Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref
Open access

Phytopathological Note

Etiology of brown rot in strawberry (*Fragaria x ananassa*) in the State of Mexico

Hugo Velasco-Montaño, Victoria Ayala-Escobar, Daniel Téliz-Ortiz, Programa de Fitosanidad-Fitopatología, Colegio de Postgraduados, Campus Montecillo, km 36.5 Carretera México-Texcoco, Montecillo, Texcoco, edo. México. C.P. 56230, México; **Nadia Landero-Valenzuela*,** Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro. Calzada Antonio Narro 1923, Buenavista, Saltillo Coahuila. C.P. 25315; **Santos Gerardo Leyva-Mir,** Departamento de Parasitología Agrícola, Universidad Autónoma Chapingo, km 38.5 Carretera México-Texcoco, edo. México. CP.56230.

***Corresponding Author:**
Nadia Landero Valenzuela
nadialandero00@gmail.com

Section:
Periodical Issue

Received:
07 June 2024
Accepted:
17 November 2024
Published:
27 December 2024
Early Access 2025

Citation:
Velasco-Montaño H, Ayala-Escobar V, Téliz-Ortiz D, Landero-Valenzuela N and Leyva-Mir SG. 2025. Etiology of brown rot in strawberry (*Fragaria x ananassa*) in the State of Mexico. Mexican Journal of Phytopathology 43(1): 45. https://doi.org/10.18781/R.MEX.FIT.2406-3

ABSTRACT

Background/Objective. In a strawberry crop established in a greenhouse in Montecillo, Texcoco, State of Mexico, in 2022, brownish brown leaf spots and rotting of fruits with asymmetric sunken lesions were observed, which extended and acquired a brown color. The objective of the present work was to identify the causal agent of brown rot in fruits and strawberry plants.

Materials and Methods. Symptomatic fruits and leaves were collected, from which fungal isolates were obtained to perform pathogenicity tests on plants and fruits, in plants by two inoculation methods: spraying via foliar and via root; in fruits by immersion. Concentrations of 2×106 conidia mL-1 were used. The ITS region of the rDNA was amplified and sequenced by PCR with the universal primers ITS1-ITS4.

Results. *Pilidium concavum* was morphologically and molecularly identified as the causal agent of brown spot and brown rot on strawberry. It was found to be pathogenic in strawberry fruits cv. Aromas and in plants less than two months old. It showed variation in virulence, in affected plants it varied from 40 to 50%, in fruits it reached 100%.

Conclusion. The result determines that *Pilidium concavum* is a pathogen that produces brown leaf spot and brown rot in strawberry fruits. It allows new lines of research related to the impact of the disease on strawberry production, yield and quality in Mexico. This research is the first report of *Pilidium concavum* as a strawberry pathogen in the State of Mexico.

Keywords: *Pilidium concavum*, strawberry, pathogenicity, virulence.

INTRODUCTION

Strawberry (*Fragaria* x *ananassa*) is one of the most important fruits worldwide from an economic, commercial, and nutritional perspective (Giampieri *et al*., 2012; Ikegaya, 2023). However, several pathogens affect its life cycle, including fungi, bacteria, viruses, and nematodes (Petrasch *et al*., 2019). Fungi have the greatest impact on reducing yield and nutritional, commercial, and economic quality. They can infect all parts of the plant, causing severe damage and even plant death (Garrido *et al*., 2011; Azam *et al*., 2019). Among the most damaging fungal and oomycete pathogens affecting strawberries are *Botrytis cinerea*, *Colletotrichum* sp., *Sphaerotheca macularis*, *Phytophthora* sp., *Verticillium* sp., and *Diplocarpon earlianum* (Reddy, 2016). Additionally, new emerging pathogens have gained increasing economic and scientific significance over time.

Emerging pathogens pose a significant threat to strawberry productivity, as their incidence and severity can increase rapidly, resulting in substantial losses (Milgroom, 2017; Ristaino *et al*., 2021). According to Pedraza Herrera *et al*. (2022), emerging diseases are caused by pathogens that have undergone changes in their incidence, geographic distribution, or host range, as well as alterations in their pathogenesis, evolution, or have been newly discovered or recognized. Among the new diseases caused by emerging pathogens are leaf spot and root and crown rot caused by *Neopestalotiopsis* sp. (Rebollar-Alviter *et al*., 2020; Baggio *et al.*, 2021), fruit rot caused by *Neofusicoccum* sp. (Zhan *et al*., 2021), leaf spot and anthracnose caused by *Pestalotiopsis* sp. (Morales-Mora *et al*., 2019), and brown leaf spot and fruit rot caused by *Pilidium concavum* (Fernández-Ortuño *et al*., 2014).

The presence of brown necrotic spot and rot on strawberry has been reported to date in only nine countries: India, Venezuela, Poland, Brazil, Belgium, China, the USA, Iran, and South Korea (Fernández-Ortuño *et al*., 2014; Park *et al*., 2017). The severity of the damage caused by this disease varies considerably, ranging from 3% to 50% infection in greenhouse plants, and up to 70% in stored fruit (Lopes *et al*., 2010; Debode *et al*., 2011). The pathogen responsible for this disease is *Pilidium concavum*, which has a broad host range in both wild and cultivated plants. Affected wild plants include *Fallopia japonica*, *Hieracium caespitosum*, *Aesculus hippocastanum*, *Greyia radlkoferi*, while cultivated hosts include *Olea europaea*, *Fragaria×ananassa*, *Paeonia suffruticosa*, *Bergenia crassifolia*, *Rosa rugosa*, *Eucalyptus spp.*, *Prunus domestica*, *Vaccinium corymbosum*, *Vitis vinifera*, and *Ilex paraguariensis* (Aguin *et al*., 2016; Karimi *et al*., 2016; Lopez *et al*., 2020). The spread of emerging pathogens to new geographical areas presents additional challenges for governments, farmers, and scientists, as they must be prepared to confront an expanding range of diseases that threaten food security and economic stability. Due to the incidence of brown toasted rot on strawberry fruit and leaves in Montecillo, Texcoco, Mexico, this study was designed to identify the causal agent of brown toasted rot in strawberry fruit plants through pathogenicity tests, morphological characteristics, and molecular assays.

A random sampling was conducted, and ten strawberry plants showing symptoms of leaf spot, flower peduncle necrosis, and fruit rot were collected. This was carried out in October and November of 2022 in the greenhouse at the Colegio de Postgraduados, Montecillo Campus, Texcoco, Mexico, located at coordinates 19°27'37"N 98°54'12"W. The samples were labeled, packaged in paper bags, and transported in a cooler for processing in the Phytosanitary-Phytopathology Laboratory at the Colegio de

Postgraduados, Montecillo Campus, State of Mexico. The samples were cut into 1 cm square tissue pieces, disinfected with 1% sodium hypochlorite for three minutes, and five tissue pieces from each sample were placed on potato-dextrose-agar (PDA; Bioxón®, Becton Dickinson de México) and in a humid chamber. Isolations were obtained from fruit and leaves. The reisolation of the microorganism associated with symptomatic leaflets and fruit from the humid chamber was done on agar-water (AA; BD Bioxón®, Becton Dickinson de México) following the procedure described by the National Service of Health, Safety, and Agro-food Quality (SENASICA, 2018). The cultures were incubated under natural white light at a laboratory temperature of 25±2 °C for 72 hours until mycelial growth was observed. To purify the isolation, a hyphal tip was transferred to PDA. Monosporic isolations were obtained through serial dilutions, which were plated on AA at 1.6% and incubated for 18 hours; then, a germinated conidium was transferred to PDA medium, and the cultures were incubated at 25±2 °C. The isolates were stored at 15°C in test tubes with slanted PDA, supplemented with sterile mineral oil (Montesinos et al., 2015). Morphological identification was carried out using taxonomic keys reported by Barnett and Hunter (2006) and species-specific keys by Palm (1991) and Rossman et al. (2004). Semi-permanent preparations with 50% glycerol on glass slides were made to observe them under a BX51 compound microscope (Olympus, Japan). Mycelial coloration and conidial morphology and morphometry were determined by examining 100 conidia per isolate at 14 days after plating.

For the pathogenicity tests, inoculations were performed on mature strawberry fruits that were uniform in size and color, as well as on strawberry seedlings of the *Aromas* variety. Pathogenicity was assessed on both wounded and non-wounded fruits, which were previously disinfected. Wounds were made using a sterile needle to a depth of 1 cm in each fruit. Five fruits were used per treatment. Inoculation was carried out by immersing the fruits for 3 minutes in a conidial suspension of 2×10^6 conidia mL^{-1}, adjusted using a hemocytometer. Control fruits were immersed in sterile distilled water for 3 minutes. The fruits were placed in a plastic dome with sterile wet towels as humid chambers and incubated at 25±2 °C under natural white light. Incidence was evaluated as the percentage of fruits affected by the pathogen, and severity was evaluated as the percentage of tissue affected.

For the plant tests, seedlings aged 1-2 months, 3-4 months, and older than 4 months were used. They were inoculated by spraying with spore suspensions of 2×10^6 conidia mL^{-1}, both foliar and root inoculations. Ten plants were assigned per treatment. Control plants were sprayed with sterile distilled water. The plants were incubated in a growth chamber with a 12-hour light and 12-hour dark photoperiod, at 80% relative humidity, for seven days. Incidence and severity were evaluated every 24 hours up to 20 days after inoculation. Incidence was assessed as the percentage of leaflets affected by the pathogen, and severity was measured using a visual scale with seven severity classes (Figure 1). Asymptomatic plant leaflets were disinfected and incubated in a humid chamber for five days, after which incidence was evaluated. The pathogenicity tests were performed in triplicate. Incidence and severity data were adjusted to a normal distribution, and the Tukey test was applied for mean comparison ($P \leq 0.05$).

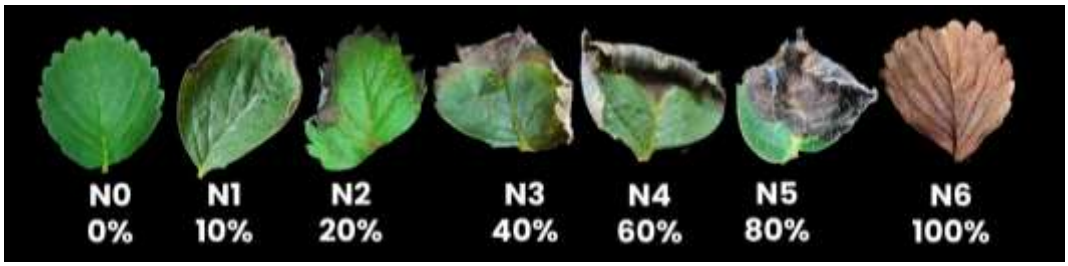

Figure 1. Visual scale for evaluating the severity of brown toasted leaf spot on strawberry. Designed by the authors.

For molecular identification, mycelium and conidia were collected to extract genomic DNA, following the method of Ahrens and Seemüller (1992). The ribosomal gene (rDNA) regions of the internal transcribed spacers ITS1 and ITS2 were then amplified by PCR using the universal primers ITS5 and ITS4 (Martin and Rygiewicz, 2005), using the Sanger method at Macrogen (Korea). The obtained sequences were purified and aligned with sequences deposited in the Gene Bank of the National Center for Biotechnology Information (NCBI) based on BLAST searches and literature. Sequences with 100% similarity were aligned in MEGA11 software, version 11.0.13 (Tamura *et al*., 2021), using the Clustal algorithm, and compared with sequences in GenBank at NCBI, supported by the BLAST tool. The most similar sequences were extracted for phylogenetic analysis, constructing a neighbor-joining tree using the Bootstrap method and the Tajima-Nei model. *Chaetomella raphigera* (accession number MH860747) was used as the outgroup, and the sequences of the isolates were deposited in the NCBI Gene Bank. Symptoms observed on the fruits of strawberry cv. *Aromas* include asymmetric, sunken, wet lesions that are pink in color, without a chlorotic halo on the epicarp. The lesions expand, turning brown with masses of white conidia that turn pink-red and eventually brown on the lesion surface (Figure 2A-B). On the leaves, lesions begin at the margin or apex as brown, asymmetric spots, progressing toward the leaf base, characteristic of "toasted" leaves (Figure 2D-E).

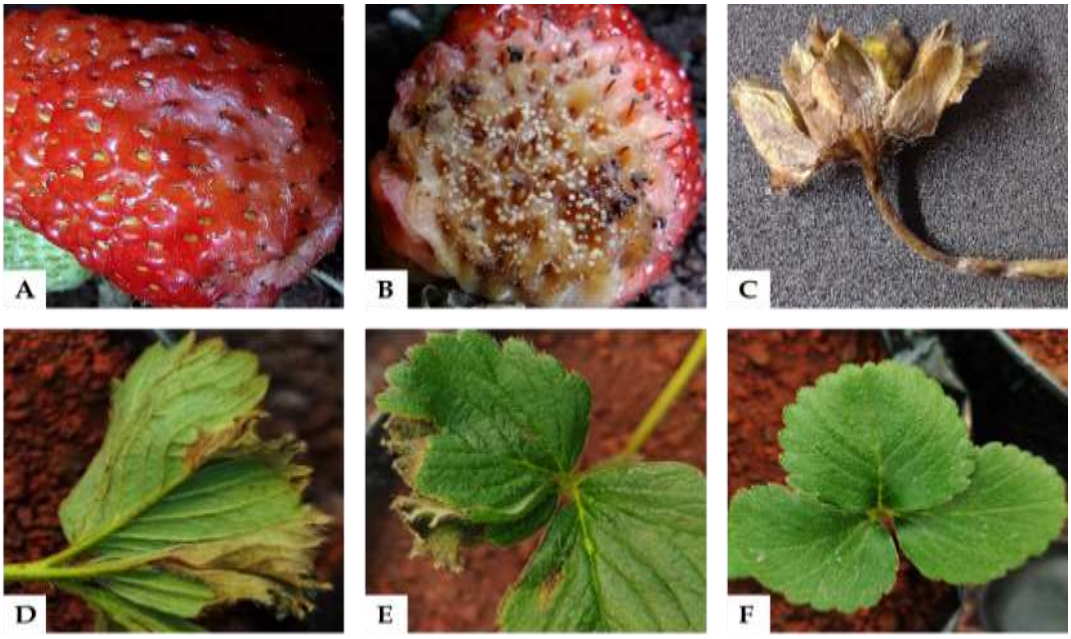

Figure 2. A) Fruit with sunken, wet lesions. B) Fruit with brown rot and fungal structures. C) Brown constriction on the flower peduncle. D-E) Leaves with toasted brown edges. F) Healthy leaves.

The isolate on PDA medium formed colonies with circular shapes, ranging from regular to irregular, and the presence of white mycelium was minimal to absent (mass-like form) with regular edges. The base color of the colony grew from cinnamon to brown. After 12 days of incubation on PDA medium, the colony edges developed a light to dark brown pigmentation. After 14 days of incubation, sporodochia were observed, forming in concentric circles with a gelatinous appearance, a black base, and disc-shaped to hemispherical structures, ranging from light brown to dark brown, suborbicular, measuring 354 to 658 µm in length and 370 to 688 µm in width. The conidiophores were hyaline, unicellular, cylindrical, and filiform, measuring 17.64 to 48.04 µm in length and 0.83 to 2.49 µm in width. Conidia were hyaline, aseptate, fusiform, canoe-shaped to allantoid, measuring 5.84 to 10.95 µm in length and 1.29 to 3.63 µm in width. (Figure 3). No sexual stage was observed. These characteristics correspond to those described by Palm (1991) and Rossman (2004) for *Pilidium*, reported as a facultative parasite with symptoms similar to those described by Debode *et al.* (2011) and Fernández-Ortuño *et al*. (2014).

The sequence alignment in the GenBank database (NCBI) showed 100% similarity with *Pilidium concavum* and *P. lythri*. In the phylogenetic tree (Figure 4), the isolate grouped with the reference isolates of *P. concavum* and *P. lythri*. Additionally, it was confirmed that *P. concavum* is more closely related to *P. pseudoconcavum*, with a support value of 94%. The isolates were registered in the GenBank database with accession numbers OR568456 and OR568457. The presence of *P. concavum* as a pathogen of leaves and fruits has been reported in several countries, including India (Phatak and Payak, 1965), Venezuela (Cedeño *et al.*, 2001), Poland (Gołębniak and Jarosz, 2003), Brazil (Lopes *et al*., 2010), Belgium (Debode *et al.*, 2011), China (Geng *et al*., 2012), the USA (Fernández-Ortuño *et al.*, 2014), Iran (Ayoubi *et al*., 2016), and Korea (Park *et al*., 2017). However, no reports of damage caused by this pathogen had been made in Mexico.

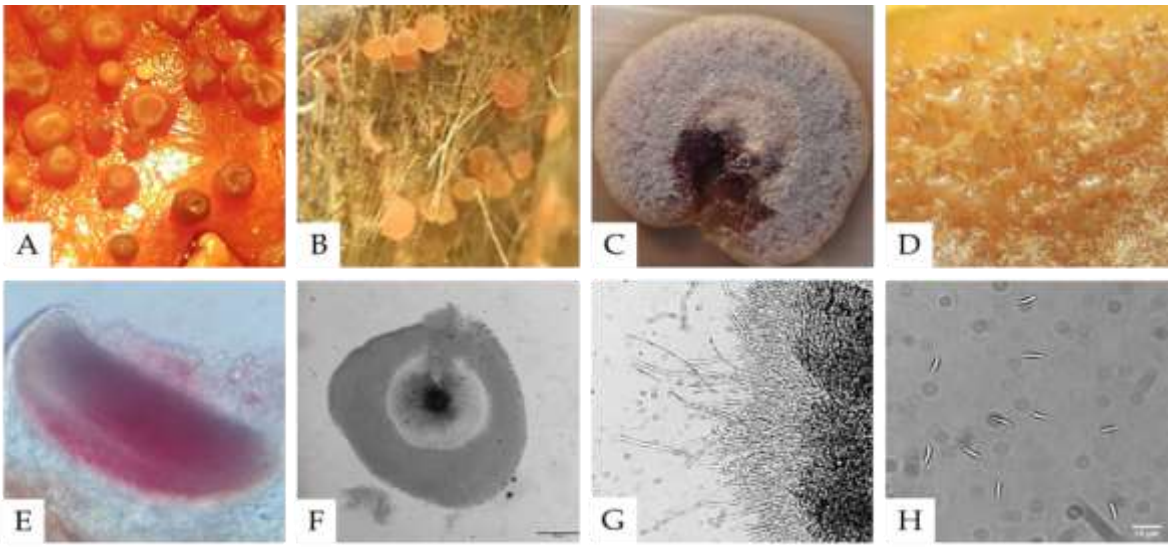

Figure 3. A) Sporodochia on strawberry fruit. **B)** Sporodochia on the underside of the leaf. **C-D)** Gelatinous colony with conidial mass on PDA medium. **E-F)** Longitudinal and transverse sections of the sporodochium. **G)** Hyaline, cylindrical, filiform conidiophores. **H)** Hyaline, aseptate, allantoid conidia.

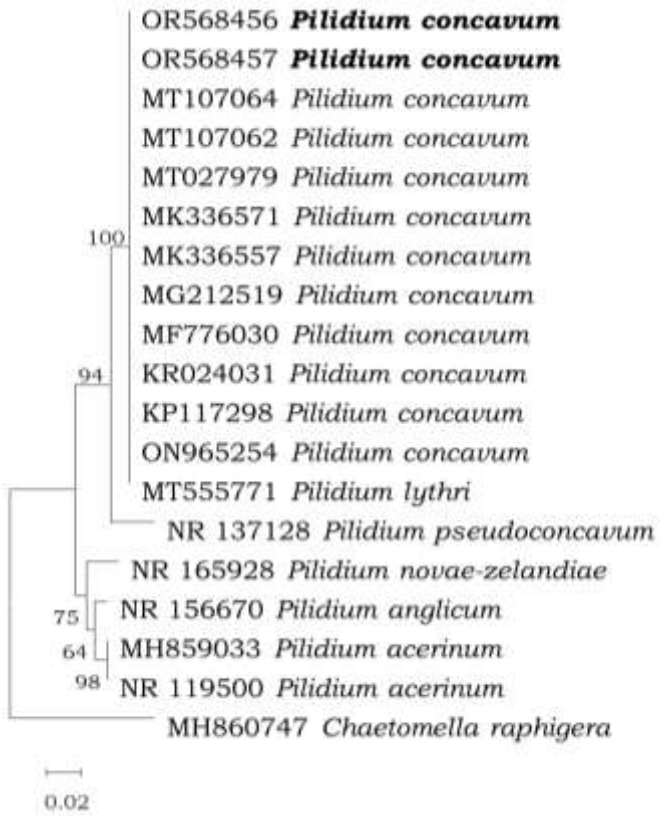

Figure 4. Phylogenetic tree based on Neighbor Joining of the ITS rDNA sequence, showing a phylogenetic affinity of the Mexico isolate (in bold) with *Pilidium concavum* above 95% of the node. Bar scaling 0.02 represents nucleotide substitutions per site.

The taxonomy of this fungus is not well defined and has undergone reclassifications based on recent morphological characteristics and phylogenetic analyses. The genus *Pilidium* of the family *Chaetomellaceae*, class *Leotiomycetes*, was first described as *P. acerinum* (Alb. and Schwein) (Rossman *et al*., 2004). Currently, the recognized species in the genus are *P. acerinum*, *P. lythri* (also known as *P. concavum*), *P. pseudoconcavum*, *P. eucalyptorum*, and *P. septatum* (Rossman *et al*., 2004; Kirk *et al*., 2011; Crous *et a*l., 2013; 2015). According to Palm (1991) and Rossman *et al*. (2004), *P. concavum* was shown to be related to the anamorph *Hainesia lythri*, *Dacryomyces lythri* (teleomorph *Discohainesia oenotherae*), *Peziza oenotherae*, *Pezizella oenotherae*, and *Sclerotiopsis testudinacea* as morphotypes of the same species, and thus are considered synonyms. However, *Pilidium* is the oldest generic name used and includes several phytopathogenic species. Recently, Rossman (2014) transferred *P. concavum* as a synonym of *P. lythri*, so referring to either name is now considered valid for the same species (Johnston *et al.*, 2014).

In the pathogenicity tests, three days after inoculation, sunken lesions were observed on wounded fruits, ranging in color from opaque pink to brown, with initial white conidial masses that turned pink and eventually brown (Figure 5A). The incidence and severity were both 100%. On non-wounded fruits, symptoms with fungal structures appeared on the fifth day after inoculation, with incidence and severity also at 100% (Figure 5D). The structures and spores on the symptomatic fruits were morphologically identical to the colonies originally used for inoculum production. The control fruits showed no symptoms of the disease. These tests confirmed that the causal agent of brown rot in strawberry fruit is *P. concavum*, as reported in similar studies by Debode *et al*. (2011) and Karimi *et al*. (2016) on strawberry fruit.

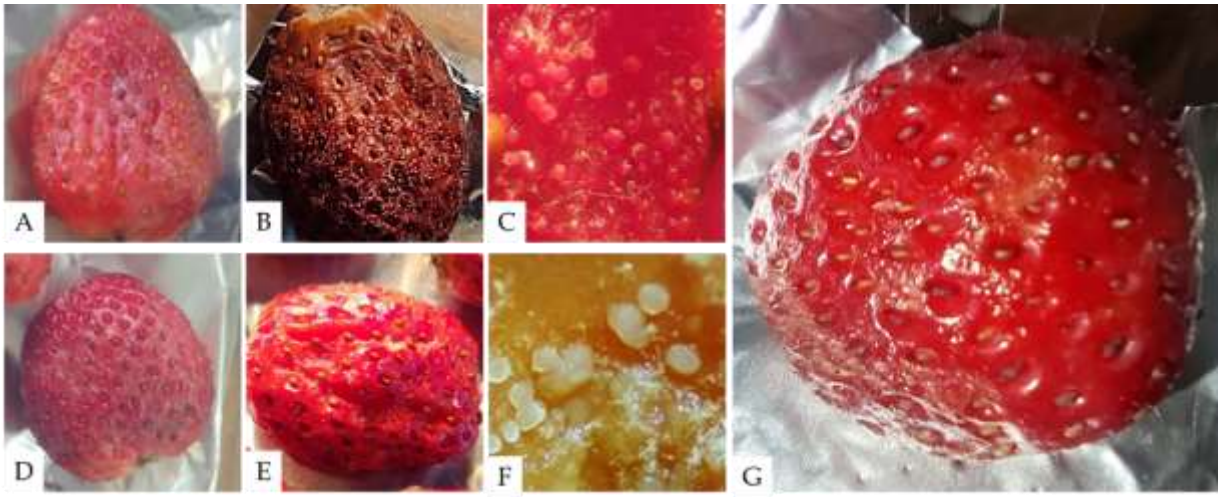

Figure 5. Pathogenicity tests on strawberry fruit inoculated with a concentration of 2×10^6 conidia mL^{-1} of *Pilidium concavum*. A) Fruit with a wound inoculated with the fungus. B) Wounded fruit showing symptoms 72 hours post-inoculation (hpi). C) Sporodochia of the fungus on the wounded fruit. D) Unwounded fruit inoculated with the fungus. E) Unwounded fruit showing symptoms 96 hours post-inoculation. F) Sporodochia of the fungus on the unwounded fruit. G) Control inoculated with sterile distilled water, showing no symptoms at 96 hours post-inoculation.

The inoculated seedlings, approximately 1 to 2 months old, showed mild symptoms on new shoots and leaves (Figure 6) 20 days after inoculation. Inoculation on leaves showed 50% incidence with 20% severity. Most symptoms were observed on new shoots (new leaves) and senescent mature leaves. In contrast, in the plants inoculated via the roots, the incidence was 40%, and symptoms appeared on senescent leaves and new leaves with 10% severity. Asymptomatic leaflets, after 5 days in a humid chamber, showed orange to brown sporodochia on the leaves (Figure 7). Fungal structures were observed in 90% of the leaf-inoculated treatment and 80% of the root-inoculated treatment. No structures were observed on stems or roots, only on leaves. The microorganism was reisolated, and its identity was confirmed through morphological identification. It is inferred that the fungus is a potential pathogen for nursery seedlings. This aligns with reports by Debode *et al*. (2011) and Fernández-Ortuño *et al*. (2014), who reported infections in nurseries and transplant batches. The infection was systemic. Plants inoculated through the roots showed structures on the leaves when exposed to a humid chamber, results similar to those obtained by Lopez *et al*. (2020), who inoculated *Ilex paraguariensis* plants via roots and observed damage on the leaves. In this study, plants at the fruiting stage showed fruit rot symptoms 6 months after inoculation, suggesting that the inoculum remained latent in the plants and caused damage when conditions became favorable. According to Hipol *et al*. (2014), *P. concavum* is part of the endophytic fungi group, as it belongs to the Leotiomycetes class of the Pezizomycotina subphylum, which includes common endomycorrhizal endophytic fungi. Therefore, it may have the ability to be endophytic in strawberry plants. Inoculation of 3-month-old and older-than-4-month-old strawberry plants did not induce brown rot symptoms on the leaves. However, when these plants developed and fruited 6 months after inoculation, the fruits and flower buds showed symptoms with fungal structures of *P. concavum*.

Figure 6. Strawberry plants (*Fragaria* x *ananassa*) cv. Aromas less than three months old, 15 days post-inoculation (dpi) with a suspension of $2x10^6$ conidia per mL of *Pilidium concavum*. A) Inoculated via foliar; B) Inoculated via root routes; C) Control inoculated with sterile distilled water.

Figure 7. A) Asymptomatic strawberry leaves after 28 days post-inoculation (dpi), placed in a humid chamber. **B)** Formation of sporodochia on the leaves after 5 days in the humid chamber.

P. concavum is a fungus with a wide host range. Its importance lies in its ability to affect economically valuable crops, especially olive fruit (Arzanlou *et al*., 2013), *Ilex paraguariensis* (López *et al*., 2020), grape clusters (Aguin *et al*., 2016), and strawberry (Debode *et al*., 2011). Thus, the evolution of this pathogen, its geographical distribution, host range, and its interaction with plants have increasing relevance in the context of global climate change. This phenomenon affects plant communities in all aspects, from growth and reproduction to resistance and susceptibility (Zhao *et al*., 2017; Tito *et al*., 2018). Additionally, climate change alters the population and behavior of microorganisms, modifying their interactions with hosts (Cohen and Leach, 2020). This may lead to the adoption of new invasion strategies through changes in the virulence system, which could, in turn, compromise plant resistance (Cheng *et al*., 2019). As a result, plant-microorganism interactions may shift from a mutualistic to a pathogenic association, or vice versa (González *et al*., 2021). This study reports *Pilidium concavum* as the species responsible for brown toasted rot in strawberry fruits and leaves in Montecillo, Texcoco, State of Mexico. Morphological characteristics, pathogenicity tests, molecular characterization, and a literature review showed no genetic diversity between the isolates from leaflets and fruits, although further studies are needed, along with monitoring regions where strawberries are cultivated. Symptoms were more frequently associated with fruits, which contributed to the difficulties in replicating symptoms in leaves. Additional research is needed to clarify the origin of the new pathogen, its disease cycle, and to develop integrated management strategies for the disease in nurseries and postharvest.

ACKNOWLEDGMENTS

The authors would like to thank the Colegio de Postgraduados, the Phytosanitary-Phytopathology program, and CONACYT for the scholarship provided.

REFERENCES

Aguin O, Ferreiroa V, González-Jartín J, Alfonso A, Mansilla JP, y Sainz MJ. (Eds.). 2016. *Pilidium concavum*, ascomiceto asociado a podredumbre de racimo en *Vitis vinifera* "Albariño". Sociedad Española de Fitopatología. XVIII Congreso de la Sociedad Española de Fitopatología.

Arzanlou M, Torbati M and Jafary H. 2013. Fruit rot on olive caused by *Pilidium concavum* in Iran. *Australasian* plant disease Notes *8*(1): 117–121. https://doi.org/10.1007/s13314-013-0111-0

Ayoubi N, Soleimani MJ and Zare R. 2016. *Pilidium concavum*, causing tan brown rot on strawberry in Iran. Journal of plant pathology: an international journal of the Italian Phytopathological Society 98(3): 667–669. http://www.jstor.org/stable/44280519

Azam M, Ejaz S, Naveed URR, Khan M and Rashad Q. 2019. Postharvest quality management of strawberries. *In* Strawberry-pre and post-harvest management techniques for higher fruit quality. Toshiki A y Md A y (eds.). IntechOpen: Rijeka, Croatia, 2019; p. 22. http://dx.doi.org/10.5772/intechopen.82341

Baggio JS, Forcelini BB, Wang N-Y, Ruschel RG, Mertely JC, and Peres NA. 2021. Outbreak of leaf spot and fruit rot in Florida strawberry caused by *Neopestalotiopsis* spp. Plant disease 105(2): 305–315. https:// doi.org/10.1094/pdis-06-20-1290-re

Barnett HL and Hunter BB. 2006. llustrated genera of imperfect fungi. 4th Edition. The American Phytopatological Society. St. Paul Minnesota, 218 p

Cedeño L, Carrero C, Castro Y, Calderón J, and Quintero K. 2001. Tan brown rot caused by *Discohainesia oenotherae* on strawberry fruits in Merida-Venezuela. Intercencia 26(3): 113–116.

Cheng YT, Zhang L and He SY. 2019. Plant-microbe interactions facing environmental challenge. Cell Host y Microbe 26(2): 183–192. https://doi.org/10.1016/j.chom.2019.07. 009

Cohen SP, and Leach JE. 2020. High temperature-induced plant disease susceptibility: more than the sum of its parts. Current Opinion in Plant Biology 56:235–241. https://doi.org/ 10.1016/j.pbi.2020.02.008

Crous PW, Wingfield MJ, Guarro J, Cheewangkoon R, van der Bank M, Swart WJ, Stchigel AM, Cano-Lira JF, Roux J, Madrid H, Damm U, Wood AR, Shuttleworth LA, Hodges CS, Munster M, Yáñez-Morales MJ, Zúñiga-Estrada L, Cruywagen EM, De Hoog GS, … Groenewald JZ. 2013. Fungal planet description sheets: 154–213. Persoonia 31(1): 188–296. https://doi.org/10.3767/003158513x 675925

Crous PW, Wingfield MJ, Roux JJL, Richardson DM, Strasberg D, Shivas RG, Alvarado P, Edwards J, Moreno G, Sharma R, Sonawane MS, Tan YP, Altés A, Barasubiye T, Barnes CW, Blanchette RA, Boertmann D, Bogo A, Carlavilla JR, … Groenewald JZ. 2015. Fungal planet description sheets: 371–399. Persoonia 35(1): 264–327. https://doi.org/ 10.3767/003158515x 690269

Crous PW, Verkley GJM, Groenewald JZ and Houbraken J. (Eds.). (2019). Westerdijk Laboratory Manual Series No. 1. *Fungal biodiversity*. Second edition. Utrecht, The Netherlands, 438p.

Debode J, Van Hemelrijck W, Heungens K, Maes M and Creemers P. (2011). First report of *Pilidium concavum* causing tan brown rot on strawberry fruit in Belgium. *Plant Disease*, *95*(8), 1029–1029. https://doi.org/ 10.1094/ pdis-10-10-0752

Fernández-Ortuño D, Bryson PK and Schnabel G. 2014. First report of *Pilidium concavum* causing tan brown rot on strawberry nursery stock in South Carolina. Plant Disease 98(7): 1010–1010. https://doi.org/ 10.1094/ pdis-01-14-0048-pdn

Garrido C, Carbú M, Fernández-Acero FJ, González-Rodríguez VE and Cantoral JM. 2011. New insight in the study of strawberry fungal pathogens. Genes, genomes and genomics 5(1): 24–39.

Geng WL, Hu P, Ma Z, Zhao XY and Wei YM. 2012. First report of *Pilidium concavum* causing tan brown leaf spot on strawberry in China. Plant Disease 96(9): 1377–1377. https://doi.org/10.1094/pdis-03-12-0310-pdn

Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B and Battino M. 2012. The strawberry: Composition, nutritional quality, and impact on human health. Nutrition (Burbank, Los Angeles County, Calif.) 28(1): 9–19. https://doi.org/10.1016/j.nut.2011. 08.009

Gołębniak B and Jarosz A. 2003. First report of tan brown rot *(Hainesia lythri)* on strawberry fruits in Poland.

González R, Butković A, Escaray FJ, Martínez-Latorre J, Melero Í, Pérez-Parets E, Gómez-Cadenas A, Carrasco P and Elena SF. 2021. Plant virus evolution under strong drought conditions results in a transition from parasitism to mutualism. Proceedings of the National Academy of Sciences of the United States of America 118(6). https://doi.org/10.1073/ pnas.2020990118

Hipol RM, Magtoto LM, Tamang SMA and Damatac AM-II. 2014. Antioxidant activities of fungal endophytes isolated from strawberry *Fragaria* x *ananassa* Fruit. Electronic Journal of Biology 10(4). https://ejbio.imedpub.com/antioxidant-activities-of-fungal-endophytes-isolated-from-strawberry-fragaria-x-ananassa-fruit.php?aid=6001

Ikegaya A. 2023. Composition of free sugars and organic acids in Japanese strawberry cultivars and their influence on the perception of sweetness and sourness. Journal of Food Science. https://doi.org/10.1111/1750-3841.16842

Johnston PR, Seifert KA, Stone JK, Rossman AY and Marvanová L. 2014. Recommendations on generic names competing for use in Leotiomycetes (Ascomycota). IMA Fungus 5(1): 91–120. https://doi.org/10.5598/imafungus.2014.05.01.11

Karimi K, Arzanlou M, Babai-Ahari A and Pertot I. 2016. Biological and molecular characterization of *Pilidium lythri*, an emerging strawberry pathogen in Iran. Phytopathologia Mediterranea 55(3): 366–379. https://doi.org/10.14601/Phytopathol_ Mediterr-18391

Kirk PM, Cannon PF, Minter DW, and Stalpers JA. (Eds.). 2011. Dictionary of the fungi (10a ed.). CABI Publishing.

Lopes UP, Zambolim L, Lopes UN, Pereira OL, and Costa H. 2010. First report of *Pilidium concavum* causing tan-brown rot in strawberry fruits in Brazil. Plant Pathology 59(6): 1171–1172. https://doi.org/10.1111/ j.1365-3059.2010.02331.x

Lopez AC, Alvarenga AE, Zapata PD, Luna MF, and Villalba LL. 2020. Aislamiento e identificación de hongos asociados a *Ilex paraguariensis* St. Hil. Revista de ciencia y tecnología 33: 20–26. https://doi.org/10.36995/j.recyt.2020.33.003

Martin KJ, y Rygiewicz PT. 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiology 5: 28. https://doi.org/10.1186/1471-2180-5-28

Milgroom MG. (Ed.). 2017a. CHAPTER 1: Introduction to population biology and evolution. *In* Population Biology of Plant Pathogens: Genetics, Ecology, and Evolution (pp. 1–11). The American Phytopathological Society.

Montesinos-Matías R, Ayala-Zermeño MA, y Berlanga-Padilla AM. 2015. Manual para la conservación y mantenimiento de hongos entomopatógenos. Centro Nacional de Referencia de Control Biológico. Dirección General de Sanidad Vegetal. SAGARPA. SENASICA. Tecomán, Colima, México. Pp. 59. ISBN: 978-968-5384-08-7

Morales-Mora LA, Martínez-Salgado SJ, Valencia de Ita MA, Andrade-Hoyos P, Silva-Rojas HV, and Romero-Arenas O. 2019. First report of leaf spot and anthracnosis caused by *Pestalotiopsis* sp. On strawberry in Puebla, Mexico. Plant Disease 103(10): 2668–2668. https://doi.org/ 10.1094/ pdis-05-19-1010-pdn

Palm ME. 1991. Taxonomy and morphology of the synanamorphs *Pilidium concavum* and *Hainesia lythri* (coelomycetes). Mycologia, 83(6): 787–796. https://doi.org/10.1080/ 00275514.1991. 12026084

Park MJ, Back CG, Park JH, and Han KS. 2017. Occurrence of tan brown leaf spot caused by *Pilidium concavum* on *Fragaria ananassa* in Korea. Han'guk Kyunhakhoe chi 45(4): 377–380. https://doi.org/ 10.4489/KJM.20170044

Pedraza-Herrera LA, Sánchez F, Arias-Avila V, Moreno MF y Sánchez-Leal LC. 2022. Enfermedades emergentes y reemergentes de plantas en Latinoamérica: una revisión. Revista de Investigación Agraria y Ambiental 13(2): 15–51. https://doi.org/10.22490/21456453. 4639

Petrasch S, Knapp SJ, van Kan JAL y Blanco-Ulate B. 2019. Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. Molecular Plant Pathology 20(6): 877–892. https://doi.org/10.1111/mpp.12794

Phatak HC, and Payak MM. 1965. *Hainesia lythri* on strawberry. A new disease record for India. Indian Phytopathology 237–239.

Rebollar-Alviter A, Silva-Rojas HV, Fuentes-Aragón D, Acosta-González U, Martínez-Ruiz M, and Parra-Robles BE. (2020). An emerging strawberry fungal disease associated with root rot, crown rot and leaf spot caused by *Neopestalotiopsis rosae* in Mexico. *Plant Disease*, *104*(8), 2054–2059. https://doi.org/10.1094/pdis-11-19-2493-sc

Reddy PP. 2016. Strawberry. En Sustainable Crop Protection under Protected Cultivation (pp. 245–260). Springer Singapore.

Ristaino JB, Anderson PK, Bebber DP, Brauman KA, Cunniffe NJ, Fedoroff NV, Finegold C, Garrett KA, Gilligan CA, Jones CM, Martin MD, MacDonald GK, Neenan P, Records A, Schmale DG, Tateosian L and Wei Q. 2021. The persistent threat of emerging plant disease pandemics to global food security. Proceedings of the National Academy of Sciences of the United States of America 118(23). https://doi.org/10.1073/pnas.2022239118

Rossman AY, Cathie-Aime M, Farr DF, Castlebury LA, Peterson KR and Leahy R. 2004. The coelomycetous genera *Chaetomella* and *Pilidium* represent a newly discovered lineage of inoperculate discomycetes. Mycological Progress 3(4) 275–290. https://doi.org/ 10.1007/s11557-006-0098-4

Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria (SENASICA).(2018. Protocolo de diagnóstico: *Fusarium oxysporum* f. sp. *Cubense* Raza 4 Tropical (Marchitez por Foc R4) [Versión 1.0]. Tecámac, México

Tamura K, Stecher G and Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/ molbev/msab120

Tito R, Vasconcelos HL and Feeley KJ. 2018. Global climate change increases risk of crop yield losses and food insecurity in the tropical Andes. Global Change Biology 24(2). https://doi.org/10.1111/gcb.13959

Zhan Y, Peng W, Xu Z, Feng X and Xu H. 2021. First report on *Neofusicoccum kwambonambiense* causing strawberry root rot on strawberry in China. Journal of Plant Pathology: An International Journal of the Italian Phytopathological Society 103(3): 1003–1004. https:// doi.org/10.1007/s42161-020-00720-1

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand JL, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, … and Asseng S. 2017. Temperature increase reduces global

yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences of the United States of America 114(35): 9326–9331. https://doi.org/ 10.1073/pnas.1701762114