml spore suspension of 10^6 pycnidiospores/ml in sterile water was placed on PDA medium in plastic petri dishes. The inoculum was evenly distributed on the surface of the medium by gentle shaking and tilting the plates. The cultures were then incubated at 25°C under continuous light (Picinini 1978) for 15 days.

Experiment 2

In this trial the effect of adding a disc of filter paper (Whatman n. 4) on the top of solidified media was tested. The same culture media as in experiment 1 were used. The spore suspensions were poured on sterilized 9 cm diameter filter paper discs and spread on its surface by using a glass rod.

Spore suspension in water for the media without filter paper were prepared by gently brushing two agar cylinders per plate cut out with a cork borer. For the media with filter paper the whole surface was brushed to make the spore suspensions. The number of spores per cm^2 was counted using a Neubauer chamber.

A randomized complete block design with five replicates was used, and Duncan's multiple range test was used to compare means.

RESULTS AND DISCUSSION

In experiment 1, Fries agar medium produced the largest number of spores of *S. glycines* (Fig. 1). Soybean leaf extract agar, tomato extract agar, Sabouraud dextrose agar, and oat meal agar produced all significantly less spores than Fries agar medium. The smallest number of spores was obtained on PDA, corn meal agar, Czapek agar, and wheat flour agar.

In experiment 2 (Fig. 2) the filter paper disc placed on the surface of the media increased sporulation only for Fries agar medium. For oat meal agar this technique reduced sporulation. The increase in sporulation may be explained by the increase in the surface area. The filter paper may also serve as a carbon source as shown by Bean & Wilcoxson (1964), Kimati (1975), McDonald & Martens (1963) and Reis (1973).

The effect of Fries agar medium may be due to the presence of yeast extract. The benefits of this extract on the sporulation of *Nomuraea rileyi* has been reported by Loch (1978). Yeast extract is a complex substance and it has several growth factors in its composition such as vitamins which are also required by *S. glycines* for sporulation.

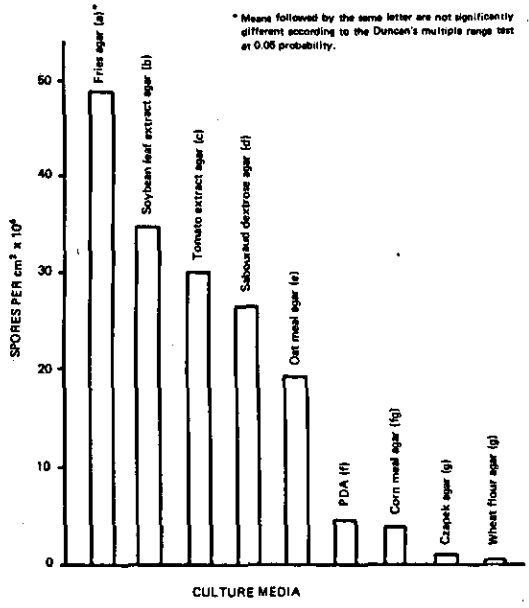

FIG. 1. Effect of culture media on the sporulation of *Septoria glycines*.

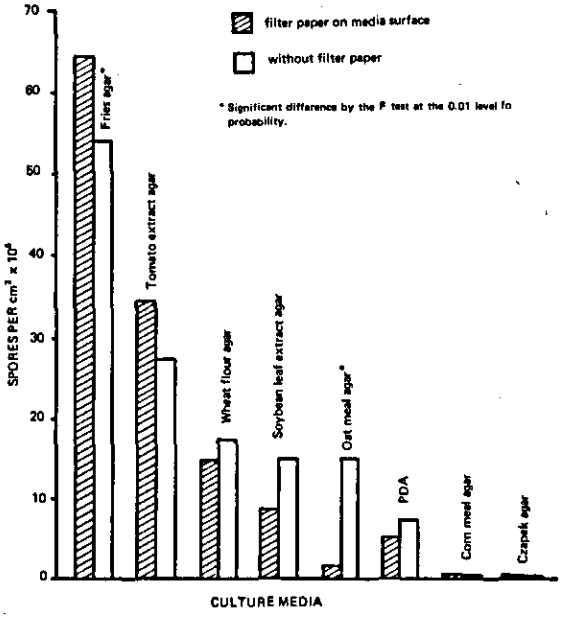

FIG. 2. Effect of adding a filter paper disc on the surface of culture media on the sporulation of *Septoria glycines*.

It is possible that the yeast extract was responsible for the food performance of the Fries agar medium.

Fries agar, soybean leaf extract agar, tomato extract, agar Sabouraud dextrose agar, and oat meal agar were all superior to PDA, when used by several authors (Lim 1979, 1980), Pataky & Lim (1981a, b) Ross (1982) and Young & Ross (1979). So far it is not known which are the best environmental conditions for the sporulation of *S. glycines* on the media tested, except for PDA (Picinini 1978).

CONCLUSIONS

1. The Fries agar is a good medium for fast and abundant pycnidiospore production of *S. glycines*.

2. This medium can, therefore, be used when large quantities of pycnidiospores are needed for artificial inoculation of soybeans.

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