Influence of propagules and inoculation method on the development of potato early dying caused by *Verticillium* spp.

Influencia de los propágulos y el método de inoculación en el desarrollo de la marchitez temprana de la papa causada por *Verticillium* spp.

Karolin Dariana Suárez-Arengas[1], Kelly Johana Sandoval-Silva[1], and Sandra Gómez-Caro[1]*

ABSTRACT

Potato early dying is a significant disease in potato-producing countries. However, the development of the disease under different pathogenic conditions and at different times of inoculation is unclear. In this study, the infection of potato plants var. Diacol Capiro by two types of *Verticillium* spp. propagules, conidia, and microsclerotia (MS), was assessed. Conidia were evaluated based on inoculation of the soil at the sowing time of the tuber, or in soil drench and root immersion 49 days after sowing (das). Microsclerotia were evaluated for their incorporation into soil at different densities at the time of sowing. The tests were carried out under a complete factorial design in a completely randomized arrangement with five repetitions per treatment; the unit experiment was a plant. Non-inoculated plants grown in sterile soil were used as controls in both cases. Variables measured included disease severity, area under the disease progress curve (AUDPC), incubation period, phenological development of the plants, and yield components. Disease incubation periods ranging from 65 to 70 d were recorded when *Verticillium* spp. was inoculated at the time of sowing. With inoculation at 49 das, the incubation period was reduced to 23 days after inoculation (dai), with lower disease severity than at the time of sowing. The results showed that all inoculation methods, times, and strains of *Verticillium* spp. caused the typical symptoms of the disease and led to the development of early death. Infection of potato plants was possible with the inoculation of conidia or MS of the pathogen; however, in the latter case, it depended on the inoculum density. With different inoculation methods, the tuber weight increased, but the number of tubers per plant decreased.

Keywords: conidia, microsclerotia, potato diseases, soil-borne pathogens, vascular wilt.

RESUMEN

La marchitez temprana es una enfermedad importante en los países productores de papa. Sin embargo, el desarrollo de la enfermedad bajo diferentes propágulos del patógeno y momentos de la inoculación no es claro. En este estudio, se evaluó la infección de plantas de papa variedad Diacol Capiro con dos tipos de propágulos de *Verticillium* spp., conidias y microesclerocios (MS). Las conidias se evaluaron en inoculación al suelo al momento de la siembra de los tubérculos, o en drench e inmersión de raíces a los 49 días después de la siembra (dds). La infección por MS se evaluó mediante incorporación al suelo en diferentes densidades al momento de la siembra. Los experimentos se realizaron bajo un diseño factorial completamente al azar con cinco repeticiones por tratamiento; la unidad experimental fue una planta. En ambos casos como controles se utilizaron plantas no inoculadas sembradas en suelo estéril. Como variables se evaluaron la severidad de la enfermedad, el área bajo la curva de progreso de la enfermedad (AUDPC), el período de incubación, el desarrollo fenológico de las plantas y componentes de rendimiento. Los períodos de incubación de la enfermedad variaron entre 65 y 70 d cuando *Verticillium* spp. se inoculó al momento de la siembra. Con la inoculación a los 49 dds, el periodo de incubación se redujo a 23 días después de la inoculación (ddi) con menor severidad con respecto a la inoculación al momento de la siembra. Los resultados mostraron que todos los métodos, momentos de inoculación y aislamientos de *Verticillium* spp. causaron síntomas típicos y llevaron al desarrollo de la marchitez temprana. La infección de las plantas de papa fue posible con la inoculación de conidias o MS, siendo estos últimos dependientes de la densidad de inóculo. Con la inoculación por los diferentes métodos, la enfermedad redujo el peso de los tubérculos, pero no afectó el número de tubérculos por planta.

Palabras clave: conidios, microesclerocios, enfermedades de papa, patógenos del suelo, marchitamiento vascular.

Received for publication: April 5, 2025. Accepted for publication: July 10, 2025.

Doi: 10.15446/agron.colomb.v43n2.119693

[1] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Departamento de Agronomía, Bogotá (Colombia).

* Corresponding author. sgomezc@unal.edu.co

Introduction

The potato is the fourth most important crop in the world. It has a tremendous economic impact due to its nutritional contribution of carbohydrates, minerals, and vitamins (Lima *et al*., 2018). However, its performance is affected by various factors, including genotype, environmental conditions, agronomic practices, and limiting pests and diseases (Jakubowski *et al.,* 2024). One of the diseases affecting this crop is early dying caused by *Verticillium* spp., which may reduce plant performance by 50% or more (Nieto, 1988). For this reason, it has attracted interest in potato-producing areas worldwide. Early dying of potatoes has become one of the most economically significant diseases affecting the crop, not only because of its negative impact on yield and tuber quality but also due to the accumulation of inoculum in the soil, which can render infested fields unsuitable for future potato production (Li *et al*., 2019; Simko & Haynes, 2017). Among the pathogenic *Verticillium* species in cultivated plants, the most noteworthy include *V. albo-atrum* Reinke & Berthold, *V. dahliae* Kleb (Daami-Remadi *et al*., 2011), and *V. tricorpus* Isaac, all of which infect stems, vascular tissue, and tubers (Nair *et al*., 2019). Of these, *V. albo-atrum* and *V. dahliae* are widely distributed and are the most common species (Klosterman *et al*., 2009; Nair *et al*., 2019; Powelson & Rowe, 1993). In Colombia, *V. albo-atrum* and *V. dahliae* have been reported to be associated with premature potato maturity in Antioquia, Boyacá, Nariño, Norte de Santander, and Cundinamarca (Gómez-Caro & Mendoza-Vargas, 2020; Nieto, 1988).

Verticillium dahliae has a wide host range, including more than 200 plant species. It can survive for long periods in the soil, even in the presence of plants, by forming resistance structures called microsclerotia (MS) (Johnson & Dung, 2010). Microesclerotia can be spread by crop residues, transportation of contaminated seed, air currents, irrigation, and other agricultural activities (Zhang *et al*., 2023) and can survive for up to 10 years in soil (Steere & Kirk, 2015). Meanwhile, *V. albo-atrum* has a more limited host range, encompassing 30-40 plant species. It survives for shorter periods through melanized hyphae that can remain viable for 3 to 5 years in soil (Gómez-Caro & Mendoza-Vargas, 2020; Johnson & Dung, 2010). Finally, *V. tricorpus* produces resistance structures, including chlamydospores, resting dark mycelium, and large, irregularly shaped MS. However, it is a less aggressive species than *V. dahliae* and *V. albo-atrum* (Nair *et al*., 2019).

Other species of the pathogen that until now have been of lesser importance but have attracted increasing interest are *V. nonalfalfae* and *V. alfalfae*. These refer to pathotypes of *V. albo-atrum sensu lato*, named based on a taxonomic revision of the genus (Inderbitzin *et al*., 2011; Inderbitzin & Subbarao, 2014). *Verticillium nonalfalfae* is morphologically indistinguishable from *V. alfalfae* but differs in host range and DNA characters (EPPO, 2020). The presence of *V. nonalfalfae* has been reported in Canada, Cuba, Germany, Japan, Slovenia, and the United Kingdom, and it infects several hosts, including hops, petunia, spinach, and potato (Inderbitzin *et al*., 2011). On the other hand, Li and Li (2021) report *V. alfalfae* causing disease on seven non-alfalfa plant species including bluish dogbane, common vetch, cotton, erect milkvetch, potato, sainfoin, and sunflower; it is reported in Canada, France, Germany, Iran, Japan, New Zealand, Russia, Sweden, and the USA (Pegg & Brady, 2002).

The MS or melanized hyphae formed by *V. dahliae* and *V. albo-atrum,* respectively, can colonize roots in response to plant exudates, which they use as signals to recognize their hosts and initiate infection (Klosterman *et al*., 2009). The fungus penetrates the plant's roots directly or through wounds (Gómez-Caro & Mendoza-Vargas, 2020). Once the fungus enters the root, it colonizes the bark, and the mycelium can enter the xylem vessels. Once there, the fungus travels to the upper third of the plant via the vascular system as conidia. As the fungus colonizes the vascular system, symptoms such as chlorosis, necrosis, and leaf wilting begin to appear (Steere & Kirk, 2015). Early potato dying caused by the pathogen can lead to premature plant senescence and tuber production (Rowe & Powelson, 2002).

To date, research on this disease caused by *Verticillium* spp. has mainly focused on countries such as Australia, Canada, China, and the USA. These studies have helped to understand infection by this pathogen in various economically important plant species, such as alfalfa, cotton, eggplant, lettuce, maple, okra, olive, potato, rapeseed, sunflower, spinach, strawberry, tomato, and watermelon (Rowe & Powelson, 2002; Wu *et al*., 2022), and recently on avocado in Colombia (Ramírez-Gil & Peterson, 2019). Some studies on *Verticillium* spp. have been conducted under field conditions, using *in situ* inoculum in the plot's soil as the source of the pathogen. These include studies by Platt and Sanderson (1987), Uppal *et al*. (2008), Trapero, Serrano *et al*. (2013), and Mulero-Aparicio *et al*. (2020), who used different types of *Verticillium* propagules and inoculum densities varying between $4x10^6$ and $1x10^7$ conidia ml^{-1}, 5 and 21 MS g^{-1} soil, and 35 UCF g^{-1}, respectively. In these studies, the inoculum densities varied, and Mulero-Aparicio *et al*. (2020), for example, do not specify which *Verticillium*

structure they are referring to. Although these studies have contributed significantly to knowledge of *Verticillium* disease in various crops, results may vary due to inoculum density, the type of propagule used, and abiotic and biotic factors that affect assay development under field conditions.

In the specific case of potatoes, research has been carried out using artificial inoculation methods in varieties 'Russet' and 'Kennebec' type in the USA and Canada (Bae *et al*., 2007; Platt & Sanderson, 1987), 'Shepody' in China (Zhang *et al*., 2023), and 'Victoria' and 'Tasmania' in Australia (Nair *et al*., 2019). In Colombia, Nieto (1988), Guerrero *et al*. (1991), and Benavides *et al*. (1995) have studied the disease in varieties such as ICA Nariño, ICA Tequendama, and Parda Pastusa.

In studies using artificial inoculation, a concept known as pathogenicity testing is used, which is based on Koch's postulates. Despite their secular history, the postulates have stood the test of time because of their philosophical essence and conceptual stability (Volcy, 2008). The importance of pathogenicity tests in the study of *Verticillium* spp. is evident in the research carried out by Bae *et al*. (2007). Daami-Remadi *et al*. (2011) used concentrations of 1×10^7 and 8×10^6 conidia ml^{-1}, respectively, and successfully reproduced the disease in potato varieties.

Meanwhile, in Colombia, some work has been carried out with inoculations of the pathogen at the time of plant emergence using concentrations of $5x10^5$ and $5x10^7$ conidia ml^{-1} (Guerrero *et al*., 1991), and with 5 g of wheat previously inoculated with *Verticillium* (Benavides *et al*., 1995). Regarding the use of MS of the pathogen as inoculum, Land *et al*. (2017) conducted a study in commercial cotton lots, using 250 ml of a suspension containing $1x10^7$ MS ml^{-1} that was incorporated into the substrate within the first 12 cm pots. In all these trials, disease symptoms were observed, and the pathogen was confirmed.

As described, various studies have separately documented the use of pathogenicity tests with different inoculation methods and *Verticillium* spp. inoculum densities. Among the most frequently used methods are the application of a suspension of the pathogen to the soil (drench) (Leon-Ttacca *et al*., 2018), immersion of roots in the suspension of the pathogen (Serrano *et al*., 2023; Trapero, Díez *et al*., 2013), incorporation of propagules into substrate (wheat) (Benavides *et al*., 1995) or into the soil (Platt & Sanderson, 1987), and inoculation by puncture or wound (Leon-Ttacca *et al*., 2018; Zhang *et al*., 2023). Although the disease has been reproduced using different methods and inoculum densities, no studies have shown how the pathogenesis process varies across different infective structures, such as conidia, microsclerotia, or melanized mycelium formed by the different *Verticillium* species reported in potato.

Since no comparative studies on the timing of pathogen inoculation and its effects on potato early-dying parameters exist, the possible outcomes of disease development under variations in inoculation time, propagule type, and inoculation method remain unresolved. This information would not only provide insight into the development of potato early dying under different inoculation methods, but also into the mechanisms underlying it. But it would also facilitate the interpretation and discussion of the results obtained. Additionally, it is necessary to clarify whether the disease can occur due to pathogen infection at more advanced stages of plant development, rather than only at the sowing time. Therefore, this work sought to evaluate different inoculation methods, using conidia and microsclerotia from *Verticillium* spp. obtained from infected plants in commercial fields in Cundinamarca (Colombia) on potato early dying development.

Materials and methods

The study was carried out in a greenhouse at the Facultad de Ciencias Agrarias of the Universidad Nacional de Colombia (UNAL), Bogotá campus, located at 4°38'17.3 N and 74°05'20.3" W, at an altitude of 2630 m a.s.l. The study took place over the second half of 2021 and the first half of 2022. The environmental conditions during the experiments were an average daily temperature of ± 23°C, 50% and 70% relative humidity, and a natural photoperiod of 12 h. The volume required for watering the plants was determined by daily quantification of their evapotranspiration requirements, as described by Hainaut *et al*. (2016).

Plant material and *Verticillium* strains

As plant material, certified potato seed tubers var. Diacol Capiro was used. The seed tubers were planted in 3 kg plastic pots containing soil from non-agricultural areas previously sterilized through autoclaving for 2 cycles of 30 min at 121°C and 21 psi, followed by 20 min of drying. Soil fertilization was carried out at the time of sowing by applying the commercial fertilizer Sir 24 (Precisagro SAS, Colombia) containing $12N\text{-}24P_2O_5\text{-}12K_2O+10CaO+1.5S$ at a dose of 40 g per plant.

In the study, two monosporic strains of *Verticillium* spp. obtained from infected potato plants in commercial fields in Cundinamarca (Colombia), and one reference strain of

V. albo-atrum from potato was used. The *V. albo-atrum* strain was provided by the microbiology laboratory of the Colombian Agricultural Research Corporation (Agrosavia). The other two strains were obtained from plants of the Diacol Capiro variety with symptoms of potato early dying collected in commercial plots in Funza (4°44'56.0" N, 74°12'04.0" W) (Funza 104 strain) and Mosquera (4°39'48.0" N, 74°14'42.0" W) (Mosquera 98 strain). These two strains (Funza 104 and Mosquera 98) were selected based on the high disease severity of the commercial crop plants from which they were isolated and their contrasting *in vitro* characteristics, including colony appearance, growth rate, and the propagules formed by the pathogen. The strain Funza 104 was characterized by the formation of conidia and melanized mycelium, and the strain Mosquera 98 by the profuse production of microsclerotia (MS); *V. albo-atrum* (Agrosavia) was characterized by the production of conidia only. Previously, the strains Funza 104 and Mosquera 98 were morphologically and molecularly identified by Mendoza-Vargas *et al.* (2025) as belonging to the genus *Verticillium,* with greater phylogenetic proximity to the species *V. nonalfalfae* and *V. alfalfae* according to phylogenetic analysis, which have not been previously reported in Colombia.

Multiplication of *Verticillium* strains

The inoculum of the three *Verticillium* strains [Funza 104, Mosquera 98, and *V. albo-atrum* (Agrosavia)] for the tests was produced on sterile rice, following the protocol proposed by Guerrero *et al.* (1992), and adjusted by the laboratory. For this purpose, flasks containing 30 g of rice and 20 ml of sterile distilled water (SDW) were autoclaved for 20 min in two cycles at 121°C, 21 psi, followed by 20 min of drying. The flasks, with rice as substrate, were inoculated with 1 ml of a conidial suspension ($1x10^7$) of Funza 104 or Agrosavia strain, separately, and placed in an incubator (Incucell® V, Medcenter, Planegg, Germany) at 21°C for 11 d. To prepare each conidial suspension, a plug of Potato Dextrose Agar (PDA) medium (Oxoid®, Thermo Scientific, USA) with seven-day-old mycelial growth colonies of each strain was added to sterile tubes containing 12 ml of SDW, shaken in a Vortex® for 2 min, and filtered with sterile gauze. Conidia were counted in a Neubauer chamber (Neubauer, VWR, Darmstadt, Germany), and the suspensions of $1x10^7$ conidia ml^{-1} were adjusted.

For the essays involving microsclerotia, the strain Mosquera 98 was multiplied using the same methodology described above for conidial production. Still, approximately 15 days were required to obtain microsclerotia (MS). After this period, the MS were separated following the methodology proposed by Hawke and Lazarovits (1994), which consisted of preparing sand (50 g) with 37% HCl on watch glasses and allowing this to act for 24 h. Subsequently, this mixture was thoroughly washed with abundant SDW to neutralize its acidity. The MS were recovered by sieving (100, 50, and 25 µm), collected on filter paper, and stored in sealed Falcon® tubes until inoculation.

Pathogenicity tests

Different methods and times of inoculation of the pathogen were evaluated. These varied according to the propagules formed by each *Verticillium* strain of interest. Conidia as inoculum were assessed from the Funza 104 and Agrosavia strains, and MS from Mosquera 98. Two moments of inoculation of conidia were evaluated, at the sowing time and 49 das. In this case, plants were inoculated at 49 das, since the time period between 45 and 55 das in potato coincides with an active root growth, beginning of tuberization, and the maximum vegetative growth stage, when the pathogen may strongly impair water and nutrient transport (Klosterman *et al*., 2009; Steere & Kirk, 2015).

For conidia inoculation (Funza 104 and Agro) the treatments were: (i) incorporation of propagules in the substrate (rice) into the soil at the time of sowing (IPS) and (ii) 49 das (IPS 49); (iii) incorporation of conidia into the soil at the time of sowing (CS); (iv) inoculation by immersion of roots in the suspension of conidia 49 das (150 ml $plant^{-1}$; 10 min) (IR); and (v) inoculation of the conidial suspension by soil drench 49 das (120 ml $plant^{-1}$) (DS). For these treatments, a concentration of $1x10^7$ conidia ml^{-1} was used (Ashraf *et al*., 2012; Mendoza-Vargas *et al.,* 2025). In the case of propagules into the substrate (rice), the amount of rice needed to achieve the desired conidia density for inoculation was previously determined (data not shown). In each inoculation method evaluated for conidia, five potato plants var. Diacol Capiro were inoculated, and five non-inoculated plants were used as controls. The assessed treatments are explained in Table 1.

For MS (Mosquera 98 strain), test plants were inoculated with five different concentrations: 2, 5, 10, 30, and 60 MS g^{-1} of soil. The suspension of MS was prepared by counting the required number of MS in a Neubauer chamber and suspending them in 600 ml of SDW. The inoculation was carried out at sowing by incorporating the MS suspension directly into the soil and thoroughly mixing to ensure uniformity. In each MS concentration evaluated, five potato plants var. Diacol Capiro was inoculated, and five non-inoculated (treated only with SDW) were used as controls. Previously, the viability of MS, measured as the germination rate, was assessed in PDA medium following the method of Coley-Smith and Javed (1970). The MS

TABLE 1. Inoculation methods, strain of *Verticillium* spp., and amount of conidia used as inoculum in potato plants var. Diacol Capiro.

Inoculation method	Strain of the pathogen	Amount of substrate or suspension per plant	Final concentration
Incorporation of propagules in the substrate (rice) into the soil at the time of sowing (IPS)	Funza	4.35 g of rice	$1x10^7$ conidia ml^{-1}
	Agrosavia	0.8 g of rice	
Incorporation of propagules in the substrate (rice) into the soil 49 das (IPS 49)	Funza	4.35 g of rice	
	Agrosavia	0.8 g of rice	
Incorporation of conidia into the soil at the time of sowing (CS)	Funza	120 ml	
	Agrosavia		
Inoculation by immersion of roots in the suspension of conidia 49 das (IR)	Funza	150 ml	
	Agrosavia		
Inoculation of the conidial suspension by soil drench 49 das (DS)	Funza	120 ml	
	Agrosavia		

viability was determined to be 70%, a value used to adjust the amount of MS per gram of soil for each inoculum density assessed.

Monitoring of disease development and plant phenology

Disease monitoring was carried out using the severity scale for *Verticillium* in potatoes proposed by Hunter *et al*. (1968). This is a five-level scale, where 0: no symptoms of the disease; 1: slight wilting and discoloration of leaves; 2: moderate wilting affecting less than half of the leaves on the plants; 3: severe wilting affecting more than half of the leaves on the plants; and 4: plant death due to wilting. The results were used to calculate the disease severity index (DSI) according to Equation 1 (Chávez-Arias *et al*., 2020):

$$DSI = \frac{\Sigma(nv)}{V} \quad (1)$$

where n is the level of infection according to the scale, v is the number of plants present in each level, and V is the total number of evaluated plants.

Evaluation was conducted twice a week for 17 weeks (120 d), by visually inspecting all plants in each pot across all treatments. Using the data obtained for each treatment, the incubation period (Simko & Haynes, 2017), disease incidence, and the area under the disease progress curve (AUDPC) were calculated according to Campbell and Madden (1990). The formula used for calculating the AUDPC is presented in Equation 2.

$$AUDPC = \sum_i^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i) \quad (2)$$

where n is the number of evaluations, y_i and y_{i+1} are the values of the severity scale that were obtained at every evaluation time, and $(t_{i+1} - t_i)$ is the time interval between evaluations.

To confirm the presence of the pathogen in the symptomatic plants at the end of the study, stem explants from each treatment were isolated on PDA media according to the protocol described by EPPO (2007). Pure culture isolations from each treatment were prepared and visually confirmed as *Verticillium* by microscopic observations (Olympus CX31) at 10X and 40X of 7-d cultures according to the morphology described by Barnett and Hunter (1998).

The phenological monitoring of the plants was carried out weekly using the BBCH scale (Meier, 2018)—the German acronym for Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie— selecting from the outset the most vigorous stem in each pot. With these data, the effect of the disease on the phenological development of the potato plants was determined. Once the plant cycle in each treatment was completed, the inoculated and control plants were harvested. The number of tubers per plant, tuber weight, and caliber were evaluated in accordance with Colombian technical standard 341 (ICONTEC, 2018). The experiments were conducted until harvest (120 das), and harvest time was carefully monitored to ensure consistency among treatments.

Experimental design

The test was carried out using a complete factorial design in a completely randomized arrangement with five replicates (plants) per treatment. Two trials were conducted based on the type of *Verticillium* propagules used as inoculum: conidia or MS. The first trial, which involved conidia, included two inoculation timings: (i) at sowing and (ii) at 49 d after sowing (das). The factor evaluated was the inoculation method, comprising five different methods, while the levels considered were the *Verticillium* strains (Funza 104 and Agrosavia). The second trial, focusing on MS, involved inoculating with varying MS densities. Here,

the factor evaluated was the inoculum density, with five different densities tested, and the level was represented by the single Mosquera 98 strain.

Data analysis

The free software RStudio version 4.4.1 (Core R Team 2024) was used to analyze the data. The Shapiro-Wilk (P>0.05) and Bartlett (P>0.05) tests were used to assess normality and homogeneity of variances. ANOVA analysis of variance (P<0.05) was performed, along with Tukey's multiple comparison tests (P<0.05). A multivariate analysis of variance (MANOVA) (P<0.05) was utilized to analyze the harvest data, facilitating the examination of correlations among response variables originating from the same experimental units. This approach enabled a simultaneous assessment of the effects of various factors. In addition, cluster analysis was performed to further explore treatment relationships.

Results

Characteristic early potato-dying symptoms were observed in plants inoculated with the Funza 104, Agrosavia, or Mosquera 98 strains, regardless of inoculation method, timing, or the propagule type used (MS or conidia). The symptoms resembled those typically reported for potato early dying, loss of turgor, unilateral chlorosis of the lower leaves extending from the edge to cover the entire leaf blade, subsequent necrosis starting from the borders of the leaflets that progress to the complete leaves advancing from the base of the stem towards the upper part of the plants, accompanied by wilting and subsequent necrosis of the stems (Ashraf *et al*., 2012; Gómez-Caro & Mendoza-Vargas, 2020; Trapero, Serrano *et al*., 2013). For all conidial inoculation methods and for most MS densities evaluated, the same pattern of symptom development was observed over time. The differences between the evaluated treatments were associated with the number of affected plants and the severity of the disease.

In the treatments inoculated with conidia (Funza 104 and Agrosavia), a 100% incidence of the disease was achieved, confirming that the evaluated inoculation methods successfully induced pathogen infection and reproduced the disease (Fig. 1). The inoculation with MS (Mosquera 98), a 20% incidence of the disease was obtained with the density of 5 and 30 MS g^{-1} of soil, and 40% incidence was obtained with the density of 10 and 60 MS g^{-1} of soil. For the density of 2 MS g^{-1} of soil, there was no development of symptoms associated with potato early dying caused by *Verticillium* (Fig. 2).

Development of the disease from the inoculation of *Verticillium* conidia

Regarding the incubation period (Fig. 3A), the results showed that all treatments inoculated at the time of sowing presented higher values [59-71 d after inoculation (dai)] than treatments inoculated at 49 das, where the symptoms appeared between 17-32 dai (Fig. 3B), this being the shortest incubation period observed. The therapy with conidia in the soil (CS) presented significant differences (P<0.05) compared to the treatment with inoculation of propagules in substrate (rice) (IPS), for both Funza 104 and Agrosavia. The incubation period for the treatment with incorporation of conidia into the soil (CS) with the Agrosavia strain was the longest (71 das) of all the treatments evaluated. On the other hand, plants inoculated at 49 das did not show significant differences in the incubation period (Fig. 3B). In the control treatment plants, disease symptoms did not develop during the study.

The AUDPC values in Figure 4 represent the amount of disease per treatment and *Verticillium* over 120 d (from the appearance of the first disease symptoms until the end of the evaluations). Significant differences (P<0.05) in AUDPC are observed between inoculation methods and the two inoculation timings for all treatments. The results show that the disease caused by the strains Funza 104 and Agrosavia in potato plants inoculated at sowing presents higher AUDPC values (110-160, respectively). Additionally, these plants showed shorter incubation periods and a faster increase in disease severity in plants inoculated at 49 das (65-110 d, respectively) (Fig. 6A-B). Inoculation of propagules in the substrate (rice) (IPS) at the time of sowing with the strain Funza 104 presented the highest AUDPC value (160.82). Additionally, this treatment showed significant differences (P<0.05) with respect to the treatments with incorporation of conidia into the soil (CS) with Funza 104 and Agrosavia strains at the time of sowing (Fig. 4A). In contrast, the treatment with inoculation of conidia in the substrate 49 das (IPS 49) of the Agrosavia strain presented the lowest AUDPC value (70.55) of all the treatments (Fig. 4B).

The results for treatments with inoculation of the pathogen's conidia into the substrate (rice) at 0 and 49 das showed significant differences (P<0.05) in AUDPC values (Fig. 4B), despite using the same inoculation method and differing only in the time of inoculation. In this case, potato early dying progressed more rapidly when the pathogen was inoculated at sowing. The results showed that plants

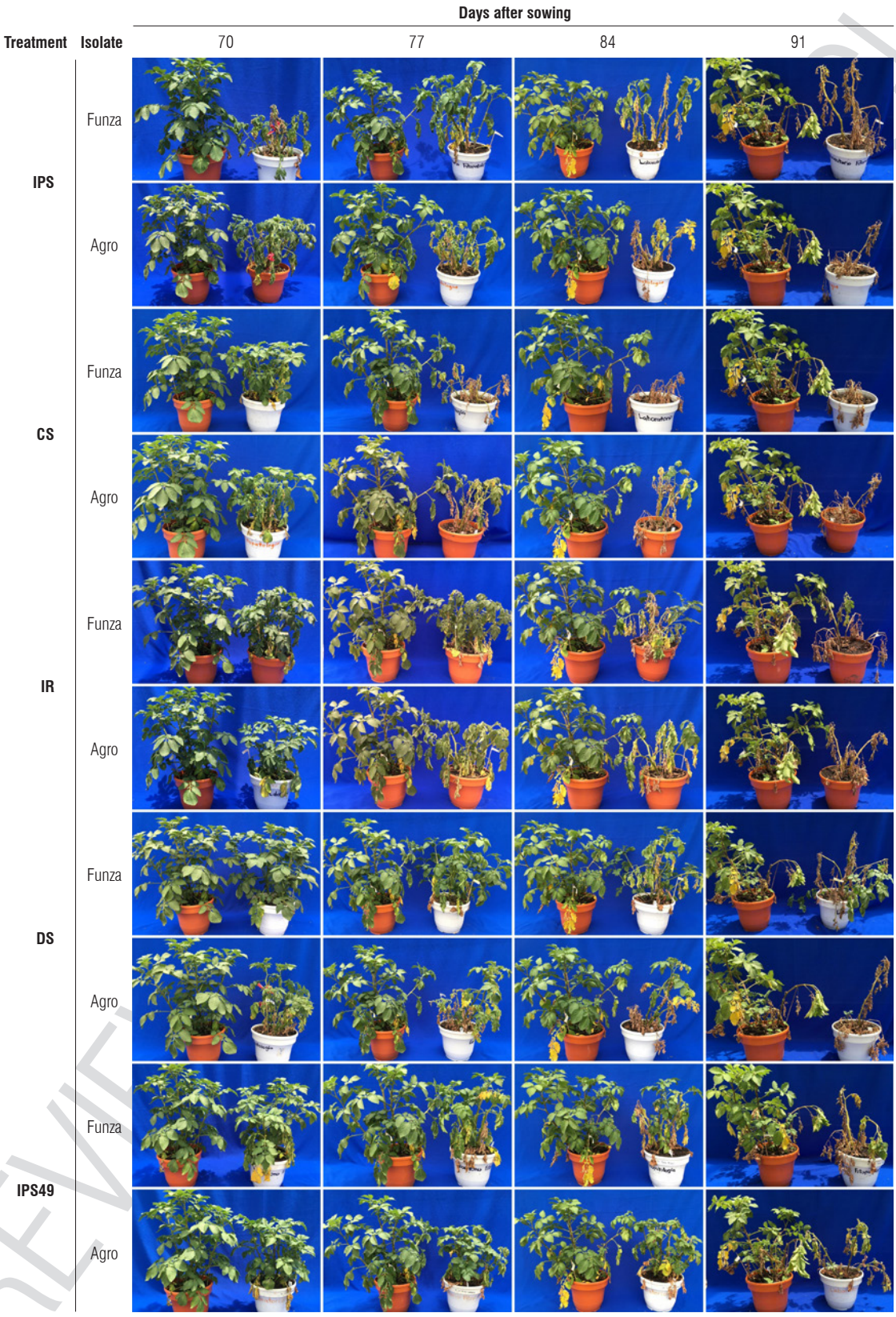

FIGURE 1. Development of potato early-dying caused by *Verticillium* in the plant var. Diacol Capiro under different inoculation methods at 70, 77, 84, and 91 d after sowing (das). IPS: incorporation of propagules in substrate (rice); CS: incorporation of conidia into the soil; IR: immersion of roots in a suspension of conidia; DS: applications of conidia in drench to the soil; IPS 49: incorporation of propagules in substrate (rice) at 49 das. Agro: Agrosavia strain; Funza: Funza 104 strain. The pots on the left of each image correspond to the control.

FIGURE 2. Development of potato early dying caused by *Verticillium* at 76, 84, 99, and 111 d after sowing in the plants var. Diacol Capiro inoculated at the sowing time with different densities of microsclerotia (2, 5, 10, 30, and 60 MS g^{-1} of soil) of the Mosquera 98 strain. The pots on the left of each image correspond to the control.

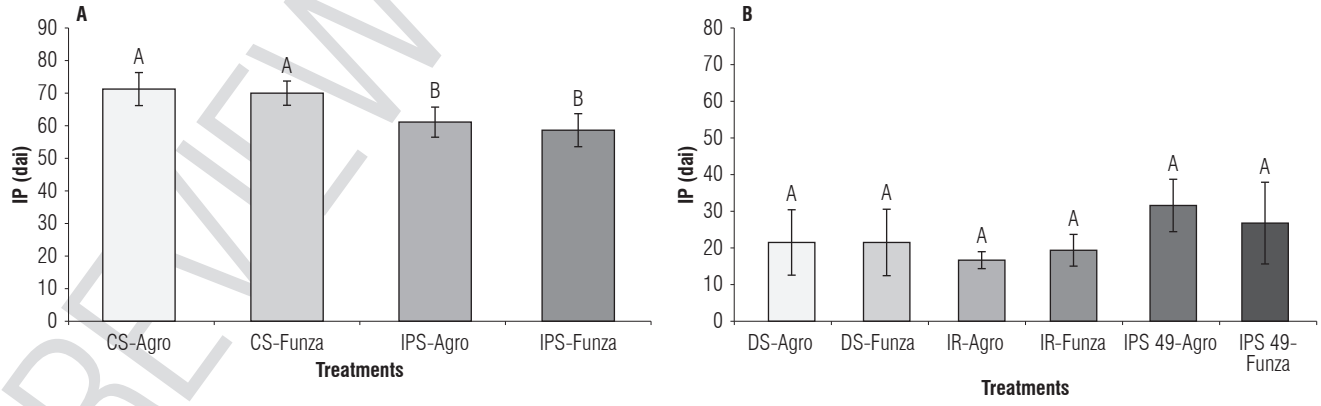

FIGURE 3. Incubation period (IP) of potato early-dying in plant var. Diacol Capiro after the inoculation of *Verticillium* conidia under different methods and strains of the pathogen. A) at the time of sowing and B) 49 d after sowing (das). Treatments at the time of sowing: CS: incorporation of conidia into the soil; IPS: incorporation of propagules in substrate (rice). Treatments 49 das: DS: applications of conidia in drench to the soil; IR: immersion of roots in a suspension of conidia; IPS 49: incorporation of propagules to substrate (rice) at 49 das. Agro: Agrosavia strain; Funza: Funza 104 strain. Bars represent the mean of five values ± SE (n=5). Different letters indicate significant differences according to the Tukey test ($P \leq 0.05$).

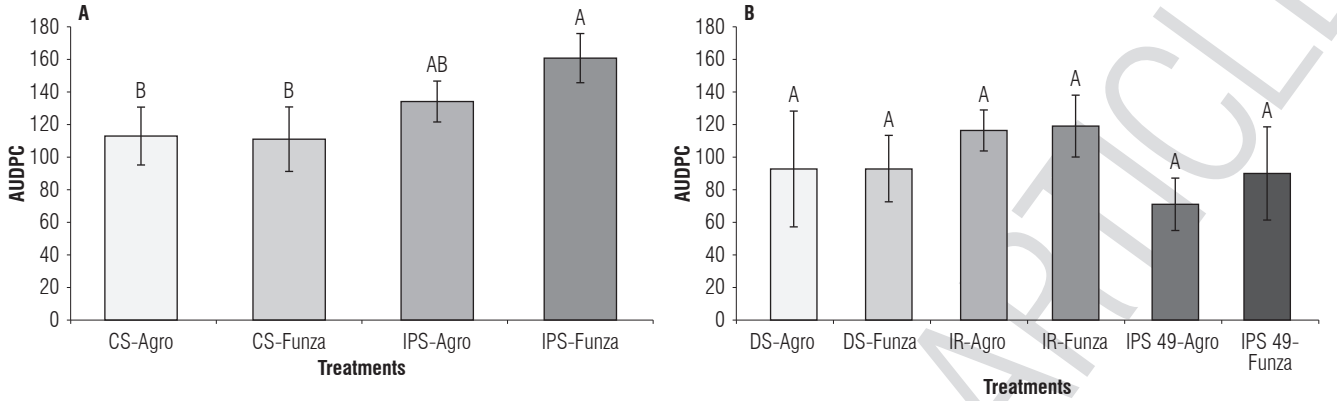

FIGURE 4. Area under the disease progress curve (AUDPC) of potato early dying in plants var. Diacol Capiro after the inoculation of *Verticillium* conidia under different methods and strains of the pathogen. A) at the time of sowing and B) 49 d after sowing (das). Treatments at the time of sowing: CS: incorporation of conidia into the soil; IPS: incorporation of propagules in substrate (rice); DS: applications of conidia in drench to the soil. Treatments 49 das: IR: immersion of roots in a suspension of conidia; IPS 49: incorporation of propagules in substrate (rice) at 49 das. Agro: Agrosavia strain; Funza: Funza 104 strain. Bars represent the mean of five values ± SE (n=5). Different letters indicate significant differences according to the Tukey´s test ($P \leq 0.05$).

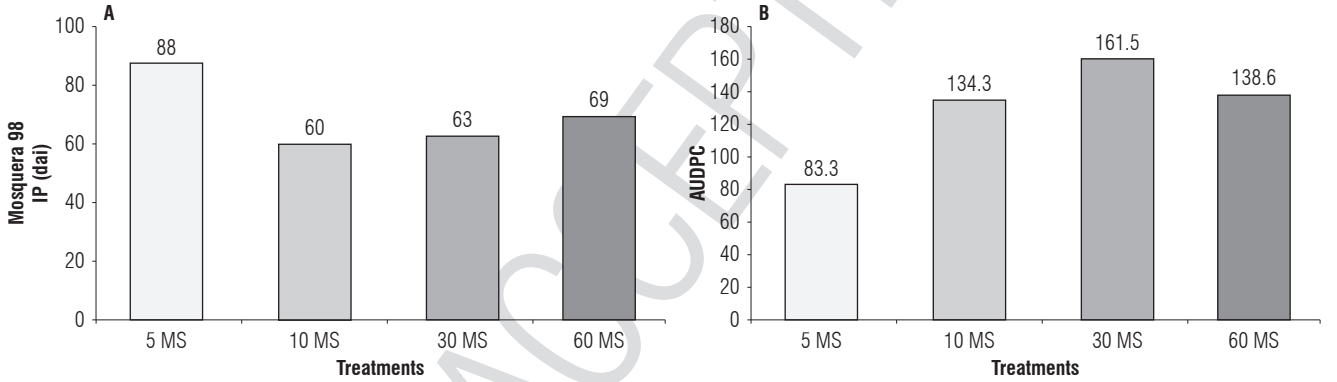

FIGURE 5. Development of potato early-dying in plant var. Diacol Capiro from inoculation of *Verticillium* microsclerotia (Mosquera 98). A) Incubation periods (IP), B) Area under the disease progress curve (AUDPC) for each density of *Verticillium* microsclerotia (MS). The data presented only includes information on diseased plants.

inoculated with *Verticillium* conidia by soil drench (DS) and incorporation of propagules into the substrate (rice) 49 das (IPS 49), with the Funza 104 and Agrosavia strains, generated the lowest severity values for potato early dying (Fig. 6A and 6B).

Development of the disease from inoculation of *Verticillium* microsclerotia

The IP of potato early-dying in plants inoculated with MS of Mosquera 98 was in the range of 60 to 88 das, the longest being the treatment with a density of 5 MS g^{-1} of soil (Fig. 5A). Regarding the AUDPC values, the highest was found with the density of 30 MS g^{-1} of soil. Due to the small number of plants affected by this inoculation method, it was not possible to perform a statistical analysis of the data obtained.

Development of the plants

Regarding the phenological development of potato plants, var. Diacol Capiro with the two types of propagules (conidia and MS), the first disease symptoms were observed after 45 das at phenological stage 4 (tuberization) and the disease severity increased 60-90 das at phenological stages 5 (tuber development) and 6 (flowering), regardless the inoculation method, timing of inoculation, strain or type of inoculum used (Fig. 6). After 90 das, the potato plants reached values over 2.5 and the maximum disease severity of 4.0 at 120 das with conidia as inoculum (Fig. 6A and B). In the case of MS, a similar tendency was observed, but with lower disease severity values (Fig. 6C). necrosis of leaves caused by *Verticillium* infection led to leaf fall and subsequently to the affected stems dying, altering the phenological development of the plants. Upon inoculation

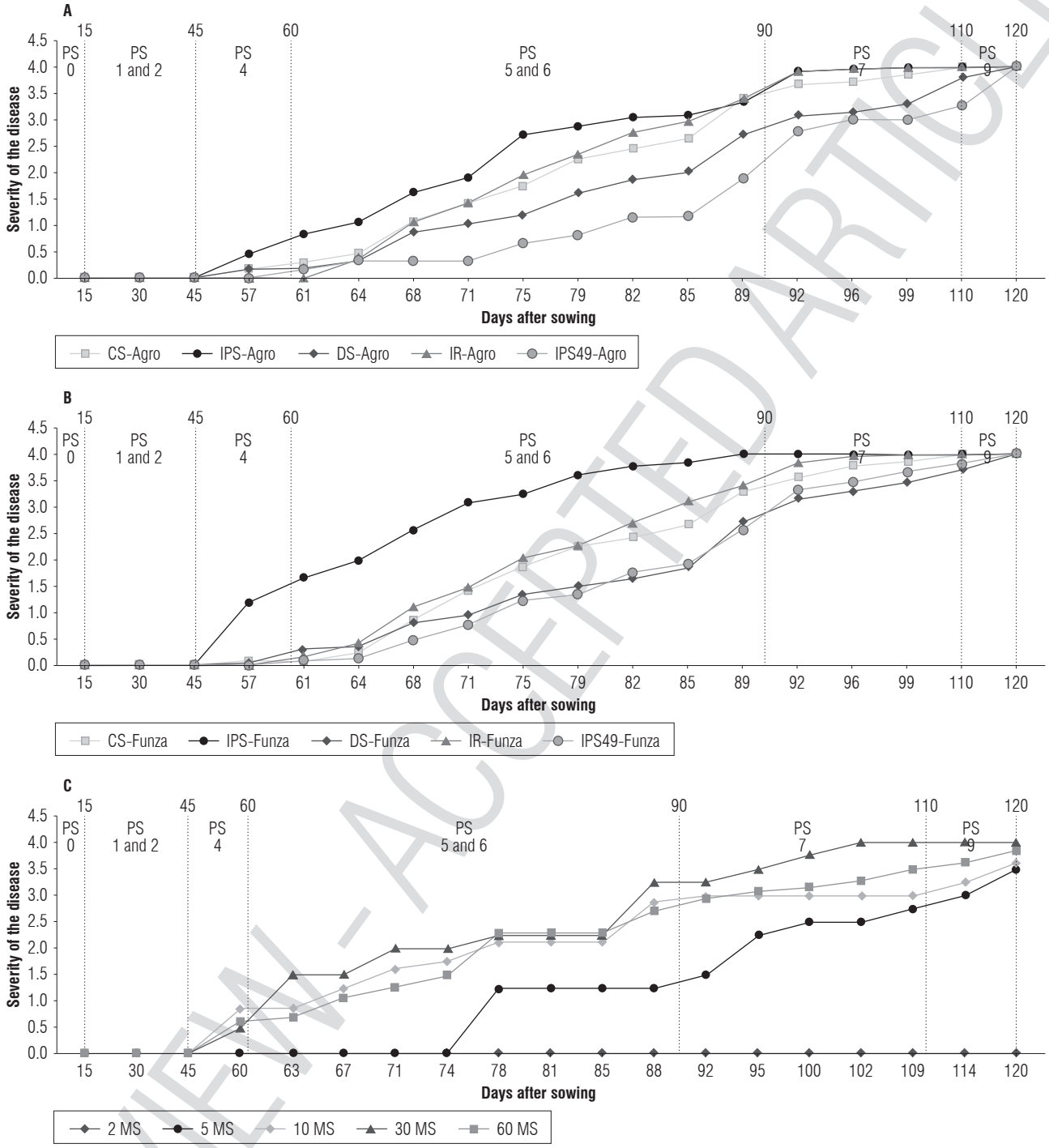

FIGURE 6. Severity of potato early-dying and phenological stages of plant var. Diacol Capiro was inoculated with three different sources of *Verticillium inoculum*. A) Inoculation of conidia Agrosavia strain (Agro) under different methods, B) Inoculation of conidia Funza 105 strain (Funza) under different methods, C) Inoculation of microsclerotia Mosquera 98 strain (Mosquera) under different densities of MS (2, 5, 10, 30, and 60 MS g^{-1} of soil). Treatments: CS: incorporation of conidia into the soil at the time of sowing (st); IPS: incorporation of propagules in substrate (rice) at st; DS: applications of conidia in drench to the soil 49 d after sowing (das); IR: immersion of roots in a suspension of conidia 49 das; IPS 49: incorporation of conidia in substrate (rice) at 49 das. Dashed vertical lines indicate the transition between main phenological stages according to the BBCH scale. Stage 0 (PS0): sprouting (0-15 das); stage 1 (PS1): leaf development (15-30 das), stage 2 (PS2): development of lateral stems (30-45 das), stage 4 (PS4): tuberization (45-60 das), stage 5 (PS5): tuber development (60-90 das), stage 6 (PS6): flowering (60-80), stage 7 (PS7): tuber filling/initial ripening (90-110 das) and stage 9 (PS9): senescence (110-120 das).

with conidia, the plants showed senescence at 60-67 das, and plant death was observed from 84 das. Premature senescence of potato plants inoculated with the pathogen was consistently observed across all inoculation methods and *Verticillium strains* evaluated. Plants inoculated with conidia reached phenological stage PS9 according to the BBC scale, as early as 67 das in most inoculation methods with the Agrosavia strain (Fig. 7) or the Funza 104 (Fig. 8).

These results show that the plants inoculated with Agrosavia and Funza 104 strains senesced 28-35 d earlier than the control plants (non-inoculated), which flowered (PS6)

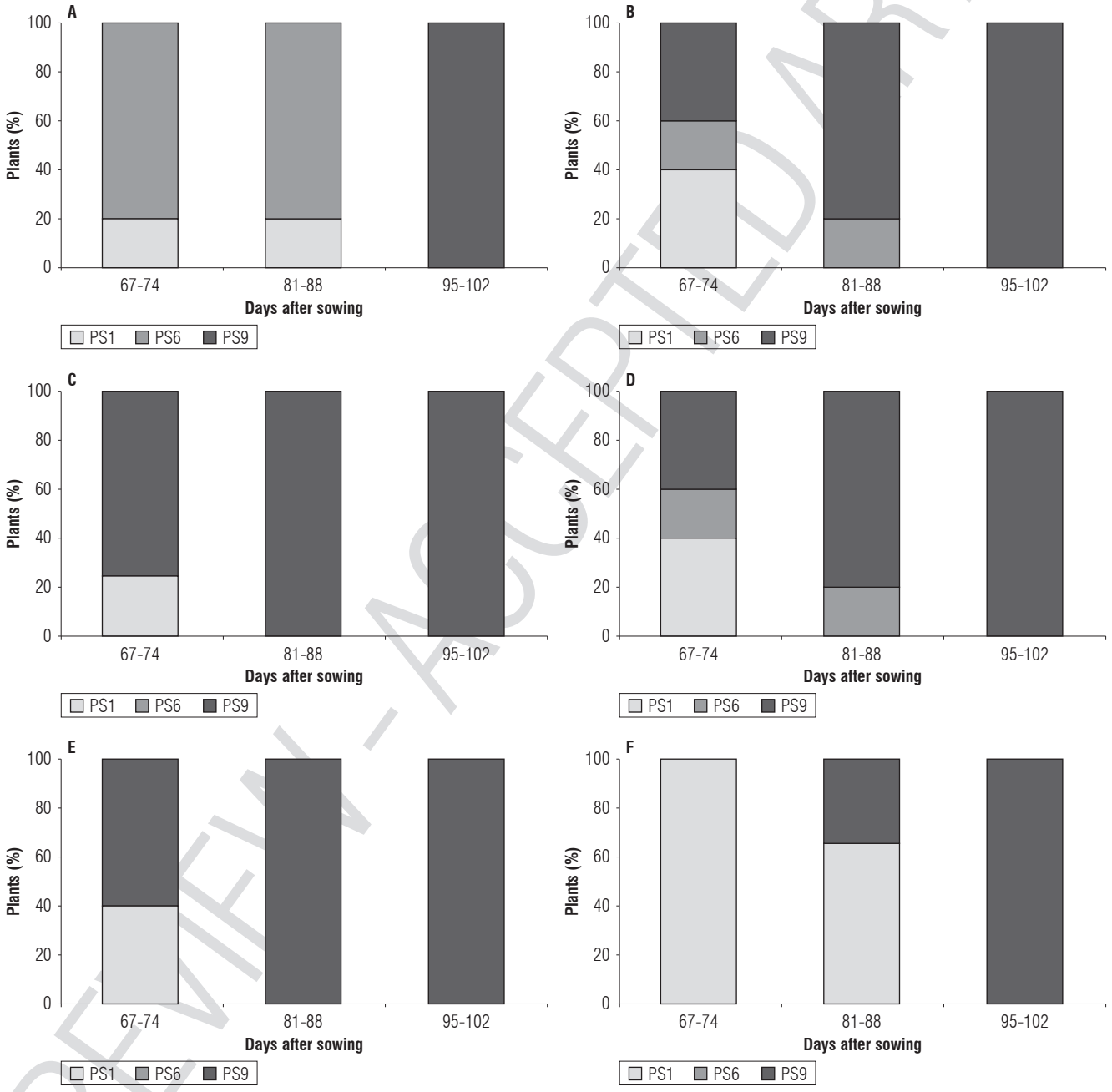

FIGURE 7. Developmental status of potato var. Diacol Capiro during the final phenological stages of plants inoculated with conidia of the strain Agrosavia (Agro). The figure illustrates the percentage of plants in each phenological stage (PS), for leaf development (PS1), flowering (PS6), and senescence (PS9) according to the BBCH scale. A) Control plants non-inoculated, B) Incorporation of propagules in substrate (rice), C) Incorporation of conidia into the soil, D) Immersion of roots in the suspension of conidia, E) Application of conidia by soil drench, F) Incorporation of propagules in substrate (rice) at 49 days after sowing (das). Note the early dying of plants under *Verticillium* inoculation (PS9) starting at 64-67 das, compared to control plants (A), which reached PS9 (senescence) at 95-102 das.

between 67 and 88 das and began to show senescence symptoms at 95 das (Figs. 7A and 8A). In the case of plants inoculated with MS (Mosquera 98 strain), the alteration of the phenological development of the plants was less remarkable; however, leaf fall associated with wilting was also recorded, which affected the phenology of the plants, causing them to enter the senescence stage around 20 d earlier than the control plants.

Isolation of *Verticillium* from plants

In the symptomatic potato plants, var. Diacol Capiro, inoculated with the two types of propagules of the pathogen

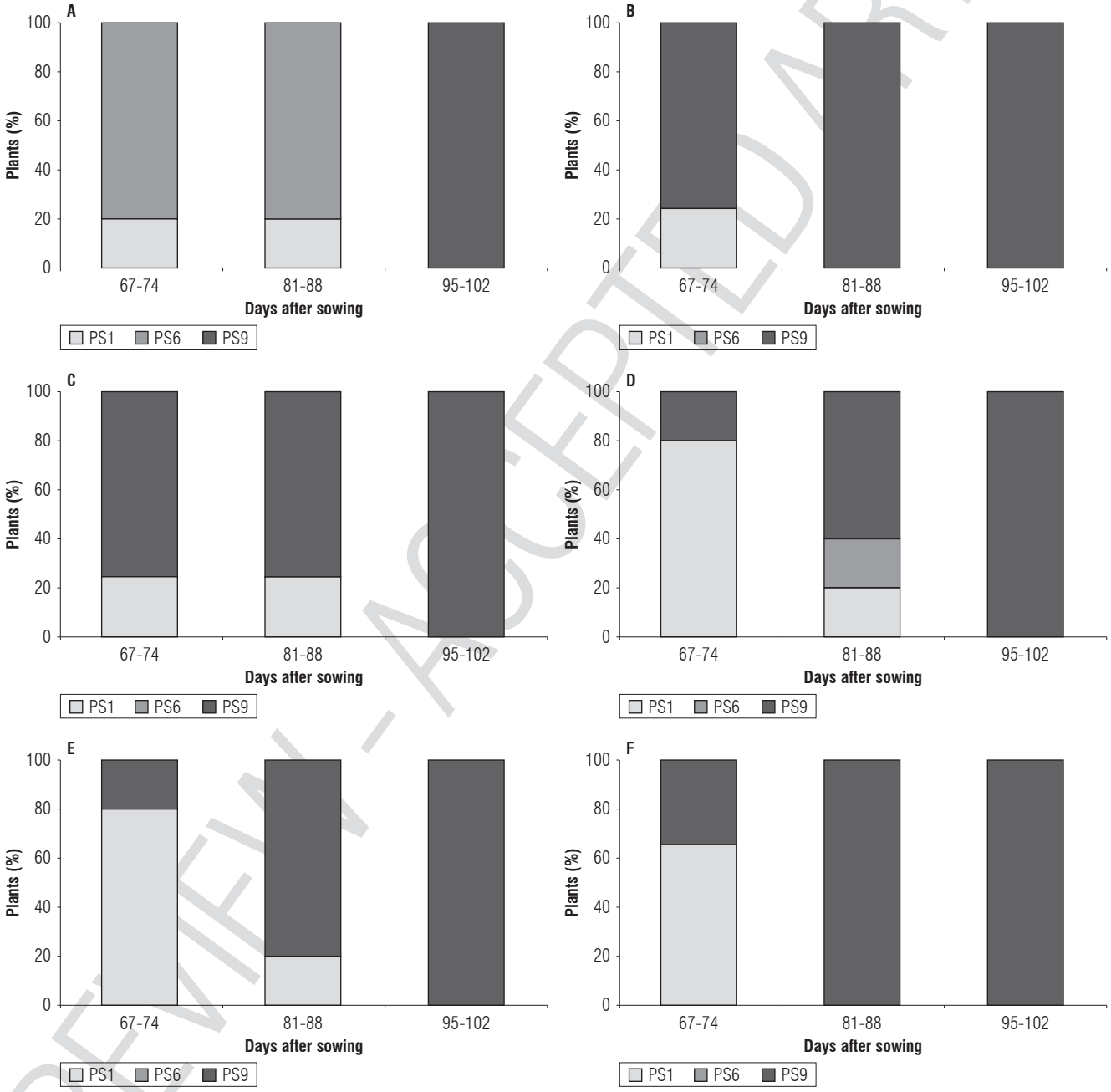

FIGURE 8. Developmental status of potato var. Diacol Capiro during the final phenological stages of plants inoculated with conidia of the strain Funza 104 (Funza). The figure illustrates the percentage of plants in the phenological stages (PS), leaf development (PS1), flowering (PS6), and senescence (PS9) according to the BBCH scale. A) Control plants non-inoculated, B) Incorporation of propagules in substrate (rice), C) Incorporation of conidia into the soil, D) Immersion of roots in a suspension of conidia, E) Application of conidia by soil drench, F) Incorporation of propagules in substrate (rice) at 49 days after sowing (das). Note the early dying of plants under *Verticillium* inoculation (PS9) starting at 64-67 days after sowing (das, compared to control plants (A), which reached PS9-senescence at 95-102 das).

(conidia and MS) and the three strains of *Verticillium* evaluated, the browning of the vascular bundles of the stems reported for the disease was observed in cross stem sections taken from the base of the diseased plants (Daami-Remadi *et al*., 2011; Powelson *et al*., 1993; Simko & Haynes, 2017). In isolations carried out in PDA medium from these stems, the growth of *Verticillium* colonies was confirmed, with typical mycelial growth of this genus as reported by Klosterman *et al*. (2009), Rubilar (2010), and Leon-Ttacca *et al*. (2018). In the colonies obtained, the characteristic whorled conidiophores were observed (Barnett & Hunter, 1998; Leon-Ttacca *et al*., 2018). In stems of control plants, the presence of vascular browning was not observed, and no *Verticillium* colonies were isolated. These results allowed us to confirm successful infection by the pathogen and that the symptoms presented in the potato plants var. Diacol Capiro, inoculated and maintained under greenhouse conditions, corresponded to potato early dying disease caused by *Verticillium* spp.

Harvest

From the MANOVA analysis of harvest data from plants inoculated with conidia, the inoculation method had a highly significant effect (P>0.05) on the set of response variables, and neither the strain nor the interaction had significant effects. From the clusters obtained, two possible groups were observed. The first group comprised the drench methods, application of conidia to the soil, and incorporation of propagules into the substrate for 49 das. The second group included root immersion and the incorporation of propagules into the substrate at sowing. Group 1 had the highest average tuber weights, and Group 2 had the lowest. Regarding the MS test, the analysis showed no significant differences in density; however, the treatments could not be distinguished.

Regarding the number of tubers per plant, for the two types of inoculated propagules (conidia and MS), no significant differences (P>0.05) between inoculation method, inoculum density, *Verticillium* strain, or the control treatments. Regarding tuber quality, in the conidia test, the most significant proportion of these (a range of 67% to 98% of the total tubers) corresponded to third-class quality, and no tubers classified as zero or first-class quality were obtained. In the case of MS, no zero-class quality tubers were recorded. Still, a small number of first-class quality tubers (maximum 2) were observed. In contrast, between 90% and 95% of the total tubers were classified as second or third-class quality, with similar proportions for the two classes. In both cases (conidia and MS), the control treatment presented the same behavior as the inoculated treatments for this variable.

Discussion

Under natural conditions, wilting of potato plants due to *Verticillium* begins during tuberization, as a result of infection by the inoculum present in the soil (Busch & Edgington, 1967), and that it can also originate from inoculum present in seed tubers (Ayers, 1952; Robinson & Ayres, 1961). In this study, a comparative evaluation was carried out of the development of potato early-dying under different methods, inoculation timing and types of *Verticillium* propagules (conidia and microsclerotia), in order to expose the host plants (seed tubers and newly emerged plants) to inoculum that can be found on the surface of the tuber, in the soil near the seed, and near the root system of developing plants. Our research revealed that almost all plants inoculated with the three *Verticillium* strains and treated with the different methods developed the characteristic symptoms of early wilting. The results also confirmed that *Verticillium* can infect established plants (49 das), and was not limited to early root from potato tuber seed at the time of sowing.

Characteristic symptoms of the disease developed under all the inoculation methods evaluated. These results are in accordance with previous studies, in which *Verticillium* inoculation methods such as soil drench (Leon-Ttacca *et al*., 2018), root immersion (Trapero, Díez *et al*., 2013), incorporation of propagules (conidia) in substrate (wheat) and application of propagules (conidia) to the soil (Platt & Sanderson, 1987) were applied to potatoes; the results find that it is possible to induce potato early dying by *Verticillium*. Based on the results, this method of inoculating a pathogen into the soil is recommended, as it allows the exact inoculum to be determined. The pathogen is similar to what is found in natural conditions. Additionally, due to its practicality, incorporating propagules into the substrate (rice) underlines the effectiveness of rice as a substrate for the multiplication and growth of *Verticillium* spp., but it also demonstrates the ease of adjustment to the required inoculum concentration. Regarding the timing of inoculation, it is suggested that it be done at sowing, as this best represents the *Verticillium* infective process under natural commercial cultivation conditions.

In this study, a progressive increase in the severity of the disease was observed over time, ending with the wilting and death of the plants, which is in accordance with what was reported by Guerrero *et al*. (1991), Bae *et al*. (2007), Johnson and Dung (2010), and Gómez-Caro and Mendoza-Vargas (2020). These symptoms appear once the pathogen has penetrated and invaded the xylem, where it generates numerous conidia that move systemically through the host's

vascular system, causing chlorosis, Necrosis, and, finally, leaf wilting (Johnson & Dung, 2010; Steere & Kirk, 2015).

Observing the development of the disease from the inoculation of *Verticillium* conidia demonstrated that the incubation period was shorter (23 dai) in the treatments where the pathogen was inoculated at 49 das. However, although symptom onset in these treatments was faster, disease progression was less severe than in treatments with conidia applied to the soil and propagules in substrate (rice), where the pathogen was inoculated at the time of sowing. Johnson and Dung (2010) state that, although *Verticillium* infection in potatoes can occur in the early stages of crop growth, wilting symptoms usually appear in the last phase of development, coinciding with the rapid tuber growth stage. Therefore, the treatments (*Verticillium* inoculation) applied at sowing exhibited a more extended incubation period and higher disease than those inoculated at 49 das. This difference is probably because, when the *Verticillium* inoculum reaches the crop after it has been established and its root system is developed, infection and the subsequent appearance of symptoms occur more rapidly. Moreover, although studies quantifying phenological susceptibility are limited, Johnson and Dung (2010) found that *Verticillium* inoculation at sowing resulted in more severe disease than inoculation at a later stage. This implies heightened susceptibility during early plant development, which likely contributes to the more extended incubation period and greater disease levels observed in these treatments.

In the development of potato early-dying through MS inoculation, we observed that the incubation periods coincided with the intervals reported by Trapero, Díez *et al.* (2013) in three of the five densities evaluated (10, 30, and 60 MS g^{-1} of soil) for this pathogen, which range between 60 and 80 d. Furthermore, we observed an increase in disease (higher AUDPC) with increasing MS levels in the soil. However, at soil densities greater than 30 MS g^{-1}, the AUDPC decreased. Similar results are reported by Xiao and Subbarao (1998) in cauliflower, where the incidence of wilting due to *V. dahliae* is 16%, even at 4 MS g^{-1}, and inoculum densities greater than 20 MS g^{-1} of soil did not cause a further reduction in plant growth. Likewise, these authors point out that the minimum MS concentration in soil required to cause wilting, as well as the number of infected plants at increasing MS density, depends on the cultivated plant species. In the present work, we found that infection with the pathogen and development of the disease in potato var. Diacol Capiro was at a density of 5 MS g^{-1} of soil. The results obtained in this and other studies reflect the difficulty of working with *Verticillium* resistance propagules such as MS, due to the limited protocols available for them. Although numerous methods have been described for their quantification, in most cases, sample processing is complex and laborious, MS recovery rates are low, and isolates are often inconsistent. In summary, the low efficiency of these techniques has been attributed, among other factors, to the possible dormancy that MS may present in the semi-selective media used for their production (López-Escudero *et al*., 2003). Moreover, MS have been reported to survive in soil for 10 to 15 years as melanized microsclerotia in the absence of a suitable host (Duressa *et al*., 2013).

Pullman and DeVay (1982) report that cotton plants inoculated with *V. dahliae* primarily exhibit growth and development. This finding is consistent with observations of potatoes in the current study, in which leaf fall and vascular wilting affected plant phenology. In addition, this would lead to a lower photosynthetic rate, directly due to wilting and leaf drop, and indirectly through vascular blockage or pathogen, resulting in reduced tuber production. In this regard, in the 1960s, it was speculated that the pathogen's toxins are responsible for leaf necrosis in plants infected by *V. dahliae* (Stoddart & Carr, 1966). However, the proteins secreted by *V. dahliae* are mostly intrinsic toxins that cause leaf wilting, fulfilling two main functions: altering the host's physical barriers through their enzymatic activity, and manipulating its defense system through their synergistic contribution to virulence (Chen *et al*., 2021). In *V. dahliae,* the best-known toxin is the necrosis and ethylene-inducing protein (VdNEP), which induces wilting in cotton leaves (Wang *et al*., 2004) and cell death in tobacco (Santhanam *et al*., 2013; Zhou *et al*., 2012) and is a virulence factor in tomato and *Arabidopsis thaliana*. Potato plants var. Diacol Capiro inoculated with *Verticillium senescens* approximately 20 d after plants were free of the pathogen showed results coinciding with those reported by Rowe and Powelson (2002), in which the death of potato plants affected by this pathogen was accelerated between 20 and 30 d. These findings underscore the altered phenological development of potato plants caused by pathogen infection.

Studies carried out by Steere and Kirk (2015). Gómez-caro and Mendoza-Vargas (2020) report that early potato dying caused by *Verticillium* in potatoes indicates that plants cannot develop tubers of significant size, resulting in a lower proportion of tubers in the zero and first-class categories, which negatively affects the performance of diseased plants and makes early dying a limiting disease for the crop. In this study, a greater number of tubers was found in the second and third-class categories, along with a reduction in tuber weight in the affected plants. According to Botseas

and Rowe (1994), tuber size may have been affected by a decrease in photosynthetic rate resulting from the premature loss of foliage, a consequence of chlorosis that progresses to necrosis and ultimately causes the plant's death. Plants subjected to methods of inoculation of *Verticillium* conidia by soil drench (DS), incorporation of conidia into the soil (CS), and incorporation of propagules into the substrate at 49 das (IPS 49) had a greater tuber weight. In contrast, plants subjected to root immersion (IR) 49 das and those inoculated by incorporating propagules into the substrate at the time of sowing (IPS) had lower weights. According to the results, the pathogen inoculation methods that showed the highest AUDPC values (CS and IPS), which resulted in premature plant death around 30-35 d before the control plants, presented the lowest harvested tuber weights.

Given the increasing importance of early mortality caused by *Verticillium* spp. in potato cultivation in Colombia, the need to conduct studies that consider melanized hyphae as a source of inoculum is evident, as these resistance structures, which form some *Verticillium* species, have been little studied. Likewise, the results of the present study could be expanded by evaluating other potato varieties, different inoculation processes with various propagules, and their mixtures in the development of the disease under different soil conditions, including pH, texture, and humidity. This would broaden understanding of the effects of soil conditions, including on the development of early- dying caused by *Verticillium* in potatoes, within the framework of studies involving a pathogen that produces different infective propagules potato early dying that is a complex disease to study and to control.

Conclusions

This study revealed that potato early-dying caused by *Verticillium* may occur due to plant infection at sowing time or during later growth stages, such as the onset of tuberization and the maximum vegetative growth stage, 45 to 55 days after sowing. However, the results suggest that the disease is more severe in plants inoculated at sowing. It was found that the first symptoms in potato plants var. Diacol Capiro can be observed between 65 and 70 d, regardless of the type of *Verticillium* propagule used as inoculum. Although either conidia or microsclerotia can infect potato plants, the density of microsclerotia plays an essential role in plant infection, with densities of 5 MS g^{-1} of soil.

Additionally, it was found that plants affected by the pathogen senesce prematurely, 28 to 35 d earlier than pathogen-free plants. This finding highlights the altered phenological development of infected plants, leading to reduced tuber production and quality, with a more pronounced effect when infection occurs at sowing. To further understand the disease's development, evaluations using melanized hyphae as a source of inoculum are necessary, as it is a typical structure of the pathogen for persisting in soil. Finally, this study lays the groundwork for a systematic evaluation of potato varieties in Colombia and the Andean region. A similar approach could be employed to screen *Solanum* species related to *Solanum tuberosum* or *Solanum phureja* to identify resistant sources.

Acknowledgments

We thank the Fondo Nacional del Fomento de la Papa (FNFP), administered by FEDEPAPA, for financing the study, as well as the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia. We also thank the students Luis Mendoza and Sebastián Chitiva from the Master's program in Agricultural Sciences with an emphasis in Phytopathology for their support in the laboratory and greenhouse during the study. Finally, they thank the undergraduate students involved in the project "Study of *Verticillium* and a pathology of unknown origin in potato: approach to detection, epidemiology, management and economic importance" HERMES Code 49825, for their help in setting up the trials.

Conflict of interest statement

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

Author's contributions

Conceptualization: SG; Formal data analysis: KSA and KSS; Funding acquisition: SGC; Research: KSA and KSS. Methodology: SGC; Validation: KSA and KSS and SGC; Writing – original draft: KSA and KSS; Writing – review & editing: KSA, KSS, and SGC; Supervision: SGC. All authors have read and approved the final version of the manuscript.

Literature cited

Ashraf, A., Rauf, A., Abbas, M. F., & Rehman, R. (2012). Isolation and identification of *Verticillium dahliae* causing wilt on potato in Pakistan. *Pakistan Journal of Phytopathology*, *24*(2), 112–116. https://www.academia.edu/37983367/ISOLATION_AND_IDENTIFICATION_OF_VERTICILLIUM_DAHLIAE_CAUSING_WILT_ON_POTATO_IN_PAKISTAN

Ayers, G. W. (1952). Studies on verticillium wilt of potatoes. *American Potato Journal*, *29*, 201–205. https://doi.org/10.1007/BF02881339

Bae, J., Atallah, Z. K., Jansky, S. H., Rouse, D. I., & Stevenson, W. R. (2007). Colonization dynamics and spatial progression of *Verticillium dahliae* in individual stems of two potato cultivars with differing responses to potato early dying. *Plant Disease*, *91*(9), 1137–1141. https://doi.org/10.1094/PDIS-91-9-1137

Barnett, H. L., & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi* (4th ed.). The American Phytopathological Society. https://es.scribd.com/document/415060565/Barnett-Hunter-Illustrated-genera-of-imperfect-fungi-APS-pdf

Benavides, J. A., Rodríguez, R. P., Arteaga Meneses, G., & Guerrero, O. (1995). Comportamiento de dos variedades de papa a la inoculación de *Verticillium albo-atrum* Reinke & Berth y *Globodera pallida* (Stone) Beherens. *Revista de Ciencias Agrícolas*, *13*(1-2), 103–22. https://revistas.udenar.edu.co/index.php/rfacia/article/view/1177

Botseas, D. D., & Rowe, R. C. (1994). Development of potato early dying in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans*. *Phytopathology*, *84*(3), 275–282. https://doi.org/10.1094/Phyto-84-275

Busch, L. V., & Edgington, L. V. (1967). Correlation of photoperiod with tuberization and susceptibility of potato to *Verticillium albo-atrum*. *Canadian Journal of Botany*, *45*(5), 691–693. https://doi.org/10.1139/b67-076

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. John Wiley & Sons.

Chávez-Arias, C. C., Gómez-Caro, S., & Restrepo-Díaz, H. (2020). Mitigation of the impact of vascular wilt and soil hypoxia on cape gooseberry plants by foliar application of synthetic elicitors. *HortScience*, *55*(1), 121–132. https://doi.org/10.21273/HORTSCI14550-19

Chen, J. Y., Klosterman, S. J., Hu, X. P., Dai, X. F., & Subbarao, K. V. (2021). Key insights and research prospects at the dawn of the population genomics era for *Verticillium dahliae*. *Annual Review of Phytopathology*, *59*, 31–51. https://doi.org/10.1146/annurev-phyto-020620-121925

Coley-Smith, J. R., & Javed, Z. U. R. (1970). Testing the viability of fungal sclerotia. *Annals of Applied Biology, 65*(1), 59–63.

Daami-Remadi, M., Jabnoun-Khiareddine, H., Ayed, F., & El Mahjoub, M. (2011). Comparative aggressiveness of *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* on potato as measured by their effects on wilt severity, plant growth and subsequent yield loss. *Functional Plant Science and Biotechnology*, *5*(Special issue 1), 1–8. http://www.globalsciencebooks.info/Online/GSBOnline/OnlineFPSB_5_SI1.html

Duressa, D., Anchieta, A., Chen, D., Klimes, A., Garcia Pedrajas, M. D., Dobinson, K. F., & Klosterman, S. J. (2013). RNA-seq analyses of gene expression in the microsclerotia of *Verticillium dahliae*. *BMC Genomics, 14*, Article 607. https://doi.org/10.1186/1471-2164-14-607

EPPO. (2020). PM 7/78 (2) *Verticillium nonalfalfae* and *V. dahliae*. *EPPO Bulletin, 50*(3), 462–476. https://doi.org/10.1111/epp.12701

EPPO. (2007). *Verticillium albo-atrum* and *Verticillium dahliae* on hop. *EPPO Bulletin*, *37*(3), 528–535. https://doi.org/10.1111/j.1365-2338.2007.01160.x

Gómez Caro, S., & Mendoza Vargas, L. A. (2020). Madurez temprana del cultivo de papa, una enfermedad limitante que todos debemos conocer. *Revista Papa*, *51*, 32–40. http://hdl.handle.net/20.500.12324/37792

Guerrero Guerrero, O., Benavides Pazmiño, J., & Rodríguez Escobar, R. (1991). Efecto de la inoculación de *Verticillium albo-atrum* Reinke y Berth y *Globodera pallida* (Stone) Beherens en las variedades de papa ICA-Nariño y Parda pastusa. *Revista ICA*, *26*(1-2), 9–18. http://hdl.handle.net/20.500.12324/644

Guerrero, O., Benavides, J., Rodríguez, R. (1992). Pérdidas ocasionadas por *Verticillium albo-atrum* Reinke y Berth., *Globodera pallida* (Stone) Beherens y sus interacciones en dos variedades de papa. *Revista ICA*, *27*(1), 13–23. http://hdl.handle.net/20.500.12324/16882

Hainaut, P., Remacle, T., Decamps, C., Lambert, R., Sadok, W. (2016). Higher forage yields under temperate drought explained by lower transpiration rates under increasing evaporative demand. *European Journal of Agronomy,* (72), 91–98. https://doi.org/10.1016/j.eja.2015.10.002

Hawke, M. A., & Lazarovits, G. (1994). Production and manipulation of individual microsclerotia of *Verticillium dahliae* for use in studies of survival. *Phytopathology*, *84*(9), 883–890. https://doi.org/10.1094/Phyto-84-883

Hunter, D. E., Darling, H. M., Stevenson, F. J., & Cunningham, C. E. (1968). Inheritance of resistance to Verticillium wilt in Wisconsin. *American Potato Journal*, *45*, 72–78. https://doi.org/10.1007/BF02862864

Inderbitzin, P., Bostock, R. M., Davis, R. M., Usami, T., Platt, H. W., & Subbarao, K. V. (2011). Phylogenetics and taxonomy of the fungal vascular wilt pathogen *Verticillium*, with the descriptions of five new species. *PLoS ONE*, *6*(12), Article e28341. https://doi.org/10.1371/journal.pone.0028341

Inderbitzin, P., & Subbarao, K. V. (2014). *Verticillium* systematics and evolution: how confusion impedes Verticillium wilt management and how to resolve it. *Phytopathology*, *104*(6), 564–574. https://doi.org/10.1094/PHYTO-11-13-0315-IA

Jakubowski, T., Rolbiecki, S., Rolbiecki, R., Wichrowska., W., Figas, A., Jagosz, B., Atilgan, A., Pal-Fam, F., Keszthelyi, S., & Krakowiak-Bal, A. (2024). Response of *Solanum tuberosum* L. to drip irrigation and nitrogen application: productivity, nutrition composition, bioactive compounds, antioxidant activity. *Journal of Plant Protection Research*, *64*(3), 307–315. https://www.plantprotection.pl/pdf-187983-113252?filename=113252.pdf

Johnson, D. A., & Dung, J. K. S. (2010). Verticillium wilt of potato – the pathogen, disease and management. *Canadian Journal of Plant Pathology*, *32*(1), 58–67. https://doi.org/10.1080/07060661003621134

Klosterman, S. J., Atallah, Z. K., Vallad, G. E., & Subbarao, K. V. (2009). Diversity, pathogenicity, and management of Verticillium species. *Annual Review of Phytopathology*, *47*, 39–62. https://doi.org/10.1146/annurev-phyto-080508-081748

Land, C. J., Lawrence, K. S., Burmester, C. H., & Meyer, B. (2017). Cultivar, irrigation, and soil contribution to the enhancement of Verticillium wilt disease in cotton. *Crop Protection*, *96*, 1–6. https://doi.org/10.1016/j.cropro.2017.01.002

Leon-Ttacca, B., Arévalo-Gardini, E., & Bouchon, A. S. (2018). Muerte repentina de *Theobroma cacao* L. causado por *Verticillium dahliae* Kleb. en el Perú y su biocontrol in *vitro*. *Ciencia y Tecnología Agropecuaria*, *20*(1), 117–148. http://www.scielo.org.co/pdf/ccta/v20n1/en_0122-8706-ccta-20-01-00117.pdf

Lima, F. S. O., Matos, V. S., Silva, E. S., Carvalho, M. A. S., Teixeira, R. A., Silva, J. C., & Correa, V. R. (2018). Nematodes affecting potato and sustainable practices for their management. In M. Yildiz (Ed.), *Potato – from Incas to all over the world* (Ch. 6, pp. 107–124). Intech Open. https://doi.org/10.5772/intechopen.73056

Li, F., & Li, Y. (2021). Evaluation of pathogenicity, systemic colonisation, and host range of *Verticillium alfalfae* in a greenhouse. *Crop and Pasture Science*, *72*(5), 383–392. https://doi.org/10.1071/CP20449

Li, H., Wang, Z., Hu, X., Shang, W., Shen, R., Guo, C., Guo, Q., & Subbarao, K. V. (2019). Assessment of resistance in potato cultivars to Verticillium wilt caused by *Verticillium dahliae* and *Verticillium nonalfalfae*. *Plant Disease*, *103*(6), 1357–1362. https://doi.org/10.1094/PDIS-10-18-1815-RE

López Escudero, F. J., Núñez Santos, D., & Blanco López, M. A. (2003). Aislamiento de *Verticillium dahliae* del suelo y caracterización morfológica de sus microesclerocios. *Boletín de Sanidad Vegetal, Plagas*, *29*(4), 613–626. https://www.mapa.gob.es/ministerio/pags/Biblioteca/Revistas/pdf_plagas%2FBSVP-29-04-613-626.pdf

Meier, U. (Ed.) (2018). *Growth stages of mono- and dicotyledonous plants: BBCH monograph*. Blackwell. https://doi.org/10.5073/20180906-074619

Mendoza-Vargas, L. A, León-Rueda, W. A, Ramírez-Gil, J. G, & Gómez-Caro, S. (2025). Identifying the causal agent of potato early dying and key epidemiological parameters at meso-scale in the high tropics. *Tropical Plant Pathology*, *50*(1), Article 35. https://doi.org/10.1007/s40858-025-00692-6

Mulero-Aparicio, A., Varo, A., Agustí-Brisach, C., López-Escudero, F. J., & Trapero, A. (2020). Biological control of Verticillium wilt of olive in the field. *Crop Protection*, *128*, Article 104993. https://doi.org/10.1016/j.cropro.2019.104993

Nair, P. V. R., Wiechel, T. J., Crump, N. S., & Taylor, P. W. J. (2019). Seed tuber incidence, identification and pathogenicity of *Verticillium* species infecting potatoes in South East Australia. *Australasian Plant Pathology*, *48*, 637–650. https://doi.org/10.1007/s13313-019-00667-7

Nieto, L. E. (1988). La madurez prematura de la papa causada por *Verticillium* spp. en Colombia. *Revista ICA*, *23*(4), 334–340. http://hdl.handle.net/20.500.12324/532

Pegg, G. F., & Brady, B. L. (2002). *Verticillium wilts*. CABI Publishing. https://www.cabidigitallibrary.org/doi/book/10.1079/9780851995298.0000

Platt, H. W., & Sanderson, J. B. (1987). Comparison of inoculation methods for field studies on varietal response to Verticillium wilt of potatoes. *American Potato Journal*, *64*, 87–92. https://doi.org/10.1007/BF02853441

Powelson, M. L., & Rowe, R. C. (1993). Biology and management of early dying of potatoes. *Annual Review of Phytopathology*, *31*, 111–126. https://doi.org/10.1146/annurev.py.31.090193.000551

Pullman, G. S., & DeVay, J. E. (1982). Epidemiology of *Verticillium* wilt of cotton: effects of disease development on plant phenology and lint yield. *Phytopathology*, *72*(5), 554–559. https://doi.org/10.1094/Phyto-72-554

Ramírez-Gil, J. G., & Peterson, A. T. (2019). Current and potential distributions of the most important diseases affecting Hass avocado in Antioquia Colombia. *Journal of Plant Protection Research*, *59*(2), 214–228. https://doi.org/10.24425/jppr.2019.129288

Robinson, D. B., & Ayers, G. W. (1961). Verticillium wilt of potato in relation to vascular infection of the tuber. *Canadian Journal of Plant Science*, *41*(4), 703–708. https://doi.org/10.4141/cjps61-104

Rowe, R. C., & Powelson, M. L. (2002). Potato early dying: management challenges in a changing production environment. *Plant Disease*, *86*(11), 1184–1193. https://doi.org/10.1094/PDIS.2002.86.11.1184

Rubilar Rubilar, M. A. (2010). *Estudios de patogenicidad y detección mediante PCR de Verticillium albo-atrum Reinke & Berth., en Kiwi dorado (Actinidia chinensis planch) cultivar Hort16A* [Undergraduate thesis, Universidad de Chile]. https://repositorio.uchile.cl/handle/2250/112411

Santhanam, P., van Esse, H. P., Albert, I., Faino, L., Nürnberger, T., & Thomma, B. P. H. J. (2013). Evidence for functional diversification within a fungal NEP1-like protein family. *Molecular Plant-Microbe Interactions*, *26*(3), 278–286. https://doi.org/10.1094/MPMI-09-12-0222-R

Serrano, A., Rodríguez-Jurado, D., Ramírez-Tejero, J. A., Luque, F., López-Escudero, F. J., Belaj, A., Román, B., & León, L. (2023). Response to *Verticillium dahliae* infection in a genetically diverse set of olive cultivars. *Scientia Horticulturae*, *316*, Article 112008. https://doi.org/10.1016/j.scienta.2023.112008

Simko, I., & Haynes, K. G. (2017). Maturity-adjusted resistance of potato (*Solanum tuberosum* L.) cultivars to Verticillium wilt caused by *Verticillium dahliae*. *American Journal of Potato Research*, *94*, 173–177. https://doi.org/10.1007/s12230-016-9553-1

Steere, L., & Kirk, W. (2015). Potato early die complex (*Verticillium* wilt). *Extension Bulletin E-3207*. https://www.canr.msu.edu/resources/potato_early_die_complex_e3207

Stoddart, J. L., & Carr, A. J. H. (1966). Properties of wilt-toxins produced by *Verticillium albo-atrum* Reinke and Berth. *Annals of Applied Biology*, *58*(1), 81–92. https://doi.org/10.1111/j.1744-7348.1966.tb05073.x

Trapero, C., Díez, C. M., Rallo, L., Barranco, D., & López-Escudero, F. J. (2013). Effective inoculation methods to screen for resistance to Verticillium wilt in olive. *Scientia Horticulturae*, *162*, 252–259. https://doi.org/10.1016/j.scienta.2013.08.036

Trapero, C., Serrano, N., Arquero, O., Del Río, C., Trapero, A., & López-Escudero, F. J. (2013). Field resistance to *Verticillium* wilt in selected olive cultivars grown in two naturally infested soils. *Plant Disease*, *97*(5), 668–674. https://doi.org/10.1094/PDIS-07-12-0654-RE

Uppal, A. K., El Hadrami, A., Adam, L. R., Tenuta, M., & Daayf, F. (2008). Biological control of potato *Verticillium* wilt under controlled and field conditions using selected bacterial antagonists and plant extracts. *Biological Control*, *44*(1), 90–100. https://doi.org/10.1016/j.biocontrol.2007.10.020

Volcy, C. (2008). Génesis y evolución de los postulados de Koch y su relación con la fitopatología. Una revision. *Agronomía Colombiana*, *26*(1), 107–115. https://revistas.unal.edu.co/index.php/agrocol/article/view/13924

Wang, J. Y., Cai, Y., Gou, J. Y., Mao, Y. B., Xu, Y. H., Jiang, W. H., & Chen, X. Y. (2004). VdNEP, an elicitor from *Verticillium dahliae*, induces cotton plant wilting. *Applied and Environmental Microbiology*, *70*(8), 4989–4995. https://doi.org/10.1128/AEM.70.8.4989-4995.2004

Wu, M., Li, Q., Xia, G., Zhang, Y., & Wang, F. (2022). New insights into defense responses against *Verticillium dahliae* infection revealed by a quantitative proteomic analysis in *Arabidopsis thaliana*. *Functional Plant Biology*, *49*(11), 980–994. https://doi.org/10.1071/fp22006

Xiao, C. L., & Subbarao, K. V. (1998). Relationships between *Verticillium dahliae* inoculum density and wilt incidence, severity, and growth of cauliflower. *Phytopathology*, *88*(10), 1108–1115. https://doi.org/10.1094/PHYTO.1998.88.10.1108

Zhang, Y., Kang, L., Gao, J., Puri, K. D., Jia, R., Zhang, Z., Zhang, J., & Zhao, J. (2023). Systemic colonization of potato plants by *Verticillium dahliae* leads to infection of tubers and sprouting buds. *Plant Disease*, *107*(3), 750–757. https://doi.org/10.1094/PDIS-05-22-1029-RE

Zhou, B. J., Jia, P. S., Gao, F., & Guo, H. S. (2012). Molecular characterization and functional analysis of a necrosis- and ethylene-inducing, protein-encoding gene family from *Verticillium dahliae*. *Molecular Plant-Microbe Interactions*, *25*(7), 964–975. https://doi.org/10.1094/MPMI-12-11-0319

SUPPLEMENTARY TABLE S1. Analysis of variance (ANOVA) results for area under the disease progress curve (AUDPC) in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
AUDPC	9	22465	2496.08	4.9353	0.0003155
Residuals	3	16690	505.76		

Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; F value = Fisher's statistic; Pr(>F) = p-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S2. Analysis of variance (ANOVA) results for incubation period (IP) in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
AUDPC	9	19571.1	2174.57	53.589	$2.2e^{-16}$
Residuals	3	1339.1	40.58		

Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; F value = Fisher's statistic; Pr(>F) = p-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S3. Analysis of variance (ANOVA) results for average total weight of tubers harvested in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Method	4	428.91	107.226	2.0963	0.0966
Strain	2	64.37	32.187	0.6293	0.5375
Residuals	46	2352.87	51.149		

Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; F value = Fisher's statistic; Pr(>F) = P-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S4. Multivariate analysis of variance (MANOVA) results for average total weight of tubers harvested in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Method	4	0.63872	5.3959	8	92	$1.421e^{-05}$
Strain	2	0.06774	0.8064	4	92	0.5242
Residuals	46					

Df = degrees of freedom; Pillai = Pillai's trace statistic; approx F = approximate F value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Pr(>F) = *P*-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S5. Multivariate analysis of variance (MANOVA) results for the number of tubers harvested in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Method	4	0.46860	2.1288	12	138	0.01870
Strain	2	0.22414	1.8933	6	90	0.09052
Residuals	46					

Df = degrees of freedom; Pillai = Pillai's trace statistic; approx F = approximate F value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Pr(>F) = *P*. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S6. Multivariate analysis of variance (MANOVA) results for average total weight of tubers harvested in potato plants inoculated with microesclerotia of different *Verticillium* strain (Mosquera 98).

Source	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Method	5	0.67189	1.61614	15	84	0.08659
Strain	1	0.04448	0.40348	3	26	0.75170
Residuals	28					

Df = degrees of freedom; Pillai = Pillai's trace statistic; approx F = approximate F value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Pr(>F) = *P*. Significance was considered at $P<0.05$.