Reportes Científicos de la FACEN

Reportes científicos de la FACEN, Enero - Junio 2025, 16(1): 19–25

http://doi.org/10.18004/rcfacen.2025.16.1.019

Artículo Original

Evaluation of *in vitro* growth and sporulation of *Cercospora* sp. and *Septoria* sp. in different culture media

Evaluación del crecimiento y esporulación *in vitro* de *Cercospora* sp. y *Septoria* sp. en diferentes medios de cultivo

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Resumen: Este estudio tuvo como objetivo evaluar el crecimiento micelial y la capacidad de esporulación *in vitro* de los hongos fitopatógenos *Cercospora* sp. y *Septoria* sp. (causantes del Tizón Foliar y la Mancha Marrón de la Soja, respectivamente) en diferentes medios de cultivo: Agar Decocción de Hojas (ADH), Agar Decocción de Granos (ADG) y Papa Dextrosa Agar (PDA). Se midió el crecimiento micelial cada tres días hasta completar 15 días de incubación, y se cuantificó la producción de esporas al finalizar el ensayo mediante cámara de Neubauer. Se detectaron diferencias significativas en el crecimiento vegetativo entre los medios. Para *Cercospora* sp., el medio PDA mostró el mayor crecimiento desde el día 6 hasta el día 15, alcanzando un promedio de 21,44 mm, significativamente superior a ADG y ADH. Por otro lado, para *Septoria* sp., el medio ADG fue el más efectivo, alcanzando un crecimiento promedio de 8,84 mm al día 15, siendo el PDA el medio con menor crecimiento (3,65 mm). Este trabajo constituye el primer reporte del uso del medio ADG para estudiar el crecimiento in vitro de *Septoria* sp.. Los resultados demostraron que ninguno de los medios utilizados indujo la esporulación en las condiciones evaluadas, observándose únicamente crecimiento micelial estéril. Se concluye que, aunque los medios evaluados permitieron el crecimiento micelial, es necesario explorar nuevas condiciones ambientales y medios alternativos para optimizar la esporulación, dada la relevancia de estas especies en la epidemiología y manejo de enfermedades en soja.

Palabras clave: *Hongos fitopatógenos, crecimiento, in vitro, medio de cultivo, soja.*

Abstract: This study aimed to evaluate the in vitro mycelial growth and sporulation capacity of the phytopathogenic fungi *Cercospora* sp. and *Septoria* sp. (causal agents of Soybean Leaf Blight and Brown Spot, respectively) in different culture media: Leaf Decoction Agar (LDA), Grain Decoction Agar (GDA), and Potato Dextrose Agar (PDA). Mycelial growth was measured every three days for 15 days of incubation, and spore production was quantified at the end of the experiment using a Neubauer chamber. Significant differences in vegetative growth among the media were detected. For *Cercospora* sp., the PDA medium exhibited the highest growth from day 6 to day 15, reaching an average of 21.44 mm, significantly higher than GDA and LDA. In contrast, for Septoria sp., the GDA medium was the most effective, reaching an average growth of 8.84 mm by day 15, while PDA showed the lowest growth (3.65 mm). This study represents the first report on the use of the GDA medium for studying the in vitro growth of *Septoria* sp. The results demonstrated that none of the tested media induced sporulation under the evaluated conditions, with only sterile mycelial growth observed. It is concluded that, although the evaluated media supported mycelial growth, it is necessary to explore new environmental conditions and alternative media to optimize sporulation, given the relevance of these species in the epidemiology and management of soybean diseases.

Keywords: *Phytopathogenic fungi, growth, in vitro, culture medium, soybean.*

Editor responsable: Bolívar R. Garcete-Barrett*

Recibido: 03/04/2025 **Aceptado:** 01/06/2025

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Introduction

The phytopathogenic fungi *Cercospora* sp. and *Septoria* sp. are responsible for major diseases in soybean production, namely Cercospora Leaf Blight and Septoria Brown Spot, respectively. Both diseases significantly reduce the photosynthetically active leaf area, leading to substantial yield losses (Hartman *et al*., 2015). The widespread adoption of no-till farming, the extensive monoculture of soybean, and the effects of climate change have markedly increased the prevalence and severity of these diseases (Sautua *et al*., 2024).

For *Cercospora* sp., estimated average annual yield reductions are approximately 10%, with potential losses ranging between 30% and 50%, depending on environmental conditions throughout the production cycle (Lavilla & Ivancovich, 2021; Hartman *et al*., 2015). In Paraguay, the prevalence of this disease has been predominantly observed in commercial soybean crops from the R4 reproductive stage onward. Although no precise records exist regarding the specific yield losses caused by this pathogen in the country (Enciso-Maldonado, Fernández-Gamarra *et al*., 2021; Arrúa *et al*., 2021), Wrather *et al*. (2010) estimated losses of 0.7 million tons in 2006, when total soybean output was recorded at 2,227,487 tons across a cultivated area of 3,641,186 hectares (Paraguayan Chamber of Cereal and Oilseed Exporters and Traders, 2025).

With respect to *Septoria* sp., the etiological agent of Septoria Brown Spot in soybean, several studies have reported yield reductions between 8% and 34% (Hartman *et al*., 2015). In regional production systems, this pathogen is commonly observed during reproductive stages, with symptom severity levels approaching 50% and recorded yield losses of approximately 9% (Enciso-Maldonado *et al*., 2021).

Cercospora sp. infection results in symptoms affecting various plant structures, including hypocotyls, leaves, stems, petioles, pods, and seeds (Sautua *et al*., 2024). In Paraguay, the primary soybean diseases associated with *Cercospora* sp. are *Cercospora* Leaf Blight and Cercospora Purple Seed Stain (Caballero-Mairesse *et al*., 2024). Foliar symptoms initially manifest as irregular, reddish-purple or tan lesions due to cercosporin production. These lesions gradually expand and coalesce into necrotic areas, leading to early defoliation that predominantly affects upper leaves, while petioles often remain attached (Enciso-Maldonado, Fernández-Gamarra *et al*., 2021).

Similarly, *Septoria* sp. infection initially presents as small, irregular brown lesions accompanied by a characteristic yellow halo. This disease may also lead to early defoliation (Hartman *et al*., 2015).

The ability of these phytopathogens to grow and sporulate under controlled laboratory conditions has critical implications for understanding their epidemiology and designing effective management strategies. In *Cercospora* sp., the limited sporulation ability exhibited in artificial media poses a significant challenge for in-depth studies on the biology, taxonomy, and pathogenicity of the genus (Chand *et al*., 2013; Crous *et al*., 2019). Additionally, sporulation is markedly influenced by environmental factors, particularly temperature and light intensity (Silva *et al*., 2016; Brunelli *et al*., 2006). A frequent limitation is the rapid decline in the reproductive capacity of isolates, which frequently lose their ability to produce conidia when maintained *in vitro*, thereby hindering further studies (Yuliarni *et al*., 2013).

To address these challenges, several culture media have been evaluated, including Oat Agar, Malt Agar, Potato Dextrose Agar, V8 Agar, and Soybean Leaf Infusion Agar (Alloati *et al*., 2015; Uppala *et al*., 2019; Kashiwa *et al*., 2021). For *Septoria* species specifically, certain studies specifically highlight Malt Agar and Oat Agar as suitable media, emphasizing the importance of modifying environmental conditions, including light intensity and exposure to near-ultraviolet radiation, to induce sporulation (Quaedvlieg *et al*., 2013; Crous *et al*., 2019).

Given the significance of these previous findings, this study aimed to assess the growth and sporulation of *Cercospora* sp. and *Septoria* sp. isolates in different culture media to establish the optimal *in vitro* conditions for their development.

Materials and methods

Fungal Strains

The *Cercospora* sp. isolate was obtained from CEMIT-UNA and the Phytopathology Laboratory of Universidad Católica (Hohenau Pedagogical Unit) within the framework of the PINV01-152 Project, funded by CONACYT. The *Septoria* sp. isolate, in turn, was supplied by the Paraguayan Institute of Agricultural Technology (IPTA), Capitán Miranda Research Center, Itapúa, Paraguay. Both isolates are currently being subjected to molecular identification.

Preparation of culture media

For the preparation of Soybean Leaf Decoction Agar (SLDA), 200 g of fresh soybean leaves, 10 g of sucrose, 15 g of agar, and 1000 mL of distilled water were used. The soybean leaves were placed in water in a pot and heated to boiling, maintaining a gentle simmer for 15-20 minutes. The mixture was then filtered to remove solid residues, retaining only the resulting liquid. Subsequently, in a sterile medium preparation container, 10 g of sucrose and 15 g of agar were dissolved in the decoction liquid, ensuring a homogeneous mixture.

For the preparation of Soybean Grain Decoction Agar (SGDA), 200 g of soybean seeds, 10 g of sucrose, 15 g of agar, and 1000 mL of distilled water were used. The soybean seeds were placed in water in a pot and heated to a boil, maintaining a gentle simmer for 15-20 minutes. The mixture was then filtered to remove solid residues, retaining only the liquid fraction. In a sterile medium preparation vessel, 10 g of sucrose and 15 g of agar were dissolved in the decoction liquid, ensuring complete dissolution and a homogeneous mixture (SCABUSA, 2021).

Finally, for the preparation of Potato Dextrose Agar (PDA) medium, 200 g of potato, 10 g of dextrose, 20 g of agar, and 1000 mL of distilled water were used. The potatoes were placed in water in a pot and heated to a boil. The mixture was then filtered to remove solid particles, retaining only the liquid fraction. In a sterile medium preparation vessel, 10 g of dextrose and 20 g of agar were dissolved in the decoction liquid, ensuring complete dissolution and uniform mixing.

Each culture medium was transferred to autoclavable glass containers and sterilized at 121°C for 15 minutes in an autoclave. For the transfer of the medium into Petri dishes, the sterilized mixture was cooled to approximately 50°C and aseptically transferred into the Petri dishes inside a laminar flow hood. Finally, the medium was allowed to solidify at room temperature and then stored under appropriate conditions until required for use.

Growth and Sporulation Assay

Each medium (ADH, ADG, and PDA) was prepared in five replicates. A 5 mm diameter plug of actively growing mycelium, obtained from pure pathogen cultures, was placed at the center of each plate. The inoculated plates were incubated at 24 ± 1°C under controlled conditions for the entire experimental period.

Evaluated Variables

Mycelial growth and sporulation were evaluated. Colony diameter was measured every three days using a sterile ruler or a digital caliper, up to day 15. Sporulation was quantified at the end of the experiment using a Neubauer chamber, and results were expressed as the mean number of spores per cm^2 of colony.

Statistical Analysis

An analysis of variance (ANOVA) was conducted using Infostat version 2020 (Di Rienzo *et al*., 2020) to compare the mean mycelial growth among culture media. For sporulation, the mean spore production per cm^2 was determined for each medium to identify the optimal medium for spore production in each pathogen.

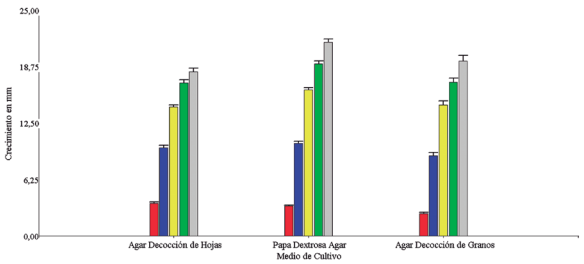

Figure 1. In vitro growth of *Cercospora* sp. in different culture media.

Results

Under the evaluated experimental conditions, none of the studied phytopathogenic fungi exhibited sporulation, as only sterile mycelial growth was observed.

Significant differences in mycelial growth were detected among the tested culture media. On day 3, *Cercospora* sp. (Fig. 1) showed variations in its development, with the ADG medium supporting the highest average growth (3.63 ± 0.11 mm), while ADH exhibited the lowest (2.50 ± 0.11 mm). From day 6 to day 15, PDA significantly promoted the most extensive mycelial expansion, reaching a final mean of 21.44 mm on day 15, surpassing the growth observed in ADG (19.39 mm) and ADH (18.19 mm).

Significant differences in colony development of *Septoria* sp. (Fig. 2) were observed from day 3 to day 15, with the ADG medium supporting the highest average mycelial expansion on day 15 (8.84 mm). In contrast, the PDA medium exhibited the lowest growth rate for this pathogen (3.65 mm). This study constitutes the first documented use of the ADG medium for assessing the *in vitro* growth dynamics of *Septoria* sp., highlighting its potential application in mycological research.

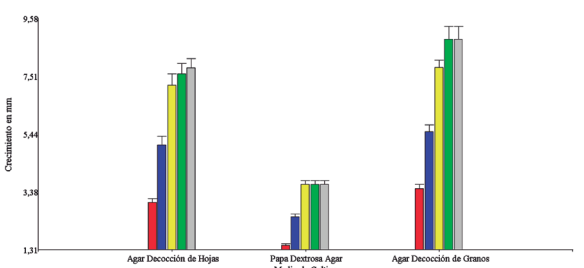

Figure 2. In vitro growth of *Septoria* sp. in different culture media.

Discussion

No sporulation was detected in any of the evaluated culture media, as only sterile mycelium was observed in both analyzed phytopathogens. This difficulty in conidia production by *Cercospora* species grown in artificial media aligns with previous literature reports (Crous *et al*., 2019).

Yeh and Sinclair (1980) reported that various culture media, including potato dextrose agar, carrot leaf decoction agar, V8 agar, and mature soybean residue decoction agar, did not significantly affect the sporulation of *Cercospora kikuchii* isolates. These authors recommended specific media (V8A, DSPT, and CLDA) under controlled environmental conditions (25°C with an alternating 12-hour photoperiod) to optimize conidia production. In contrast to their findings, none of the tested media successfully induced sporulation in our experiment.

Similar findings were reported by Beckman and Payne (1983) for *Cercospora zeae-maydis*, who indicated that serial subculturing of the fungus generally led to limited conidiation and the development of sterile mycelium. These researchers observed a significant influence of the culture medium on mycelial growth and colony morphology, noting that V8 medium favored sporulation, whereas PDA promoted mycelial growth. This latter aspect aligns with our findings, as PDA significantly enhanced the growth of *C. kikuchii.*

Vathakos and Walters (1979) reported that *C. kikuchii* exhibited vegetative growth only in media prepared with carrot leaf decoction and immature or senescent plant tissues from various crops. Additionally, these authors documented abundant sporulation under specific lighting conditions (Gro-Lux fluorescent lamps), noting that subculturing from conidia yielded greater spore production than from mycelium. Furthermore, El-Gholl *et al*. (1982) and Vathakos and Walters (1979) identified V8 agar as a suitable medium for

inducing sporulation in *Cercospora* sp.. However, since this medium is not commercially available in Paraguay, there is a pressing need to identify viable local alternatives.

Regarding *Septoria glycines*, Bertagnolli *et al*. (1986) evaluated various culture media and identified Fries medium as the most effective for inducing sporulation. Zalewska (2012), while studying related species such as *S. carvi*, suggested that both temperature and the composition of the culture medium (malt medium and malt with leaf extract decoction) are key factors for optimizing fungal growth and sporulation. Although no sporulation of *Septoria* sp. was observed in our study, ADG medium significantly promoted its vegetative growth, marking the first documented use of this medium for this species.

Saidi *et al*. (2012) also emphasized the importance of culture medium composition, temperature, and light conditions as critical factors for efficient sporulation induction in *Septoria* species. This highlights the multifactorial nature of sporulation and supports our findings, suggesting that the absence of sporulation could be attributed not only to the culture medium used but also to additional environmental factors such as temperature and photoperiod.

Whereas the growth and sporulation of *Cercospora* sp. and *Septoria sp.* are multifactorial processes influenced by a range of variables. Future research should aim at the integrated optimization of culture medium, light conditions, and temperature to enhance spore production under in vitro conditions.

Conclusions

Under the evaluated experimental conditions, the culture media allowed mycelial growth of *Cercospora* sp. and *Septoria* sp. but did not induce sporulation. PDA medium was the most effective in promoting mycelial growth in *Cercospora* sp., whereas ADG medium favored the development of *Septoria* sp.. Given the absence of sporulation, further research should investigate variations in environmental factors such as temperature and photoperiod, as well as alternative culture media formulations, to enhance in vitro sporulation in both pathogens. This study provides fundamental insights for selecting appropriate media in future research on the growth and management of these important soybean phytopathogens.

Contribution of the authors

All the authors constributed equitatively in the preparation of this paper..

Conflicts of interest

The authors declare to be free of conflicts of interest.

Funding Source

The authors were self funded.

Acknowledgements

Al Consejo Nacional de Ciencia y Tecnología del Paraguay, CONACYT con fondos del Fondo para la Excelencia de la Educación y la Investigación FEEI por el co financiamiento del Proyecto INIC01-3.

Literature cited

Alloatti, J., Li, S., Chen, P., Jaureguy, L., Smith, S. F., Florez-Palacios, L. & Rupe, J. (2015). Screening a diverse soybean germplasm collection for reaction to purple seed stain caused by *Cercospora kikuchii*. *Plant Disease*, 99(8): 1140–1146. <https://doi.org/10.1094/PDIS-09-14-0878-RE>.

Arrua, A. A., Lopez-Nicora, H. D., Fernández Ríos, D., Grabowski, C. J., Cazal-Martínez, C. C. & Kohli, M. M. (2021). El impacto de las enfermedades fúngicas en el cultivo de soja (*Glycine max*) en Paraguay ¿es posible estimar las pérdidas. *Investigaciones y Estudios - Universidad Nacional de Asunción*, 12(1): 34–46. <https://doi.org/10.47133/IEUNA2114>.

Beckman, P. M. & Payne, G. A. (1983). Cultural techniques and conditions influencing

growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. *Phytopathology*, 73(2): 286–289. <https://doi.org/10.1094/Phyto-73-286>.

Bertagnolli, P. F., Porto, M. D. D. M. & Reis, E. M. (1986). The influence of culture media on the sporulation of *Septoria glycines* Hemmi, causal agent of soybean brown spot. *Pesquisa Agropecuária Brasileira*, 21(6): 615–618. <https://doi.org/10.1590/S1678-3921.pab1986.v21.14846>.

Brunelli, K. R., Fazza, A. C., Athayde Sobrinho, C. & Camargo, L. E. A. (2006). Efeito do meio de cultura e do regime de luz na esporulação de *Cercospora zeae-maydis*. *Summa Phytopathologica*, 32: 92–94.

Chand, R., Kumar, P., Singh, V. & Pal, C. (2013). Technique for spore production in *Cercospora canescens*. *Indian Phytopathology*, 66(2): 159–163. <https://epubs.icar.org.in/index.php/IPPJ/article/view/29980>.

Crous, P. W., Verkley, G. J. M., Groenewald, J. Z. & Houbraken, J. (Eds.) (2019). *Fungal Biodiversity*. Utrecht: Westerdijk Fungal Biodiversity Institute. *Westerdijk Laboratory Manual Series*, 1: 425 pp.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M. & Robledo, C. W. (2020). *InfoStat versión 2020*. Córdoba: Centro de Transferencia InfoStat de la Universidad Nacional de Córdoba. [Consulted: 4.vii.2025]. <http://www.infostat.com.ar>.

El-Gholl, N. E., Alfieri Jr, S. A., Ridings, W. H. & Schoulties, C. L. (1982). Growth and sporulation in vitro of *Cercospora apii*, *Cercospora arachidicola*, *Cercospora kikuchii* and other species of *Cercospora*. *Canadian Journal of Botany*, 60(6): 862–868.

Kashiwa, T., Lavilla, M. A., Paleo, A. D., Ivancovich, A. J. G. & Yamanaka, N. (2021). The use of detached leaf inoculation for selecting *Cercospora kikuchii* resistance in soybean genotypes. *PhytoFrontiers™*, 1(4): 250–257. <https://doi.org/10.1094/PHYTOFR-01-21-0002-TA>.

Quaedvlieg, W., Verkley, G., Shin, H. D., Barreto, R., Alfenas, A., Swart, W. J., Groenewald, J. Z. & Crous, P. W. (2013). Sizing up *Septoria*. *Studies in Mycology*, 75: 307–390. <https://doi.org/10.3114/sim0017>.

Saidi, A., Eslahi, M. R. & Safaie, N. (2012). Efficiency of *Septoria tritici* sporulation on different culture media. *Trakia Journal of Sciences*, 10(3): 15–8.

Sautua, F. J., Pizá, M. C. P., Scandiani, M. M. & Carmona, M. A. (2024). *Cercospora* leaf blight and purple seed stain of soybean: A permanent challenge. *Plant Pathology*, 73(8): 1981–2004. <https://doi.org/10.1111/ppa.13956>.

SCABUSA. (2021). *US Wheat and Barley Scab Iniciative*. Fargo: U.S. Wheat and Barley Scab Initiative. [Consulted: 1.vii.2025]. <https://scabusa.org/>.

Silva, M. G. D., Pozza, E. A., Lima, C. V. R. V. D. & Fernandes, T. J. (2016). Temperature and light intensity interaction on *Cercospora coffeicola* sporulation and conidia germination. *Ciência e Agrotecnologia*, 40: 198–204. <https://doi.org/10.1590/1413-70542016402025915>.

Uppala, S. S., Zhou, X. G., Liu, B. & Wu, M. (2019). Plant-based culture media for improved growth and sporulation of *Cercospora janseana*. *Plant Disease*, 103(3): 504–508. <https://doi.org/10.1094/PDIS-05-18-0814-RE>.

Vathakos, M. G. & Walters, H. J. (1979). Production of conidia by *Cercospora kikuchii* in culture. *Phytopathology*, 69(8): 832–833. <https://doi.org/10.1094/Phyto-69-832>.

Yeh, C. C. & Sinclair, J. B. (1980). Sporulation and variation in size of conidia and conidiophores among five isolates of *Cercospora kikuchii*. *Plant Disease*, 64(4): 373–374. <https://doi.org/10.1094/PD-64-373>.

Yuliarni, F. F., Sjamsuridzal, W. & Hidayat, I. (2013). Multilocus sequence analysis of *Cercospora* spp. from different host plant families. *Makara Journal of Science*, 17(2): 2. <https://doi.org/10.7454/mss.v17i2.2098>.

Zalewska, E. (2012). Growth and sporulation of *Septoria carvi* Syd. in different culture conditions. *Acta Scientiarum Polonorum Hortorum Cultus*, 11(1): 93–107. <https://czasopisma.up.lublin.pl/asphc/article/view/3007/2077>.