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# Damping off on soybean (*Glycine max*) caused by *Pythium aphanidermatum* in Buenos Aires Province (Argentina)

## Damping off en soja (*Glycine max*) causado por *Pythium aphanidermatum* en la provincia de Buenos Aires (Argentina)

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**Abstract**

*Pythium aphanidermatum* has a cosmopolitan distribution in the warmer parts of the world. The aim of this study was to identify the causal agent of damping off in soybean seedlings on late planting dates under hot conditions, from different locations in Buenos Aires province. The isolates induced different levels of plant and seedling death and growth rate at different incubation temperatures (15, 25 and 35°C). In accordance with morphological, cultural and molecular characteristics, the pathogen was identified as *P. aphanidermatum*, and this is the first report of this oomycete causing soybean root and stem rot in Buenos Aires province, Argentina.

**Keywords**
oomycetes • root rot • growth rate • pathogenicity • temperature

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**Resumen**

*Pythium aphanidermatum* presenta una distribución cosmopolita, principalmente en las regiones cálidas del mundo. El objetivo de este trabajo fue identificar el agente causal del damping off en plántulas de soja en fechas de siembra tardía bajo condiciones cálidas, en diferentes localidades de la provincia de Buenos Aires. Los aislamientos indujeron diferentes niveles de mortandad de plantas y plántulas y en la tasa de crecimiento a diferentes temperaturas de incubación (15, 25 and 35°C). De acuerdo con la morfología, las características culturales y moleculares, el patógeno fue identificado como *P. aphanidermatum*, y esta es la primera cita de este oomycete causando podredumbre de tallos y raíces en soja en la provincia de Buenos Aires, Argentina.

**Palabras claves**
oomycetes • podredumbre de raíz • tasa de crecimiento • patogenicidad • temperatura

## Introduction

In Argentina, soybean diseases are responsible for 10 to 15% of the yield losses caused by plant pathologies in the production of this crop (4, 7, 8).

*Pythium* spp. cause root rot and pre and post emergence damping-off on soybean and often contribute to poor stand establishment; at least 17 species are pathogenic on soybean (19). In recent decades, climate change has caused a negative impact on crop yield and consequently, many species have modified their geographic ranges in response to this (6). Few studies have been conducted to examine oomycetes in soybean in Argentina, and these were mainly carried out in early planting dates. Only *P. ultimum*, *P. irregulare*, *P. debaryanum*, *P. rostratum* and *P. sylvaticum* were cited (12, 14, 18). Risk of disease is favored by moist soils, low temperatures, free moisture, earlier planting and no tillage (5), but in the 2014/2015 growing season some problems appeared on late planting dates and under hot conditions. Soybean plants were significantly affected, reducing seedling emergence, and in many cases, replanting was necessary (photo 1).

**Photo 1.** Field shows scarce soybean seedling emergence caused by *Pythium aphanidermatum.*

**Foto 1.** Campo con escasa emergencia de plantas de soja causado por *Pythium aphanidermatum.*

Temperature is an important factor affecting the pathogenicity of *Pythium* spp. *P. ultimum* reduces soybean seed germination below 24°C, *P. aphanidermatum* reduces seed germination above 20°C, and *P. irregulare* causes cucumber damping-off only from 20 to 24°C (3, 20). *P. aphanidermatum* is cosmopolitan in distribution and one of the most common plant pathogens of a number of different crops in warm parts of the world. The present study has been carried out with the main goal of identifying the causal agent of the previously described disease affecting soybean seedling in Argentina.

## Materials and methods

Diseased soybean seedlings were collected during 2014/15 growing season in commercial fields of Mechita, Rojas, Capitan Sarmiento, Gorostiaga (northern Buenos Aires province) and Villaguay (Entre Ríos province). The samples were transported to the laboratory of Phytopathology of Buenos Aires University to obtain pure isolates. Roots and basal stems were cut into 1-$cm^2$ pieces, washed with tap water and cultured on potato dextrose agar (PDA) and corn meal agar (CMA) + Pimaricin-ampicilin-rifampicin-pentachloronitrobenzene (PARP), in Petri plates, at 23 to 25°C, in the dark. Three colonies obtained, Py15-7 from Gorostiaga, Py15-12 from Mechita and Py15-64 from Rojas, were morphologically and molecularly characterized, used to carry out the pathogenicity tests and to measure the daily growth rate in PDA, under three different temperatures (15, 25 and 35°C). The isolates are kept in the FAUBA fungal collection (Buenos Aires University- Py15-7 accession number to the Genbank #MK514097).

**Daily growth rate**

One segment, about 1 cm diam, from a 7-day-growth in PDA, of each colony was plated in a 9 cm diam Petri plate with 15 ml of PDA. These plates were incubated in a Sanyo incubator model MIR-553. The mycelial growth was measured daily.

***In vitro* test**

One segment, about 1 cm diam, from 7-day-old PDA of each isolate, was excised and transferred to the center of a 9-cm Petri plate containing 25 ml of PDA and ten soybean seeds (cv Harosoy previously disinfected) were carefully placed towards the periphery. The plates were laid on a bench in a completely randomized experimental design, with 5 replicates/treatment. The experimental unit consisted of a Petri plate with 10 seeds, which were incubated in darkness at the different temperatures. Seven days later, the dead seeds and seedlings were registered using the severity scale proposed by Jiang *et al.* (2012).

***In vivo* test**

Pots (12 cm diam) were half-filled with a mix of twice tindalized commercial substrate. A layer of mycelial growth from the PDA cultures of the three isolates was excised from the Petri plates and carefully put on top of the substrate. The mycelia was covered with a 2-cm layer of the same substrate. Immediately, 15 soybean seeds were placed on top of the substrate and another substrate layer was again added to cover the seeds. Control plants were inoculated with agar discs that did not contain the oomycete. The pots were maintained in a climatic chamber at different temperatures, 12 h/12 h (light/dark) and the soil was kept wet by watering plants daily as required. Fifteen days after sowing, the number of emerged,

visually healthy and well developed seedlings in each pot was recorded.

The data of emergence and daily growth were subjected to ANOVA for a completely randomized design and DGC post-hoc multiple comparison test (9). For all statistical analysis, the Infostat (10) program was used.

Morphological characteristics were observed on grass blade cultures: 3-4 agar sections of 0.5 cm diam with mycelium of 7 days of growth were placed in Petri dishes containing sterile distilled water with 15-20 pieces of *Agrostis* sp. (0.5 to 1 cm in length), boiled for 10-15 min. After 48-72 h, the colonized segments were observed under microscope (1). The identification was based on Van Der Plaats-Niterink (1981).

DNA extraction was made from a pellet of the isolates grown in PDA, using the nucleon™ PhytoPure™ Genomic DNA extraction kit (GE Healthcare) according to manufacturer's instructions. The ITS region of the nuclear rDNA was amplified using primers ITS4 and ITS5 (22). Amplification was carried out in 50 µl of PCR Buffer (Boehringer Mannheim) in the presence of 150 ng of each primer, 200 mM of each dNTP (Boehringer Mannheim), 10 ng of DNA and 2 U of Taq polymerase (Boehringer Mannheim). To check the presence of PCR products, 5 mL of the PCR reaction mixture was electrophoresed on 1.5% (w/v) agarose gels run at 100 V for 1 h in tris-acetate buffer, stained with ethidium bromide, and visualized under UV light. PCR products were desalted with the ExoSAP-IT for PCR Product Cleanup kit (Affymetrix) according to the manufacturer's instructions. Desalted amplicons were sequenced at the Servicio de Genotipificación y Secuenciación, Instituto de Biotecnología del Instituto Nacional de Tecnología Agropecuaria (INTA, Buenos Aires). In order to minimize sequencing errors, both strands were sequenced. A consensus sequence was inferred from forward and reverse sequences using GeneTool Life 1.0 (16). The rDNA sequences obtained were compared with oomycete sequences deposited in GenBank, using the Basic Local Alignment Search Tool (BLAST) program (2).

## Results and discussion

After five days of incubation, 10 colonies with cultural and morphological features of a peronosporomycete, from symptomatic plants of all the surveyed fields, were transferred to PDA and CMA. Growth rate of the isolates was greater at higher temperature. The different incubation temperatures induced different levels of plant and seedling death: the higher the temperature, the higher the percentage of dead plants and seedlings, being almost not pathogenic at 15°C (figure 1, page 286). This coincides with Van Der Plaats-Niterink (1981), who cites 35-40°C as optimum temperature. Control plants remained symptomless. Koch's postulates were confirmed by re-isolating the same microorganism from diseased plants. The isolated pathogen was characterized after 10 days. Cultures on PDA and V8 quickly formed a fluffy, cottony growth with coenocytic hyaline hyphae. All isolates showed the same growth and morphological characteristics: homothallic, lobate or filamentous sporangia, one antheridium (9-12,1 µm wide by 10,6-14.0 µm long) per oogonium often intercalary, oogonia terminal 18.7-26.2 µm (av. 22.5 µm) in diam, oospores aplerotic 16.4-21.6 µm (av. 18.5 µm) in diam (photo 2, page 286).

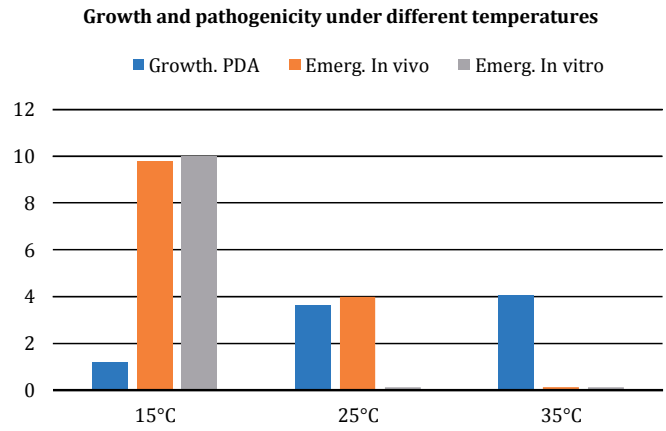


**Figure 1.** Daily growth rate in PDA and pathogenicity (*In vivo*: number of emerged seedlings of 15 soybean seeds planted. *In vitro:* germinated seedlings using Jiang *et al*. (2012) scale of 10 seeds plated under different temperatures.

**Figura 1.** Tasa diaria de crecimiento en APD y patogenicidad (*In vivo*: número de plántulas emergidas de 15 semillas sembradas. *In vitro:* plántulas germinadas utilizando la escala de Jiang *et al*. (2012) de 10 semillas sembrada bajo diferentes temperaturas.

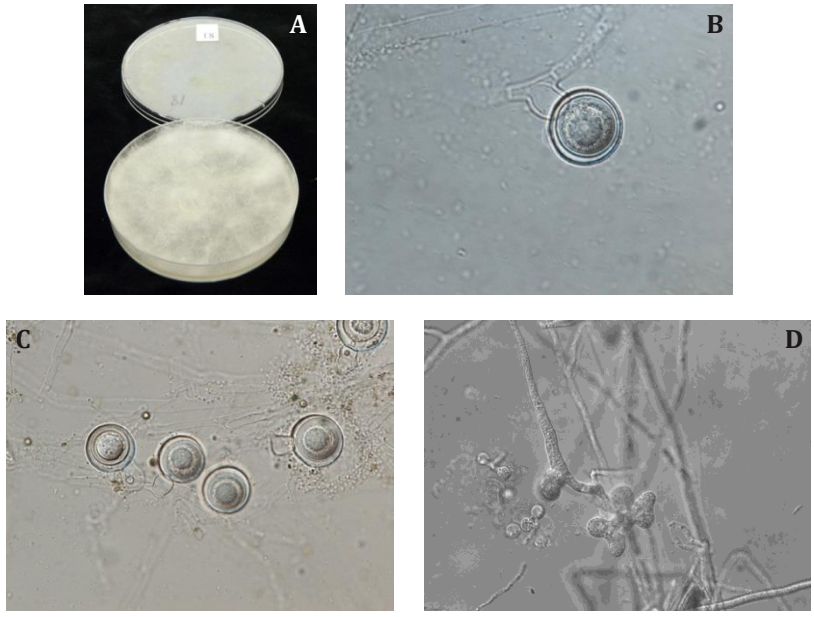


**Photo 2.** *Pythium aphanidermatum* **A**. Seven day-old colony in APD.
**B**. Oogonium and intercalary antheridium. **C**. Aplerotic oospores. **D**. Lobate sporangia. BAR= 20 µm.

**Foto 2.** *Pythium aphanidermatum* **A**. Colonia de 7 días de crecimiento en APD.
**B**. Oogonio y anteridio intercalar **C**. Oosporas apleróticas. **D**. Esporangios lobados. BAR= 20 µm.

Nucleotide sequences (867 bp) showed 100% identity with the *P. aphanidermatum* isolate deposited in GenBank (Accession #AY598622 strain CBS 118.80).

In accordance with morphological, cultural and molecular characteristics, the pathogen was identified as *Pythium aphanidermatum* (Edson). Root rot, pre and post emergence damping-off of soybean caused by *P. aphanidermatum* might be considered a potential threat to Argentinean soybean production. Rupe *et al.* (2011) identified the resistance gen *RPA1 to P. aphanidermatum,* but research is needed to determine if resistance to other species is conferred by this gene or if other genes are involved. Argentinian breeders may do future research to incorporate this gene into adapted cultivars and to determine the best way to utilize *Pythium* resistance in the field.

The optimal temperature for pathogenicity and growth of the different species of this pathogen depend on the geographical location of the field and on planting dates, which determine the dominant species in the field. On planting time, temperatures of 35°C constitute predisposing conditions and at 15°C or lower the seed and seedling would not be affected by *P. aphanidermatum*. This indicates that the incidence of the disease would be due to environmental factors. In Argentina this pathogen was first isolated from *Pisum sativum* in 1950 (10) and then recorded in many other plants, mainly in intensive crops in greenhouse conditions (13). Pastor *et al.* (2005) isolated this pathogen from soil samples, but did not test it in extensive crops.

## Conclusions

This is the first report of *Pythium aphanidermatum* causing root rot and stem rot on soybean in Buenos Aires province.

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