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INFORME BREVE

Carpogenic germination of *Sclerotinia sclerotiorum* sclerotia in Brassica oleracea var gemmifera (VN: Brussels sprouts) in North Patagonia, Argentina

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KEYWORDS
White mold;
Sexual phase;
Ascospores;
Aerial-apical rot

Abstract In August 2018, symptoms of apical and basal rot resembling those caused by *Sclerotinia sclerotiorum* infection were observed in a commercial Brussels sprouts field in North Patagonia, Argentina. The incidence of apical and basal rot was 23.30% and 2.30%, respectively. Carpogenic germination of sclerotia was detected in shaded, highly humid soil areas. To our knowledge, this is the first report of carpogenic germination of sclerotia from S. *sclerotiorum* in North Patagonia.

PALABRAS CLAVE
Moho blanco;
Fase sexual;
Ascosporas;
Podredumbre aérea – apical

Germinación carpogénica de esclerocios de *Sclerotinia sclerotiorum* en Brassica oleracea var gemmifera (VN: Brussels sprouts) en Patagonia Norte, Argentina

Resumen En agosto de 2018, se observaron síntomas de podredumbre basal y apical similares a los producidos por la infección por *Sclerotinia sclerotiorum* en un cultivo comercial de repollitos de Bruselas de la Patagonia Norte, en Argentina. La incidencia de podredumbre apical y basal fue del 23,30% y 2,30%, respectivamente. Se detectó la germinación carpogénica de esclerocios

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en áreas de suelos sombreados y muy húmedos. De acuerdo con nuestro conocimiento, este es el primer reporte de la germinación carpogénica de esclerocios de *S. sclerotiorum* en la Patagonia Norte.

Brussels sprouts are a winter crop widely cultivated in the horticultural belt of North Patagonia, Argentina. In August 2018, in Vista Alegre, Neuquén, *Brassica oleracea var. gemmifera* plants showed characteristic symptoms of white mold/rot, mainly in aerial-apical sprouts (Fig. 1). The field incidence of aerial infections was 23.3%, while the incidence of basal infections was 2.3% (n = 1925 plants).

Aerial and basal symptoms consisted of brown, soft, watery lesions with the presence of abundant white, cottony mycelium. Subsequently, sprouts exhibited abundant black sclerotia, and outer leaves turned yellow and wilted. In addition, plants with basal stem infections showed weakened leaves and neck rot near ground level (Fig. 1a-c).

In soil from areas with the highest humidity and shade conditions, sclerotia germinated to produce stipitate, pale brown, cup-shaped apothecia (Fig. 1d). Each sclerotium produced multiple (3 to 8) apothecia of 2-5 mm in diameter, with asci of 98-166 x 5-13 μm and ascospores of 9.5-14 x 3.6-8 μm (Fig. 1e-f). These morphological characteristics were similar to those reported by Domsch[3] and Ellis and Ellis[4].

Fungal isolation was performed from tissue pieces from the lower stem (basal infection) and apical sprouts (aerial infection). Samples were superficially sterilized and transferred onto potato dextrose agar (PDA). After 48 h of incubation at 25 °C in the dark, colonies of white cottony mycelium developed, and after 120 h, black sclerotia (1-40 mm) formed on the Petri plate (Fig. 1f). All fungal isolates (n = 16) reached confluence within 48 h with a count of 20-25 sclerotia/plate after 216 h. Based on cultural and morphological characteristics, the fungus was confirmed to be *Sclerotinia sclerotiorum*.

Three representative isolates, two from apical sprouts (SS1 and SS2) and one from the basal stem (SS3), were selected for molecular identification. To this end, we amplified segments of the internal transcribed spacer (ITS) region, using ITS1/ITS4 primers[14], and of the translation elongation factor 1α (TEF-1α) gene, using EF-728F/EF-986R primers[1]. The MegaBLAST analysis revealed a sequence identity ranging from 99.60%-100% for ITS fragments (GenBank KF 859933.1) and 99.00%-99.32% identity for TEF-1α sequences (GenBank AF 040087.1) against *S. sclerotiorum* sequence data. Sequences were deposited in GenBank under accessions MK527225, MK527226, and MK527227 (ITS), and MK537342, MK537343, and MK537344 (TEF-1α).

Pathogenicity tests were conducted by inoculating Brussels sprouts in pots with mycelium disks (3 mm) from young colonies of each isolate (5 days old on PDA), followed by a 5-day incubation in polyethylene bags (25 °C +/- 2 °C and 75%-80% RH). Five fungal isolate replicates were tested. Five days after inoculation, aerial white mycelium developed on the inoculated tissues, showing symptoms identical to those observed in the field. Re-isolation of the causative agent was done by transferring tissue pieces taken from symptomatic advance zones onto PDA, where *S. sclerotiorum* colonies reappeared after incubation at 25 °C for 96 h. Meanwhile, ascosporic infections were confirmed in the field under natural conditions, with symptoms originating in the upper part of the plants (~0.7 m from soil level) and the colonization of the pathogen progressing downwards.

The life cycle of *S. sclerotiorum* depends on its infection mode, which is related to the type of sclerotium germination[8]. In our region, *S. sclerotiorum* is a common pathogen of horticultural crops such as lettuce, tomato, carrot, and celery (unpublished data) that typically produces basal infections via myceliogenic germination from sclerotia. Although myceliogenic germination is more commonly associated with the small sclerotia of *Sclerotinia minor* rather than those of *S. sclerotiorum*[11], through this infectious pattern the latter pathogen causes important losses in canola (*Brassica napus*), carrot, and sunflower fields worldwide[8,12]. Carpogenic germination of sclerotia to produce apothecia requires permissive environmental conditions, primarily influenced by the temperature at which the sclerotia are formed, as well as light and water availability[5,7,10]. In our region, it is possible that the unusual agro-climatological conditions observed in recent years during the winter-spring seasons (wide temperature ranges, high rainfall intensity, excessive watering and soil waterlogging) favored the carpogenic development of the pathogen.

Upon carpogenic germination, the inoculum potential increases significantly and with it the risk of aerial infections; this mainly occurs at the end of the crop cycle, leading to substantial production and economic losses[13]. Prompted by changes in relative humidity or physical disturbance of the apothecium, *S. sclerotiorum* releases ascospores by 'puffing', i.e. the simultaneous and forceful discharge of large numbers of asci[6]. Under optimal field conditions, ascospores can thus be released continuously, at a rate of 1600 spores/h, for more than 10 days, and some can be carried several kilometers on wind currents[2,9]. This mode of dissemination hampers control strategies in the productive systems of the region, which are characterized by small-scale horticultural orchards with different species susceptible to *S. sclerotiorum* rot. Thus, it is crucial to closely monitor early signs of infection to implement adequate preventive and integrative control practices[13].

To our knowledge, this is the first report of carpogenic germination of sclerotia from *S. sclerotiorum* in *Brassica*

Figure 1 Brussels sprouts field affected by *S. sclerotiorum* (a). Plants showing aerial-apical (a) and basal stem (b) infections. Sclerotia on the ground (c). Sclerotia germinated in apothecia in soil (d). Asci with eight ascospores and paraphyses formed in the apothecium (e). Sclerotia development on PDA in a Petri plate after 7-d incubation (f).

oleracea var. gemmifera in the Alto Valle de Rio Negro and Neuquen, Argentina. These data underscore an alternative form of dissemination for *S. sclerotiorum* that might significantly increase the incidence of the disease in various horticultural crops of economic significance. Therefore, meiotic sporulation (ascospore production) and subsequent aerial dissemination must be considered in the implementation of control methods for *S. sclerotiorum* rot.

Credit author statement

Scarso Ana Gabriela: Investigation, Formal analysis. **Sosa Maria Cristina**: Conceptualization, Investigation, Formal analysis, Writing - Original Draft; Writing - Review & Editing, Supervision, **Ousset María Julia**: Data Curation, Formal analysis. **Lutz María Cecilia**: Conceptualization, Investigation, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision.

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Conflict of interests

The authors state that they have no conflict of interests.

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