# *Fusarium* species that cause corn stalk rot in the Ubaté valley of Cundinamarca, Colombia

## Especies de *Fusarium* que causan la pudrición del tallo del maíz en el valle de Ubaté en Cundinamarca, Colombia

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## ABSTRACT

In recent years, corn (*Zea mays* L.) crops in the Colombian cold tropics located in the Ubaté valley in Cundinamarca have been affected by stalk rot with incidences up to 40%. Despite the importance of this disease, accurate diagnosis has not been conducted. The objectives of the study were to determine the causal agents of corn lodging associated with stalk rot in this corn-producing region and describe the symptoms of the disease. Two plots with stalk rot reported in the municipality of Simijaca in July 2016 were sown with the regional varieties Simijaca and Sogamoso. Plants were randomly inspected on a monthly basis for describing disease symptoms and isolating the pathogen. The *Fusarium* species isolated were morphologically and molecularly identified and pathogenicity tests were conducted. The disease was detected at early plant developmental stages with the combination of chlorosis, leaf anthocyanosis, and dwarfism as the main symptoms in the two corn varieties evaluated. Crown and node necrosis in longitudinal sections of the stalk and purple colorations in the crown, nodes and internodes of plants were observed 90 d after sowing. Finally, lodging occurred at any phenological stage of the crop. *Fusarium* spp. were isolated in all stages of plant development. *Fusarium* species were identified as *F. graminearum* in the *Fusarium graminearum* species complex and *F. subglutinans* in the *Fusarium fujikuroi* species complex, which have cold-climate production zones as their ecological niche. Pathogenicity tests confirmed *F. graminearum* and *F. subglutinans* as the causal agents of stalk rot in the regional corn variety Simijaca in the Ubate valley in Cundinamarca.


**Key words:** *Zea mays* L., *Fusarium graminearum* species complex (FGSC), *Fusarium fujikuroi* species complex (FFSC), corn lodging, cold-climate corn.


## RESUMEN

En los últimos años, los cultivos de maíz (*Zea mays* L.) en el trópico frío colombiano localizados en el valle de Ubaté en Cundinamarca han sido afectados por una pudrición del tallo con incidencias hasta del 40%. A pesar de la importancia de esta enfermedad, no se ha realizado un diagnóstico preciso. El objetivo de este estudio fue determinar los agentes causales del volcamiento de maíz asociado a la pudrición del tallo en esta región productora y describir los síntomas de la enfermedad. Dos lotes con registro de pudrición de tallo en el municipio de Simijaca en el valle de Ubaté en julio de 2016 fueron sembrados con las variedades regionales Simijaca y Sogamoso. Las plantas fueron inspeccionadas aleatoriamente de forma mensual para describir los síntomas de la enfermedad y aislar el patógeno. Las especies de *Fusarium* aisladas fueron morfológicamente y molecularmente identificadas y se realizaron las pruebas de patogenicidad en maíz. La enfermedad fue detectada en estados tempranos de desarrollo de la planta como la combinación de clorosis, antocianosis de las hojas y enanismo de la planta; estos como los principales síntomas en las dos variedades de maíz evaluadas. La necrosis de cuello y nudos fue observada en cortes longitudinales del tallo y coloraciones púrpura en cuello, nudos y entrenudos de la planta fueron observados 90 d después de la siembra. Finalmente, el volcamiento ocurrió en cualquier estado fenológico del cultivo. *Fusarium* spp. fue aislado en todos los estados de desarrollo de la planta. Las especies de *Fusarium* fueron identificadas como *F. graminearum* perteneciente al complejo de especies *Fusarium graminearum* y *F. subglutinans* perteneciente al complejo de especies *Fusarium fujikuroi,* las cuales tienen las zonas de producción de clima frío como su nicho ecológico. Las pruebas de patogenicidad confirmaron a *F. graminearum* y *F. subglutinans* como los agentes causales de la pudrición del tallo en la variedad regional de maíz Simijaca en el valle de Ubaté en Cundinamarca.


**Palabras clave:** *Zea mays* L., complejo de especies de *Fusarium graminearum* (FGSC), complejo de especies de *Fusarium fujikuroi* (FFSC), volcamiento del maíz, maíz de clima frío.

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## Introduction

Corn is one of the bases of planetary food security. It is the second most important crop after wheat (Paliwal, 2016). Colombia is the fourth largest corn producer in South America, and this crop is the third most sown in the country (CIAT & CIMMYT, 2019). Simijaca is a regional corn variety of free pollination. It is adapted to cold climate production zones in Colombia. This variety was widely cultivated by the Muisca, the indigenous inhabitants of the Ubaté valley region who selected it for its culinary quality. Therefore, this corn variety has been highly accepted in Andean cities (Ligarreto, 2017). In recent years, corn crops in the municipality of Simijaca at the Ubaté valley have been affected by stalk rot but without accurate diagnosis (Gómez *et al*., 2017). This is a complex disease and its cause is difficult to determine since many fungi, bacteria, and oomycetes may appear in affected plants as secondary invaders and saprophytes (Wicklow *et al*., 2005).

Stalk rot caused by *Fusarium* spp. typically reduces yield by 10% or 30%-50% in severely affected areas (Gai *et al*., 2018). Although *Fusarium* stalk rot (FSR) is among the most economically important diseases of corn around the world (Yang *et al*., 2010; Wang *et al*., 2017), it is not frequently seen in Colombia and had not been reported in Cundinamarca until now (Buritica, 1999). In production areas around the world, at least 22 *Fusarium* species can be found in corn causing diseases (Munkvold *et al*., 2018). Two species, *Fusarium graminearum* in the *Fusarium graminearum* species complex (FGSC) and *Fusarium verticillioides* within the *Fusarium fujikuroi* species complex (FFSC) are the main causes of FSR in corn. The first species is more common in cold regions and is one of the most damaging causal agents of stalk rot, while the second *Fusarium* species is more common in hot, dry climates and is particularly damaging if it attacks before flowering (CIMMYT, 2004). In Colombia, the ancient *F. fujikuroi* has been reported causing pink rot in seeds and ears in Antioquia, Cordoba, Cundinamarca, Santander, Tolima, and Valle del Cauca, whereas *F. graminearum* has been reported as associated with stalk and ear rot in Antioquia, Cordoba, Nariño, and Valle del Cauca (Buritica, 1999).

*Fusarium sambucinum* species complex (FSAMSC) includes the FGSC, *F. cerealis*, and *F. culmorum* (Laraba *et al.,* 2021). Several taxa have been identified within the FGSC, and attempts have been made to divide this complex into phylogenetically separated species. Some of them are present in particular continents. However, it is arguable whether these taxa should all be defined as species, or if they reflect populations or lineages within a broader concept of *F. graminearum sensu lato* (Summerell, 2019). In the last 30 years, reviews of FFSC have reported 45 phylogenetic species, 10 biological species and 34 morphospecies. This complicates the identification of new isolates based only on morphological characters, generating misclassification and underestimation of species diversity (Leyva-Madrigal *et al*., 2015). The taxonomy within FFSC and FGSC is mainly based on DNA sequence analysis of *calmodulin*, elongation factor 1-α (*EF1*), and β-tubulin genes identifying most of the species in these complexes (Leyva-Madrigal *et al*., 2015). Currently EF1 is the most widely used molecular marker in phylogenetic and taxonomic studies within the *Fusarium* genus (Stakheev *et al*., 2018).

*Fusarium* spp. produce different kinds of spores that may be transported and disseminated by air, raindrops, insects and seeds and that are infected through the pistils (Windels *et al*., 1976; Ooka & Kommedahl, 1977; Munkvold *et al*., 1997; Duncan & Howard, 2010). *F. graminearum, F. verticillioides*, and *Fusarium proliferatum* (the last two in the FFSC) enter the corn plant through trichomes, leaves, xylem and stems (Nguyen *et al*., 2015; Nguyen *et al*., 2016). There is evidence of *Fusarium* spp. endophytes in wild and cultivated plants and the best example is *F. verticillioides*. In this case, *F. verticillioides* is associated with corn plants along the complete crop cycle, where plant responses to the infection depend on several factors related to plants, fungi, and the environment (Kuldau & Yates, 2000). This ancient and evolutive relationship can promote plant growth, protect the seed from infection by other 10 genera of fungi while the plant serves as a source of carbon and a pathway of vertical and horizontal transmission of the fungus (Van Wyck *et al*., 1988; Wicklow, 1988; Yates *et al*., 1997; Schulz *et al*., 1999; Kuldau & Yates, 2000).

The endophyte state is transient and *F. verticillioides* switches from an asymptomatic and biotrophic lifestyle to an hemibiotrophic one causing disease (Schulz *et al*., 1999). Stress conditions promote the disease onset and a range of virulence can be observed among different strains. However, strains that can be pathogenic are known to be asymptomatic under optimal plant growth conditions (Kuldau & Yates, 2000). Thermal stress (cold and heat), drought, high sowing density, shadow, pest attacks and the use of fertilizers with high nitrogen and low potassium content are examples of stress conditions that can promote diseases on a very well-balanced association between the plant and *F. verticillioides* (Dodd, 1980; Schulz *et al*., 1999; Kuldau & Yates, 2000; Blandino *et al*., 2009).

Given the recent problem of corn lodging caused by stalk rot in high-altitude corn-producing regions of the Ubaté

valley of Colombia, the objectives of this study were to determine the causal agents of corn lodging associated with stalk rot in this corn-producing region and describe the symptoms of the disease. Our research describes the symptoms and signs associated with stalk rot in corn plants of the regional varieties Simijaca and Sogamoso (var. Simijca and var. Sogamoso) under field conditions. The associated causal agents were morphologically and molecularly identified, and their pathogenicity was determined in corn plants of the regional variety Simijaca (var. Simijaca).

## Materials and methods

### Description of symptoms and signs

During 2016, corn seeds of the regional varieties Simijaca and Sogamoso were sown (22000 plants $ha^{-1}$) in two plots with stalk rot reports located in the municipality of Simijaca (Cundinamarca, Colombia) (5°29′49′′N; 73°49′55′′W and 5°33′11′′N; 73° 47′29′′W). These plots were selected due to the fact that the disease has been reported since 2016 and symptoms of stalk rot have been observed in corn crop cycles during recent epidemics in the region. Plants were randomly inspected on a monthly basis from sowing to tasseling to describe disease symptoms and signs of the pathogen. The inspection dates matched the developmental stages of three, six, nine true leaves, tasseling, silking, and grain with 40% of dry weight (V3, V6, V9, VT, R1, and R4, respectively) according to the scale proposed by Hanway *et al*. (1966). Diseased and healthy plants of both corn varieties in each development stage of the crop were collected and transported to the laboratory of plant pathology (Universidad Nacional de Colombia, Bogotá campus) for detailed inspection under stereoscope, processing and pathogen isolation to identify the causal agent of the disease. Data of precipitation (mm), relative humidity (%), wind speed (m $s^{-1}$), maximum, minimum and average temperatures (°C) were registered using an iMETOS® 300 climatic station (Pessl instruments, Weiz, Austria) at a 10 min frequency. Data were registered in the two experimental plots and inspected in real time. At the end of the trials, the climatic data obtained were compared with a 30-year database (1986-2016) for the municipality of Simijaca provided by the Corporación Autónoma Regional de Cundinamarca (CAR).

### *Fusarium* isolation from corn plants affected by stalk rot

*Fusarium* spp. was isolated from symptomatic plants following the Murillo-Williams and Munkvold (2008) protocol. For this purpose, tissue from roots, crown and stalk was collected, disinfected, and sown in Petri dishes with potato dextrose agar (PDA) medium (Oxoid®) acidified at 0.1% (v/v) with lactic acid. The dishes were incubated under dark conditions at 25°C for 10 d (Model FD 23, Binder®, Germany). Afterwards, the frequency of *Fusarium* isolation per corn variety and the plant's explants origin were recorded. The most representative *Fusarium* colonies were purified in PDA and monosporic cultures were obtained in 3% agar (30 g $L^{-1}$) (WA) (Oxoid®) amended with 12 ml $L^{-1}$ chloramphenicol and 20 ml $L^{-1}$ streptomycin sulfate (Leslie & Summerell, 2006). These cultures were then incubated on PDA at 25°C with a 12:12 h light/dark photoperiod for 15 d in growth chambers (MLR- 351H, Sanyo®, Japan). The resulting pure isolates were stored at -70°C in 15% glycerol. *Fusarium* frequencies, according to the corn variety and part of the plant used for isolation of the pathogen, were analyzed under a completely randomized design (n=24) and subjected to normality and variance tests; means were compared using the Tukey`s test ($P$=0.05).

### Morphological identification of *Fusarium* spp.

Morphological identification of the produced *Fusarium* isolates was performed according to Leslie and Summerell (2006). Carnation leaf piece agar (CLA), Spezieller Nährstoffarmer Agar (SNA), WA and PDA media were used and incubated in growth chambers (MLR- 351H, Sanyo®, Japan) at 25°C with a 12:12 h light/dark photoperiod for 15 d. The color of sporodochia, shape and size of macroconidia and microconidia, number of septa, type of conidiogenesis, formation of chlamydospores and perithecia were determined by light microscopy (CX 31, Olympus®, Japan). Additionally, pigmentation, appearance of the colony, and rate of mycelial growth were evaluated in PDA medium under the same incubation conditions previously described.

### Molecular identification of *Fusarium* spp.

Molecular identification was performed by sequencing the *elongation factor* 1-α (EF1) following the methodology of Stakheev *et al*. (2018). For this purpose, 100 mg samples of fresh mycelium per *Fusarium* isolate were taken from seven-day-old colonies grown on PDA and mechanically lysed with 3 mm diameter tungsten beads in a TissueLyser (Qiagen®, Hilden, Germany) (30 Hz/5 min). DNA extraction was performed using the Plant/Fungi DNA Isolation Kit (Norgen Biotek Corporation®, Canada) following the manufacturer recommendations. Species were identified using the polymerase chain reaction (PCR) of the elongation factor 1-α (EF1) using the oligonucleotides EF50Fw: 5' CGACTCTGGCAAGTCGACCAC 3' and EF590R: 5' CTCGGCTTTGAGCTTGTCAAG 3' following the methodology of Stakheev *et al.* (2018). The phylogenetic analysis was performed using the CLASSIFIER algorithm from the package MEGA version 7.0 (MacOS), using the

neighbor-joining methodology with 1000 bootstrap replicates. The phylogenetic tree for EF1 was built using the T92+G model described by Tamura (1992).

### Pathogenicity test of *Fusarium graminearum* and *Fusarium subglutinans* on corn regional variety Simijaca and their effect on plant growth

For the pathogenicity test, *F. graminearum* (26B) was multiplied following the chaff-grain methodology (Leslie & Summerell, 2006) and *F. subglutinans* (45D) was multiplied in liquid Czapek medium while stirring (Inkubator 1000 - Unimax 1010, Heidolph, Germany) at 150 rpm for 15 d at room temperature (± 20°C) (Leslie & Summerell, 2006). Conidia of each *Fusarium* species were harvested, centrifuged at 2500 rpm for 15 min (MIKRO22R, Hettich® UK), and the inoculum suspensions were adjusted to 1.0 x $10^5$ conidia $ml^{-1}$ by hemocytometer counting (Neubauer, VWR, Darmstadt, Germany).

Seeds of the regional variety Simijaca were used and treated with hot water at 52°C for 5 min in an evaporator (Water B-480, BÜCHI Labortechnik®, AG, Switzerland) following the methodology of Daniels (1983). Seeds were then inoculated with 100 ml of the previously prepared conidia suspension of *F. subglutinans* and *F. graminearum* by shaking the mixture vigorously (Wilke *et al*., 2007). Additionally, the combined inoculation with both *F. subglutinans* and *F. graminearum* was conducted using 50 ml of a suspension of each species. The inoculation of the *Fusarium* species obtained (*F. subglutinans, F. graminearum*, and the mixture) was evaluated and considered as treatments (Reid *et al*., 1999). Seeds without treatment and seeds treated with heat were used as absolute control and thermal control, respectively, and were mock inoculated with sterile distilled water (SDW).

After inoculation, the seeds were placed on plastic trays with a 5 cm layer of soil (Warham *et al.,* 1997) from a non-agricultural area from which the presence of *Fusarium* was previously ruled out according to Leslie and Summerell (2006). Once the seeds germinated, 40 seedlings were selected per treatment. These seedlings were then individually transferred to bags with 1.5 kg of soil of the same origin and taken to a greenhouse (± 25°C, ~75% relative humidity). Three months after sowing (V9), longitudinal cuts of plant stems were taken to evaluate the presence of symptoms associated with stalk rot. Plants showing apical chlorosis, leaf anthocyanosis, and dwarfism were considered diseased plants. Incidence of the disease and internal rot stalk (I) were determined using Equation 1 according to Madden *et al*. (2007).

$$I = \left(\frac{Pd}{Pt}\right) \times 100 \quad (1)$$

where Pd represents the number of plants showing the characteristic, and Pt is the total number of plants per treatment. Pathogen isolation was conducted 90 d after sowing (DAS) on PDA medium at 25°C as described above. Plant height (cm) from the stem base until the tip of the third true leaf and stem diameter at the base (mm) were also registered. Data analysis was conducted using a completely randomized design (n=40) and subjected to normality and variance tests; mean comparisons between treatments were performed using the Tukey's test ($P$= 0.05).

## Results

### Symptoms of stalk rot (FSR) and signs of the pathogen

The external symptoms of FSR in plant grown in the municipality of Simijaca at the Ubaté valley corresponded to apical chlorosis, leaf anthocyanosis, and plant dwarfism (Fig. 1A-C). Intense necrosis of the crown and plant nodes was detected in longitudinal stalk sections, and progressed towards the internodes (Fig. 1G) causing basal disintegration (Fig. 1I).

In longitudinal sections of healthy plants, the pith was cream-colored and the pith of diseased plants had occasional purple coloration (Fig. 1H-I). Lodging, as the final manifestation of corn stalk rot, occurred at any phenological stages of the crop (Fig. 1D). Initial infection was contained in the crown of the plant and then spread to all the plant levels through the nodes colonized by the pathogen at initial stages of plant development, causing a systemic infection (Fig. 1F).

### *Fusarium* isolation and morphological identification

Although there was no statistical difference of *Fusarium* frequency of isolation between corn varieties ($P$=0.091) or between the different plant organs analyzed ($P$=0.112), isolation was 44%, 58%, 78%, and 87% for developmental stages of three, six, and nine true leaves and tasseling, respectively (V3, V6, V9, and VT). Progressive colonization of the stem was observed, with *Fusarium* spp. detected at low frequencies at V3 increasing at V6, V9, and VT. Similar frequencies were observed on the crown and roots (data not shown). Two representative morphotypes of *Fusarium* were isolated from symptomatic plants, purified and morphologically identified.

*F. graminearum* (isolate 26B) showed white colonies that changed to ocher and reddish tones with a feathery appearance (Fig. 2A) on PDA medium. Brown sporodochia

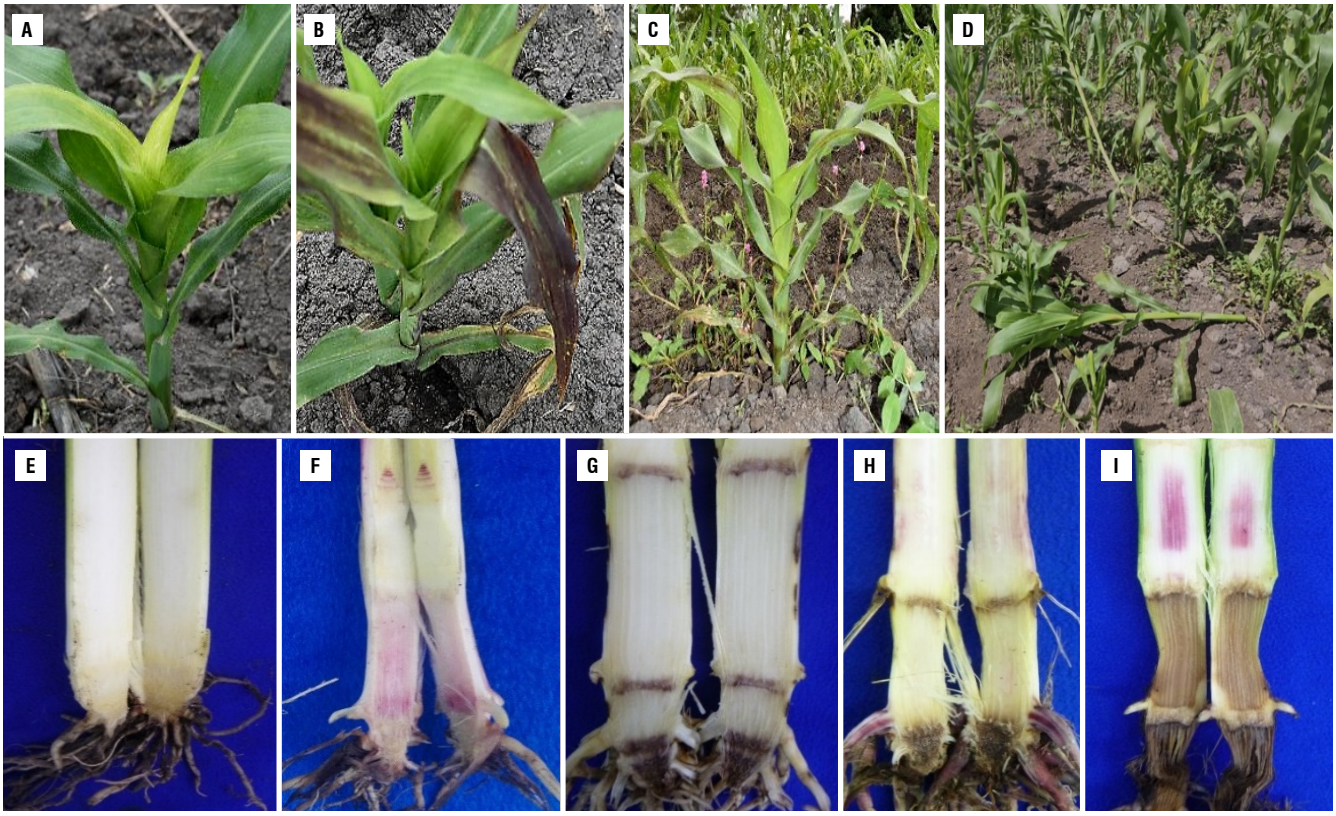


**FIGURE 1.** External and internal symptoms of stalk rot in corn plants of the regional variety Simijaca three to five months after planting (V6-R1) in the municipality of Simijaca (Cundinamarca, Colombia). A) Apical chlorosis of leaves (V9), B) leaf anthocyanosis (V9), C) plant dwarfism (V9), D) plant lodging (R1), E) healthy plant (V9), F) dissemination of the disease through nodes (V6), G) crown and node necrosis (VT), H) progress of the lesion from nodes to internodes (VT), and I) rot and stalk base disintegration (R1).

were observed on CLA medium (Fig. 2B). Macroconidia were slightly swollen in the middle, 40-50 µm x 4.5-5.5 µm in size with five or six septa moderately curved, with the ventral side straight and the dorsal side arched. The basal cell was foot-shaped, and the apical cell straight with a narrow hook or beak (Fig. 2C). Superficial perithecia of *F. graminearum* were observed on the crop debris of the regional corn varieties Simijaca and Sogamoso, mainly on the stalk nodes of diseased plants. These structures were red under lactophenol blue staining with asci containing usually eight trisected-ascospores of approximately 38 µm in length (Fig. 2D-E).

*Fusarium subglutinans* (isolate 45D) showed white cottony colonies with orange and purple colorations (Fig. 3A) on PDA medium. On CLA medium, orange sporodochia

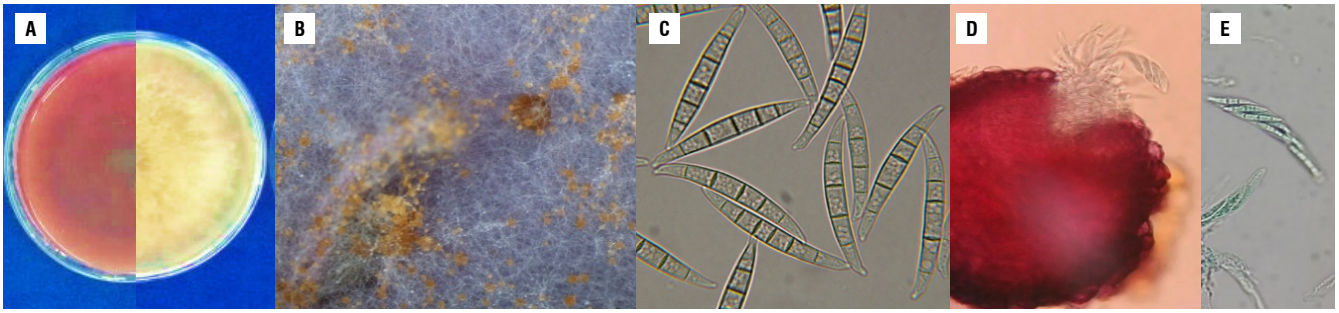


**FIGURE 2.** Morphological characteristics of *Fusarium graminearum* isolate 26B (in the *Fusarium graminearum* species complex FGSC), isolated from corn plants with stalk rot. A) Characteristics of the colonies, right: appearance of the colony and left: pigmentation on the back of the Petri dish on PDA medium 15 d after culture, B) brown sporodochia on CLA medium, C) macroconidia on CLA medium, slightly swollen in the middle, with five or six septa, moderately curved, with the ventral side straight and the dorsal side arched. The basal cell was foot-shaped, and the apical cell straight with a narrow hook or beak, D) *In situ* perithecia on CLA medium, E) asci and ascospores. PDA - Potato Dextrose Agar; CLA - Carnation Leaf Agar.

were formed (Fig. 2B) and macroconidia were typical of the FFSC, 50-60 µm x 3-4 µm in size, three to four septa, straight with curved apical cell and poorly developed basal cell. Microconidia were predominantly oval, usually without septa and (Fig. 3C) forming pseudo-heads over polyphialids (Fig. 3D). Chlamydospores were not formed on CLA, WA or SNA media. On PDA medium, the growth rate of *F. subglutinans* was less than 1.0 cm per day, whereas *F. graminearum* showed a growth rate over this value.

## Molecular identification of *Fusarium* spp. from corn plants with stalk rot

The phylogenetic trees of *EF1* is shown in Figures 4 and 5 in which isolate 26B (MT598159) was grouped with the graminearum clade containing *F. culmorum, F. cerealis* and two species belonging to the FGSC (*F. graminearum sensu stricto* and *F. ussurianum*) (72%) within the FSAMSC (Fig. 4). Isolate 45D (MT598158) was grouped with species of the FFSC (98%) and the species *F. subglutinans* (100%)

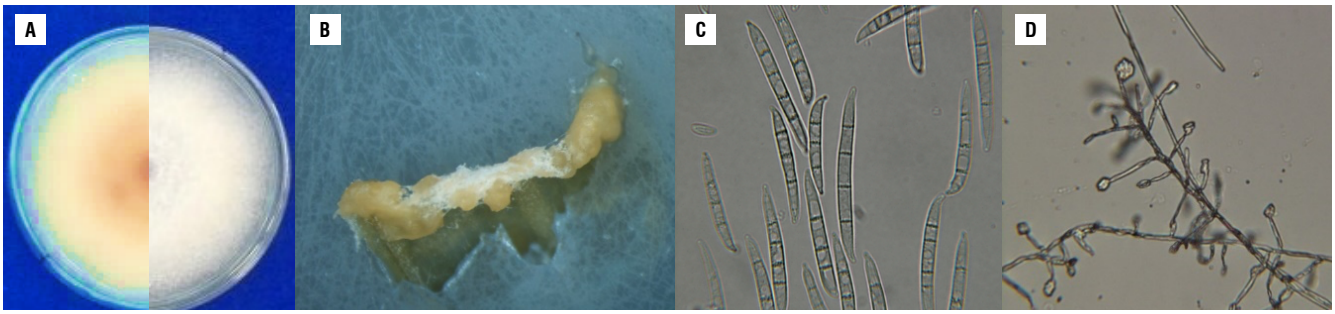


**FIGURE 3.** Morphological characteristics of *Fusarium subglutinans* isolate 45D (in the *Fusarium fujikuroi* species complex FFSC), isolated from corn plants with stalk rot. A) Characteristics of the colonies, appearance of the colony (right) and pigmentation on the back of the Petri dish on PDA medium 15 d after culture (left), B) orange sporodochia on CLA medium, C) macroconidia typical of the *fujikuroi* complex with three to four septa, straight with curved apical cell and poorly developed basal cell and microconidia predominantly oval, usually without septa, D) microconidia *in situ* on CLA medium forming pseudoheads. PDA - Potato Dextrose Agar; CLA - Carnation Leaf Agar.

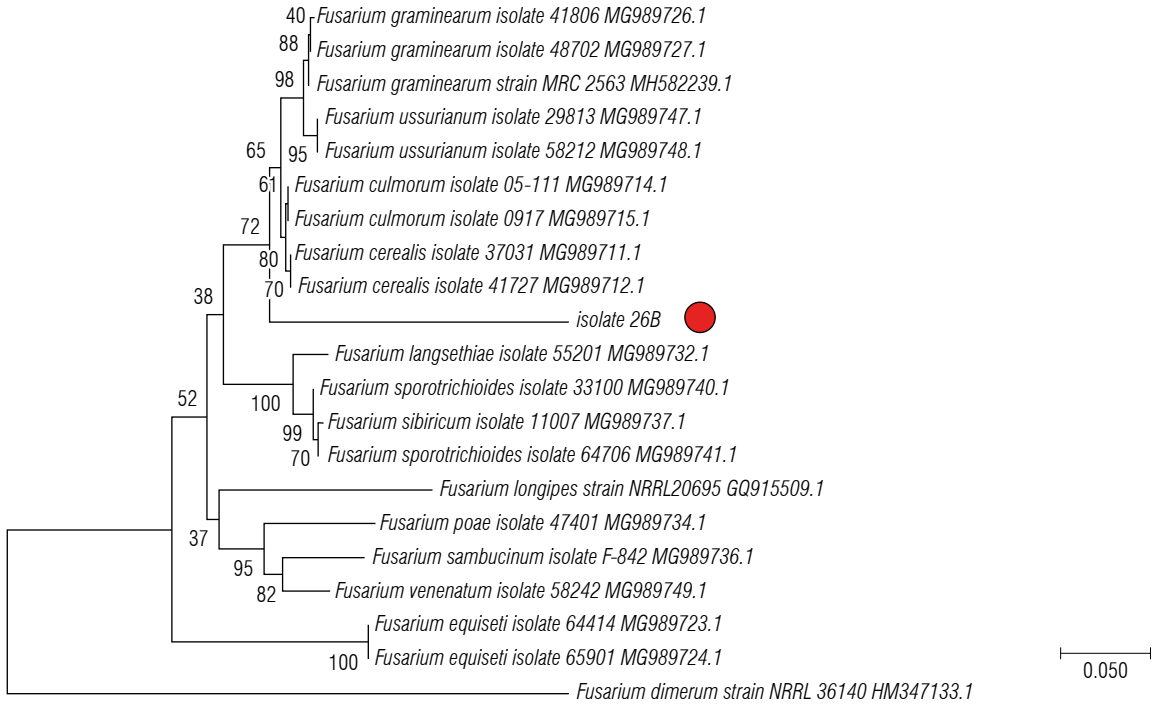


**FIGURE 4.** Phylogenetic tree of fungal isolates 26B obtained from corn plants with stalk rot symptoms and obtained by the Elongation factor 1-α (EF1). The evolutionary history was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used (0.05) to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter=3).

(Fig. 5). Finally, *EF1* sequences were annotated in the National Center for Biotechnology Information (NCBI).

### Pathogenicity of *Fusarium graminearum* and *Fusarium subglutinans* on corn regional variety Simijaca and their effect on plant growth

Isolates 26B of *F. graminearum* and 45D of *F. subglutinans* were pathogenic in corn plants of the regional variety Simijaca. At 90 d after sowing (DAS), the inoculated plants showed necrosis and reddish to purple colorations in the basal internal part of the stalk (Fig. 6). Incidence of internal symptoms was observed in 40% of the plants inoculated with *F. graminearum* and 25% of plants inoculated with *F. subglutinans*, whereas these symptoms were observed in 20% of plants inoculated with both species. *Fusarium graminearum* showed a tendency to be located mainly in

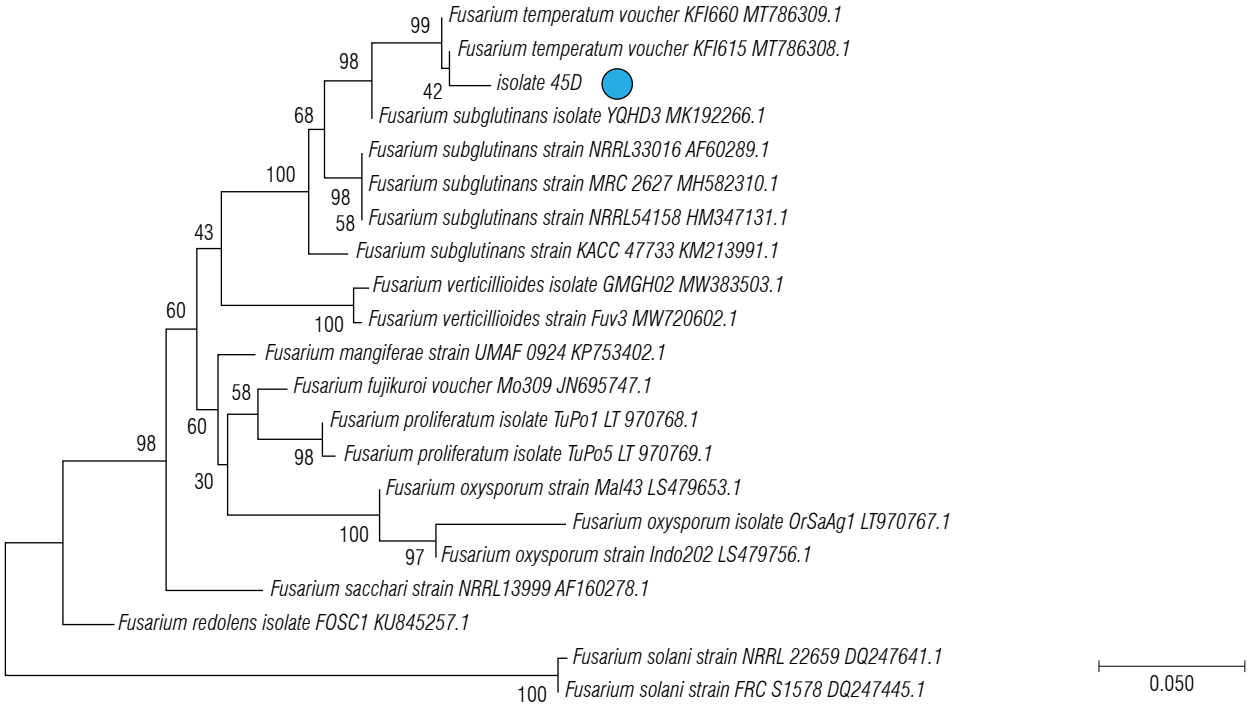


**FIGURE 5.** Phylogenetic tree of fungal isolates 45D obtained from corn plants with stalk rot symptoms and obtained by the Elongation factor 1-α (EF1). The evolutionary history was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances (0.05) used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter=3).

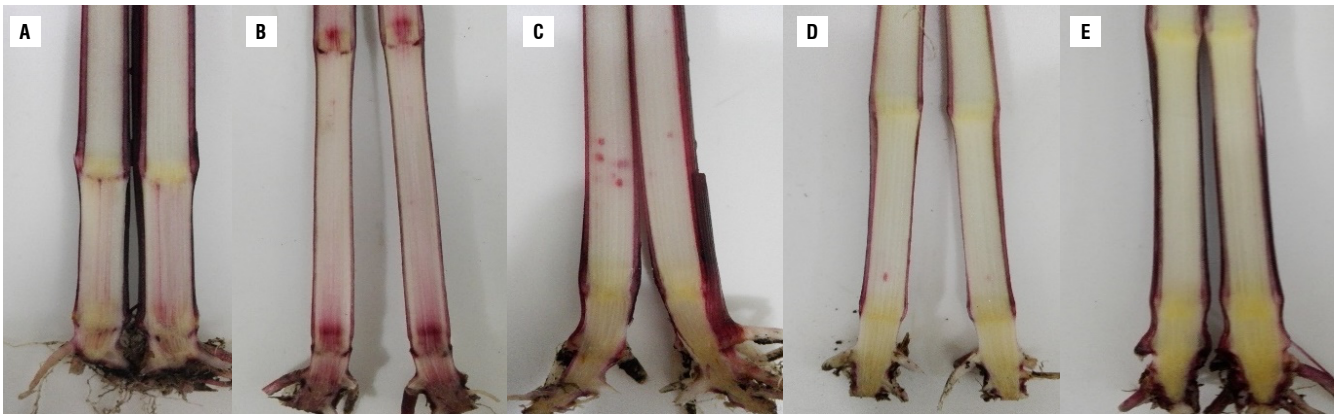


**FIGURE 6.** Pathogenicity tests of *Fusarium* spp. in corn variety Simijaca three months after sowing (V9) under greenhouse conditions. A) Longitudinal sections of plants inoculated with the mixture of *F. subglutinans* and *F. graminearum*, B) *F. graminearum*, C) *F. subglutinans*, D) absolute control (seeds without treatment), and E) thermal control (seeds treated with hot water at 52°C).

plant nodes (Fig. 6B), whereas *F. subglutinans* was located in the internodes (Fig. 6C). Necrosis of nodes and internodes occurred with the inoculation of the combination of both *Fusarium* species (Fig. 6A). The thermal control did not show symptoms (Fig. 6E), and absolute control showed a low degree of affectation (Fig. 6D). From their respective treatment, *F. subglutinans* and *F. graminearum* were isolated on PDA medium and morphologically identified fulfilling Koch postulates. *Fusarium* was not isolated from the thermal control.

Regarding plant height, the individual inoculation of *F. subglutinans* and *F. graminearum* had a significant effect generating the tallest plants (98 cm) ($P<0.0001$) at 90 DAS, whereas the joint inoculation of *Fusarium* species and the absolute control (seeds without thermal treatment) had the shortest plants (90 cm), and intermediate values were obtained on the thermal control ($P<0.0001$). Although stem diameter data were not adjusted to normality, the joint inoculation of *F. subglutinans* and *F. graminearum* suggests a higher stem diameter compared to the other evaluated treatments.

## Discussion

Pathogenicity tests carried out in corn seeds of the regional variety Simijaca developed symptoms similar to those initially observed in the field after inoculation of isolates 26B of *F. graminearum* and 45D of *F. subglutinans*. *Fusarium graminearum* appears to cause necrosis mainly in the nodes and crown of the plant, whereas *F. subglutinans* caused necrosis in the pith and crown. In this study, the joint inoculation of both *Fusarium* species caused symptoms in the crown, nodes and internodes. Therefore, the obtained results may be considered as the first report of *F. graminearum* in the FGSC and *F. subglutinans* within the FFSC causing corn stalk rot in corn crops in Cundinamarca (Colombia), specifically in the municipality of Simijaca. Internal symptoms observed in the pathogenicity tests conducted with thermal treated seeds showed the positive effect of the treatment on the reduction of symptoms. The effect of the treatment was clear in plants of thermal control (seeds treated with hot water at 52°C) that showed the normal cream-white color of the inner stem. In contrast, an internal purple color of the pith of plants was observed in plants of the absolute control (seeds without treatment). This is according to the report for contaminated seeds, as natural source of *Fusarium* spp. inoculum (Duncan & Howard, 2010). These observations are also in contrast to the more severe symptoms observed in the field study, that used untreated corn seeds. Similar positive results have been reported for the thermal treatment of corn seeds and other cereals (Clear *et al*., 2002; Coutinho *et al*., 2007; Bennett & Colyer, 2010; Piñeros-Guerrero *et al*., 2019). The lack of continuity of the necrotic area in nodes and internodes can be explained by the morphology and development of corn plants, which is used by *Fusarium* spp. for its plant colonization and dissemination. The nodes formed by the apical meristem are initially contained in the crown of the plant (Nielsen, 2008). Once the stem elongation begins, these nodes may cause a systemic infection of the plant if infected by the pathogen, as observed in this study. This is consistent with the histological observations conducted by Lawrence *et al*. (1981), who find that the fungus initially confined to the basal parts of the stalk had a rapid spread along the plant at the time of flowering.

The symptoms and signs found in this study were similar to those reported for stalk rot caused by *Fusarium* spp. in corn (CIMMYT, 2004). Additionally, superficial perithecia of *F. graminearum* were found in crop debris, mainly in stalk nodes. Unlike *F. graminearum* which is a homothallic fungus (Leslie & Summerell, 2006), *F. subglutinans* and *F. verticillioides* are heterothallic. This characteristic explains why perithecia of *F. verticillioides* are rarely observed in nature, although they are easily inducted under laboratory conditions. The same may occur with *F. subglutinans*. Blacutt *et al*. (2018) stated that in contrast to *F. graminearum*, where ascospores are the primary inoculum source, sexual reproduction in *F. verticillioides* and other species within FFSC contributes to their genetic diversity without being essential for their life cycle.

The *Fusarium* species associated with Simijaca and Sogamoso corn plants were morphologically and phylogenetically identified as *F. graminearum* in the FGSC and *F. subglutinans* within the FFSC that are reported worldwide as causal agents of stalk rot in corn (CIMMYT, 2004; Leslie & Summerell, 2006). The lineages defined as FGSC are morphologically indistinguishable (Yli-Mattila *et al*., 2009; Summerell, 2019). In this study, isolate 26B, morphologically and biologically identified as *F. graminearum,* was phylogenetically grouped with *F. culmorum*, *F. cerealis* and two species belonging to the FGSC (*F. graminearum s.s.* and *F. ussurianum*) within the FSAMSC. The *Fusarium* species described in this study, probably, belong to the homothallic species *F. graminearum* and not to *F. cerealis* or *F. culmorum*. This is supported by the fact that superficial perithecia were found on the nodes of the stalk of corn plants, and the sexual stage of *F. cerealis* and *F. culmorum* is unknown (Leslie & Summerell, 2006).

*Fusarium boothii* (in the FGSC) with the 15-acetyldeoxynivalenol (15 ADON) chemotype and *Fusarium meridionale* (in the FGSC) with the nivalenol (NIV) chemotypes are the lineages/species/chemotypes endemic to South America. However, *F. asiaticum* (in the FGSC) with its 3-acetyldeoxynivalenol (3-ADON) and NIV chemotypes has been introduced in the region. In general, *F. graminearum s.s.* 15 ADON is the most common species in Brazil and Argentina with some displacement by more aggressive 3 ADON populations (Van der Lee *et al*., 2018). Determining the species/lineages and chemotypes to which isolate 26B belongs may contribute to the knowledge of species/lineage distribution in the cold tropics of Colombia in South America. Additionally, these results may help to predict toxicological risks and aggressiveness according to the species/lineages and chemotypes present.

Isolate 45D, identified in this study as *F. subglutinans,* may represent two cryptic species distinguishable by amplified fragment length polymorphism (AFLP): *F. subglutinans s.s*. and *F. temperatum* in the FFSC (Czembor *et al*., 2015; Fumero *et al*., 2016). Determining the species to which this isolate belongs is important to predict its distribution and toxicological profile since *F. temperatum* apparently produces fumonisins (Wang *et al*., 2014), beauvericins and fusaproliferin. *Fusarium subglutinans* does not produce fumonisins, but it does produce other types of mycotoxins such as moniloformines and fusaproliferin (Fumero *et al*., 2016). *F. subglutinans* is frequent in cold areas of Peru, Mexico, and Argentina (Logrieco *et al*., 1993; Figueroa-Rivera *et al*., 2010; Reyes-Velázquez *et al*., 2011; Fumero *et al*., 2016) and *F. temperatum* has been found in Argentina and Southern Brazil (Fumero *et al*., 2016). Future studies should be carried out to document the species occurrence within the FFSC in the Colombian cold tropics of South America, where *F. verticillioides* is the most common species in warm parts of the continent (Chulze *et al*., 1996).

In this research, we found *F. subglutinans* in the FFSC and *F. graminearum* within the FGSC associated with the corn stalk rot disease on the regional varieties Simijaca and Sogamoso, which are adapted to the cold and high altitude production zones in Colombia. This result matches the effect of latitude and altitude on the distribution of *Fusarium* species reported by Munkvold *et al.* (2018) in corn. These authors observe that *F. verticillioides* prevails in warm and dry tropical and subtropical areas, whereas *F. graminearum* and *F. subglutinans* are the dominant species in cold-temperate regions as altitude increases, with reports in Europe, Asia, Oceania, and North and South America. Although *Fusarium* stalk rot is among the most economically important diseases of corn around the world, it had not been reported in the country for at least 30 years. Therefore, corn stalk rot may be considered an emergent disease in Colombia according to our findings and the epidemics occurring in corn plots in Simijaca at the Ubaté valley in the last years. The climatic conditions observed during the period of the study (2016 and 2017) were colder, with average temperatures of 13°C (below the historical average of 14°C). Additionally, dry conditions framed on a tropical El Niño episode with accumulated precipitation of 429 mm (125 mm lower than the historical) were also registered for this period of time in the Ubaté valley. Therefore, cold stress and drought conditions could have contributed to a shift in the biotrophic and symptomless association between the Simijaca corn variety and the *Fusarium* spp. migrating toward a disease-causing condition (stalk rot) (Dodd, 1980; Schulz *et al*., 1999; Kuldau & Yates, 2000; Bacon *et al*., 2008; Blandino *et al*., 2009). Because of their importance, the findings of this study need to be expanded. Therefore, further studies should be conducted with a higher number of isolates and the potential of toxin production of the species present in the field should be evaluated. Further research regarding *Fusarium* species diversity in corn along the Andean region in the Colombian cold tropic and a more robust phylogenetic analysis must be conducted to determine the species/lineages present in the country. Evaluating the toxicological profile and aggressiveness of these species/lineages may also contribute to an understanding of FSR in corn crops under cold climate conditions.

## Conclusions

*Fusarium graminearum* within FGSC and *Fusarium subglutinans* within the FFSC were found associated with corn stalk rot in the Ubaté valley throughout the entire crop cycle, and its pathogenicity was confirmed in the corn variety Simijaca. The initial infection of the pathogen was contained in the crown, but it spreads towards the upper part of the plant through the nodes previously colonized at initial stages of plant development, causing a systemic infection. Necrosis of the crown, nodes and internodes and the pith showing purple colors could be observed in longitudinal stalk sections. These symptoms caused basal disintegration and lodging, as the final manifestation of the corn stalk rot that may occur at any phenological stage of the crop. Although *Fusarium* stalk rot is well reported as an economically important disease in corn, it could be considered an emergent disease under conditions of the Ubaté valley in Colombia.

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### Conflict of interest statement

The authors declare that there is no conflict of interests for the publication of this article.

### Author's contributions

GMA conducted the research and investigation process and wrote the original draft. GMA, GLM, and SGC formulated the overarching research goals and aims, wrote, reviewed and edited the manuscript. SGC verified the overall replication/reproducibility of results. GLM and SGC obtained the financial support for the project leading to this publication. All authors reviewed the manuscript.

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