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Occurrence of *Sclerotium rolfsii* Inducing Sugar Beet Root Rot and its Sustainable Management by Acting on Soil Fertility in Western Morocco

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ABSTRACT

In Morocco, and particularly in the Doukkala irrigated perimeter, sugar beet rot caused by *Sclerotium rolfsii* is a major limiting factor for the productivity of this crop. The objective of this study was to identify the relationship between the frequency of *Sclerotium rolfsii* infestation and the quantity of viable sclerotia in the soil on the one hand, and with the different physicochemical parameters of the soil in cropped sugar beet fields on the other hand. In total, 1794 soil samples were collected during a four years period in the whole irrigated perimeter. These samples were analyzed for their sclerotial content. In addition, laboratory analysis of physico-chemical parameters was performed for 94 sugar beet fields in 2019. The study showed that the relative frequency of infestation by *Sclerotium rolfsii* and the number of viable sclerotia oscillate inversely and present a relative frequency of 45.7, 57.3, 42.3 and 49.5% for 2016, 2017, 2018, and 2019, respectively, and a number of sclerotia of 2.01, 1.65, 2.11 and 1.25 in 250 g of soil for 2016, 2017, 2018 and 2019, respectively. The study showed that the number of viable sclerotia was positively correlated with clay and silt content, soil pH, Mg, K, Fe, Cu, and Mn and negatively correlated with sand, SOM, EC, $CaCO_3$, Zn, B, Ca, NH_4 and NO_3 contents. The observed decrease in viable sclerotia between 2016 and 2019 can be attributed to the decrease of Fe and Cu in the soil. It is possible that SOM intervenes with its humified fraction in the neutralization of the redox potential of Feand Cu which are involved in the production of sclerotia under oxidative stress. The good control of fertilization, especially organic, allows limiting the production of sclerotia of *Sclerotium rolfsii*. Data analysis allowed obtaining a significant prediction model of the number of viable sclerotia in the soil according to the physicochemical soil parameters with (R^2 = 0.95 at P<0.0001) for only infested fields and (R^2 = 0.87 at P<0.0001) for all the fields.

Keywords: *Sclerotium rolfsii*, soil fertility, soil organic matter, sugar beet, Doukkala, Morocco.

INTRODUCTION

In Morocco, sugar beet (*Beta vulgaris L.*) occupies the first place as a sugar crop with an area of 58.000 ha and a production of 3.6 Mt, with an average yield of 60 t·ha^{-1}. In the Doukkala region, sugar beet is placed as the main crop in the agricultural system; it occupies an area of about 20.000 ha, and produces about 1.73 Mt of sugar, corresponding to 38% of national production (COSUMAR 2019).

The fungus *Sclerotium rolfsii* is a major constraint to sugar beet production in the Doukkala region of Morocco that can cause yield losses ranging from 50% to 80% and a deterioration in sugar quality and extraction yield once the decayed roots are introduced into the extraction process (Khattabi et al. 2004). The pathogen survives

in the soil for several years in the form of sclerotia (Tarafdar et al. 2018). These forms of conservation are characterized by a melanized cuticle that protects them against degradation factors. The disease is favored by moist soil with temperatures ranging between 25 and 30 °C (Whitney et al. 1986). In addition, its prolific growth and ability to produce large numbers of sclerotia as well as its wide host range of about 500 plant species in over 100 plant families (Leoni et al. 2014) make the control of this pathogen difficult. Therefore, control of this fungus remains difficult and must be based on the strategies that can reduce the primary inoculum in the soil or prevent infection of host plants.

In the Doukkala irrigated perimeter, early sowing in September and October of sugar beet in order to harvest earlier before the onset of high temperatures favorable to infection remains the major practice to avoid the disease. Other solutions like biological control and solarization combined with organic amendments represent promising approaches in the control of soil-borne pathogens while reducing chemical inputs and their effects on the environment (Osman et al. 2017). However, the cost of these practices and the adaptation of antagonistic species to the real conditions of the crop constitute the principal constraints for their application.

In this context, the search for other alternatives based on the manipulation of soil fertility to reduce the density of the fungus inoculum in the soil could be of great interest, especially since the investigations conducted in-vitro have given promising results. Starting from the fact that the handling of nitrogenous fertilizers quite often enables to modify the microbial balances according to (Davet 1996) and that phosphorus or potassium fertilization have a weak influence on diseases (Jayakumar et al. 2019) as well as trace elements sometimes play a role, either because they are immobilized and made inassimilable for the plant by the action of the pathogen, or because they constitute a limiting factor for the development of the parasite (Huber and Jones 2013).

On the other hand, the addition of organic matter to the soil often has beneficial effects on the health of the roots. Thus, manure and compost were found to reducethe *Rhizoctonia solani* attacks on radish and bean (Pane et al. 2011).

Concerning soil texture, the structural properties, buffer and reservoir effect of clays, give them a very important regulatory role for microbial life (Gharbi et al. 2020).

The present work aimed at evaluating under field conditions, (1) the relationship between the stock in soil of viable sclerotia of *Sclerotium rolfsii* and the infestation frequency and (2) the relationship between soil physicochemical parameters and the number of viable sclerotia in the soil at the scale of the Doukkala irrigated perimeter.

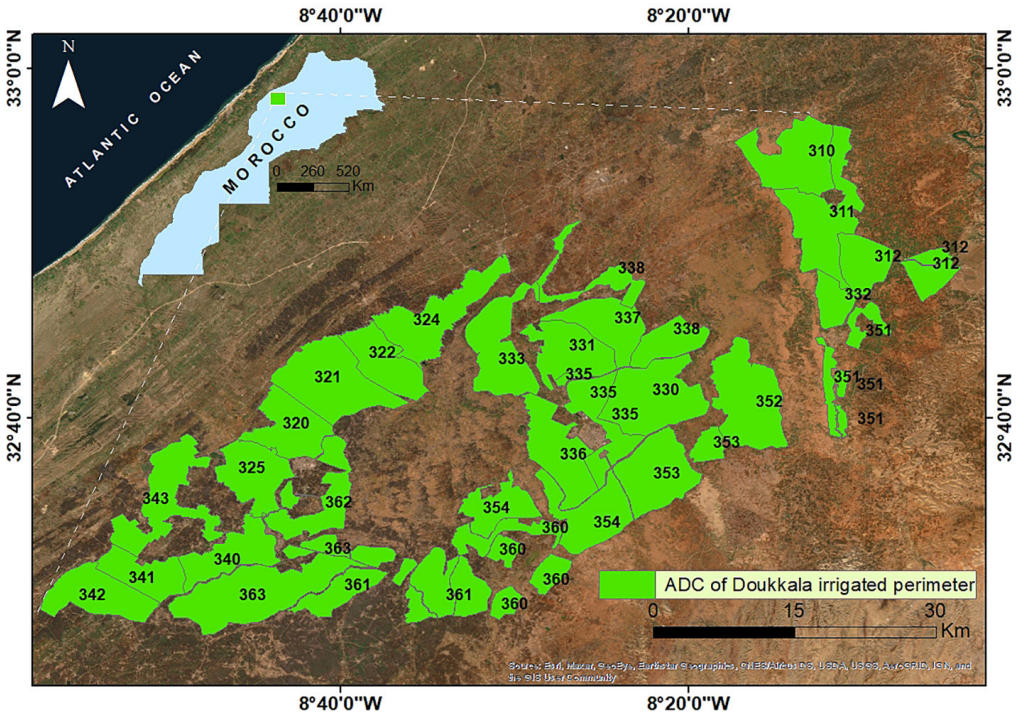

Figure 1. Location of the study area

MATERIALS AND METHODS

Study area description

The Doukkala irrigated perimeter (DIP) (Fig. 1) corresponds to a large plain south of the Atlantic coast city of El-Jadida. A relatively hot and dry summer and a moderate temperate winter characterize this semi-arid Mediterranean climate. The average annual precipitation, humidity, evaporation and temperature reaches 317 mm [125–592 mm], 80%, 1700 mm and 18 °C [4–40 °C] respectively. The main types of soils are divided into six classes, namely Aridisols, Vertisols, Calcimagnesic soils, Isohumic soils, Fersiallitic soils and Hydromorphic soils with percentage of total area of 21; 25; 12; 29; 7 and 6%, respectively (Eljebri et al. 2019).

Soil sampling and viable sclerotia germination tests between 2016 and 2019

In total, 1794 soil samples were collected and analyzed from different sugar beet fieldsfrom spread over 4 successive years, namely 2016, 2017, 2018 and 2019 with 694, 390, 433 and 277 samples, respectively. For each year, samples were taken randomly from all 28 Agricultural Development center (ADC) according to the annual area sown with sugar beets in each ADC.

The extraction of viable sclerotia (VS) was done using the method based on the stimulation of germination by methanol (Kabana 1980).

The infestation relative frequency (IF) of the ADC was calculated according to the following (Eq. 1):

$$\mathrm{IF} = \frac{\text{Number of infested samples by ADC}}{\text{Total number of samples analyzed by ADC}} \quad (1)$$

The number of viable sclerotia was calculated according to the following (Eq. 2):

$$VS = \frac{\text{Total Number of VS by ADC}}{\text{Number of infested samples in the ADC}} \quad (2)$$

Evaluation of soil physicochemical properties effect on the number of viable sclerotia

To investigate the relationship between different soil parameters and the number of VS, post-harvest soil analyses of sugar beetfields for the 2019 season were conducted for a sample of 94 fields randomly selected from fields that had undergone a sclerotia germination test during the same season.

The sampling method (0–30 cm depth) adopted for the soils is the composite sample per field. On each sugar beet field selected for analysis, a composite sample was taken from at least 5 to 10 subsamples covering the entire area. The number of sampling points depends on the size of the field and its homogeneity; if the field is heterogeneous, the sampler will take a stratified sample. The maximum area per sample was 10 ha. They were air dried, then sieved and crushed to a 2 mm size.

The particle size distribution was analyzed by Robinson pipette according to the Bouyoucos method (Beretta et al. 2014), organic matter was analyzed by the Walkey-Black method (Walkley and Black 1934), soil pH was measured according to (Bates et al. 1973) using a pH meter. Electrical conductivity was measured by conductivity meter in a 1:5 soil/water suspension; Carbonate content ($CaCO_3$, in % of DM at 105 °C) was determined by the volumetric method (ISO 10693, 1995); CEC was determined by the Metson method (Metson 1957). Nitrates (NO_3-N) by the chromotropic acid method, ammonium (NH_4-N) by colorimetry (indophenol blue), assimilable phosphorus (P_2O_5) by the Olsen method (Olsen 1954); calcium CaO, potassium (K_2O) and exchangeable sodium (Na_2O) by ammonium acetate extraction by atomic absorption and flame photometer according to (Simard 1993). Boron (B) was extracted by hot water method (Wear 1965), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) were analyzed by DTPA method (Lindsay and Norvell 1978).

Simple regression of the infestation frequency by viable sclerotia

Each pair of values, namely IF and VS of an ADC, was considered as an observation. Thus, during the four seasons, a group of 112 observations was subject of a simple linear regression of the IF according to VS.

Multiple linear regression (MLR) and validation

To model the number of VS as a function of soil properties, multiple linear regression (MLR) was performed using the following (Eq. 3):

$$y = b0 + b1X1 + \\ +b2X2 + \cdots + bnXn \quad (3)$$

where: Y – the predicted variable with regression coefficients b_1 to n and Y-intercept b_0 when the values for the predictor variables are X_1 to n.

The coefficient of determination (R^2), Root Mean Square Error (RMSE) (Eq. 4), and p-value were used to validate the selected model.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(y(i) - \hat{y}(i)\right)^2}{N}} \quad (4)$$

where: Yi and $\hat{y}i$ – the observed and predicted values of the SOM Variation, respectively, and N is the total number of observations ($N = 94$).

Statistical analysis

The means comparison (ANOVA), Simple linear regression or bivariate fit (SLR), Pearson correlation, and the Multiple Linear Regression Model (MLR) were used to statistically process the data using JMP-SAS version Pro 14.

RESULTS AND DISCUSSION

Monitoring the distribution of *Sclerotium rolfsii* between 2016 and 2019; Evolution of infestation frequency

Analysis of data from the 28 ADC of the Doukkala irrigated perimeter (Table 1) shows that the infestation frequency is characterized by a medium variation according to the season. Soil infestation by *Sclerotium rolfsii* varies from one season to another (Fig. 2):

The analysis shows that the infestation frequency average which is the number of infested sugar beet fields, was 45.7, 57.3, 42.3 and 49.5% for 2016, 2017, 2018 and 2019, respectively. The comparison of the averages shows that 2017 is marked by a significantly high infestation ($P<0.05$) compared to the years 2016, 2018 and 2019. This can be explained by the fact that the year 2016 experienced a manifestation of rot at the sugar beet fields which caused a high production of sclerotia that were probably spread to the fields analyzed in 2017.

The lowest infestation frequency without significant difference was observed in 2019 and therefore it is possible to suppose that the IF has a

Table 1. Characteristics of the infestation frequency for the 28 ADC

Parameters	Year	Min	Max	Mean	SD	Skewness	Kurtosis
Infestation frequency	2016	8.33	80	45.82	18.31	-0.11	-0.33
	2017	20	85	57.39	16.10	-0.79	0.45
	2018	22.22	71.43	42.43	10.21	0.64	1.499
	2019	0	95	49.49	25.77	-0.66	0.86
Min – Minimum; Max – Maximum; SD – standard deviation.							

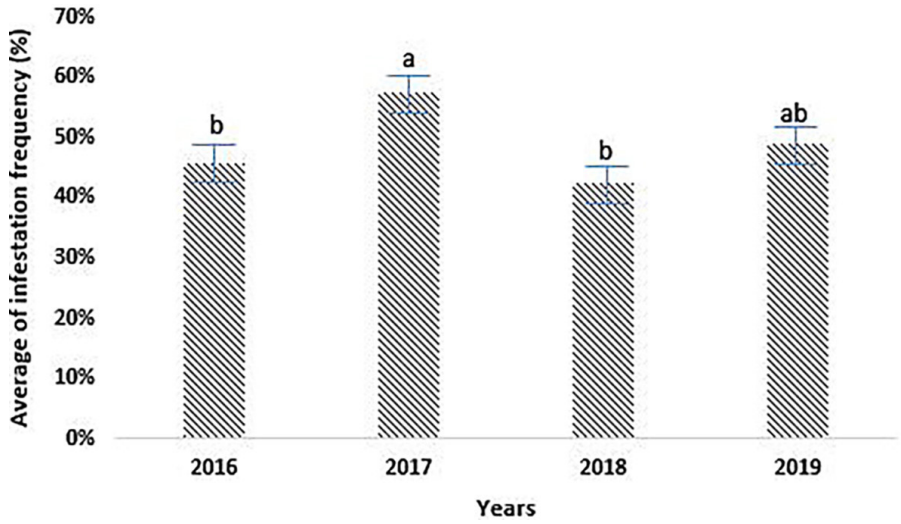

Figure 2. Evolution of the average IF by S. rolfsii between 2016 and 2019

tendency to stabilize. However, it is not possible to assume that this parameter has a decreasing tendency since 2016 was a drought year with a rainfall of 217 mm, i.e. -31% compared to the average rainfall, and an average temperature of 19.5 °C, i.e. +8% of the average annual temperature of the study area. In addition, the irrigation water allocation to the DIP during 2016 was 780 million cubic meters (Mm^3) compared to 640, 441 and 330 Mm^3 in 2017, 2018 and 2019, respectively. All of these factors created favorable conditions for the occurrence of the disease in 2016 compared to the other years of the study.

Evolution of VS average

The analysis of data from the 28 ADC of the DIP (Table 2) shows that the average number of VSis characterized by a large variability and varies from one season to the next (Fig. 3):

The analysis has shown that the average number of VS is 2.01, 1.65, 2.11 and 1.25 in 250 g of soil for 2016, 2017, 2018 and 2019, respectively. The comparison of the averages showed that the 2019 was marked by the lowest VS with a significant difference ($P<0.05$) compared to the years 2016, 2017 and 2018, and consequently, it can be assumed that the VS also has a tendency to stabilize since the other factors of rainfall, temperature and irrigation water supply were different from one year to another.

Relationship between infestation frequency and the number of viable sclerotia

It is important to note that these two parameters, namely the infestation frequency and the number of VS, have evolved inversely (Fig. 2 and Fig. 3). Thus, results during the four years of the study showed that the IF was negatively proportional to the VS according to the model (Fig. 4) characterized by ($R^2 = 0.61$, $P<0.0001$) and the statistical meanings of the coefficients (Table 3).

At the ADC scale, it is possible to use this model to estimate the density of the inoculum (viable sclerotia in 250 grams of soil) given that testing and counting is very laborious (Villeneuve et al. 2019) this inoculum which is formed by sclerotia as a unit (Gerdemann and Nicolson 1963), and which constitute 90% of the cycle with an estimated survival time of at least seven years and up to eleven years (Adams and Ayers

Table 2. Characteristics of viable sclerotia number for the 28 ADC

Petameter	Year	Min	Max	Mean	SD	Skewness	Kurtosis
Viable sclerotia	2016	0.5	6	2	1.58	1.63	1.64
	2017	0.67	3.4	1.65	0.71	0.64	0.09
	2018	1	6.86	2.11	1.47	1.99	3.52
	2019	0	4	1.25	0.97	1.4	2.27
Min–Minimum ; Max–Maximum ; SD – standard deviation.							

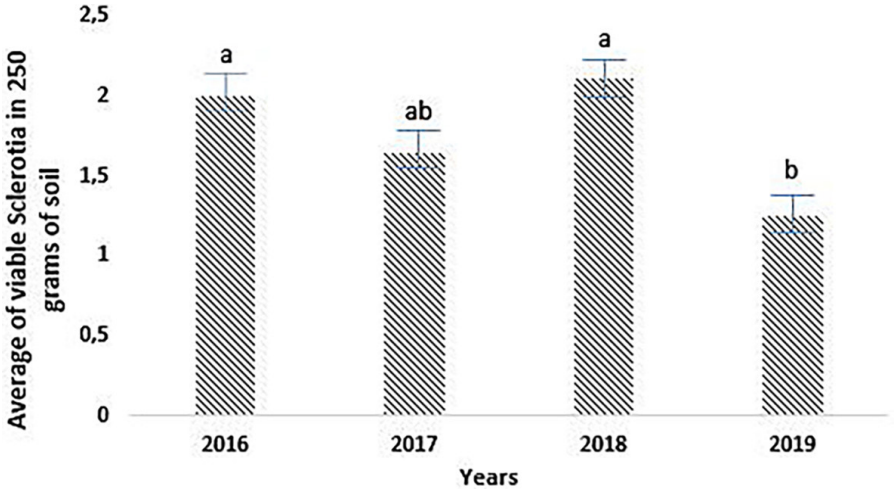

Figure 3. Evolution of the average number of VS average between 2016 and 2019

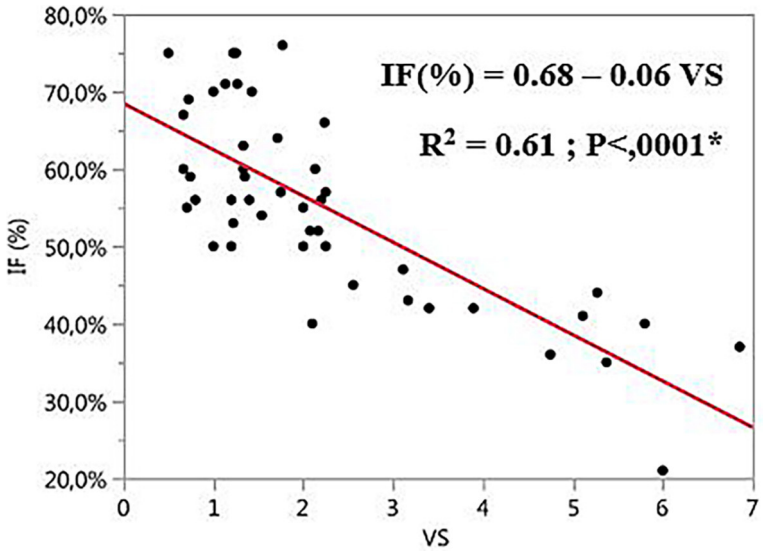

Figure 4. Bivariate fitting of infestation frequency by the number of viable sclerotia

Table 3. Estimation of the equation coefficients for fitting the infestation frequency by the number of viable sclerotia

Term	Estimate	Standard error	t ratio	Prob>|t|
Intercept	0.68	0.019	36.28	<,0001*
VS	-0.06	0.007	-8.52	<,0001*

Note: * statistically significant value.

1979), these sclerotia characterized by their density in a given volume of soil, present a relationship with the spatial distribution and the level of damage in lettuce caused by *Sclerotium minor*. Thus, the aggregation on a given surface of the root of several preserved forms or hyphae, creates an elevatedinfectious potential than the presence of a single spore (Garrett et al. 1970). Similarly, Wilkinson et al. (1985) reported that several factors can influence the infectious potential, namely the nutritional status of the inoculum and its energy reserves, its size, its genetic make-up, the limiting distance where an infectious unit must be to initiate a lesion and the environmental conditions (pH, temperature, water content, soil atmosphere, exogenous nutritional supply provided by root exudates, etc).

Venkatesh (2013) reported that the incidence of disease was maximum and reached 92.66% for 5% *Sclerotium rolfsii* inoculum level compared to 0, 1, 2 and 4% levels tested on a potted mint crop.

However, one should be careful not to draw too hasty conclusions about the biology of the infectious agents because the fields where the surveys were done do not only differ in their sclerotia densities, but in a large number of conditions, of which soil fertility is a part, that this study will try to evaluate in the following section.

Effects of soil physicochemical parameters on the number of viable sclerotia; Characteristics of the 94 fields and synthesis of Pearson correlations

The fields include a wide range of agricultural systems and soil types, from clearly sandy soils to clay soils. The characterization of the number of viable sclerotia, physico-chemical indicators of soils and micronutrients are presented in (Table 4). The different Pearson correlations found between the tested parameters, for the 94 sugar beet fields, show that there are positive and negative Pearson correlations between the number of viable sclerotia and the soil properties (Table 5) as well as concentration of elements in soil (Table 6).

The multivariate correlation of VS with the different soil parameters has shown that the statistically significant correlations are divided into two, positive correlations with clay, fine silt, coarse silt and pH and negative correlations with percentage coarse sand,fine sand, SOM and EC. $CaCO_3$ as a percentage in the soil showed no correlation with the VS (r = -0.002) (Table 7).

Table 4. Characterization of the number of viable sclerotia, physical and chemical indicators of soils and concentration elements (n = 94) in 2019

Indicator	Min	Max	Mean	SD	CV	Skewness	Kurtosis
Viable sclerotia VS	0	6	0.83	1.41	170	2.184	4.485
Na_2O mg.kg^{-1}	112	3051	607.21	423.9	70	3.39	15.861
Fe mg.kg^{-1}	3.99	55.6	10.41	6.21	60	4.679	30.124
Mn mg.kg^{-1}	5.36	107.18	20.40	13,23	65	3,272	18,847
Zn mg.kg^{-1}	0.16	3.47	0.93	0.62	67	1.332	2.08
Cu mg.kg^{-1}	0.17	1.07	0.52	0.15	28	0.46	1.084
B mg.kg^{-1}	0.1	2.34	0.49	0.34	71	2.989	11.782
MgO mg.kg^{-1}	264	3465	1464.63	674.8	46	0.447	-0.088
K_2O mg.kg^{-1}	77	1502	252.76	160.8	64	5.361	39.554
P_2O_5 mg.kg^{-1}	10	467	88.82	76.96	87	1.936	5.636
pH	6.7	8.7	8.112	0.44	5	-1.085	1.022
SOM %	1.38	2.45	1.98	0.21	11	-0.408	0.413
C. Sand %	6.8	24.5	14.8	3.95	27	0.647	-0.182
F. Sand %	10.2	69.8	37.3	11.67	31	0.311	-0.458
Sand %	19.5	89.5	52.2	14.01	27	0.324	-0.222
C. Silt %	1.7	16.7	7.09	2.92	41	0.666	0.052
F. Silt %	1	17.7	7.195	3.01	42	0.437	0.68
Silt %	2.7	28.5	14.28	4.95	35	0.107	0.064
Clay %	7.8	58.9	33.76	10.79	32	0.11	-0.397
EC ms.cm^{-1}	0.15	3.64	0.447	0.52	117	4.618	24.207
NO_3_N mg.100 g^{-1}	0.16	59.92	4.189	7.58	181	5.147	33.366
NH_4_N mg.100 g^{-1}	0.32	1.38	1.01	0.20	20	-0.913	1.224
Mineral nitrogen mg.100 g^{-1}	0.8	60.89	5.19	7.59	146	5.131	33.143
CaO mg.kg^{-1}	941	16232	7384	4173.6	57	0.332	-1.048
$CaCO_3$ %	0	21.5	2.308	3.93	170	3.179	11.388
SOM – Soil organic matter; C – coarse; F – fine; K_2O – Potassium; P_2O_5 – Phosphorus; MgO – Magnesium; CaO – Calcium; NO_3-N – Nitrates; NH_4-N – Ammonium; $CaCO_3$ – Total carbonates; Na_2O – Sodium; EC – Electrical conductivity; Zn – Zinc; B – Boron; Mn – Manganese; Fe – Iron; Cu – Copper; Min – Minimum; Max – Maximum; SD – Standard deviation; CV – Coefficient of variation.							

Table 5. Correlation of viable sclerotia number (VS) with soil properties of 94 soil samples analyzed in 2019

Indicator	VS	C.Sand	F. Sand	Sand	C. Silt	F. Silt	Silt	Clay	SOM	pH	EC	$CaCO_3$
VS	1											
C. Sand	-.346**	1										
F. Sand	-.209*	.485**	1									
Sand	-.271**	.685**	.969**	1								
C. Silt	0.175	-.419**	-.528**	-.558**	1							
F. Silt	0.110	-.446**	-.681**	-.693**	.390**	1						
Silt	0.170	-.519**	-.727**	-.752**	.828**	.839**	1					
Clay	.269**	-.647**	-.923**	-.951**	.346**	.506**	.512**	1				
SOM	-.734**	.270**	0.168	.216*	-0.129	-0.121	-0.150	-.206*	1			
pH	0.048	-.245*	-.232*	-.262*	.296**	0.026	0.191	.247*	0.041	1		
EC	-0.051	-0.101	-0.091	-0.104	-0.037	.308**	0.165	0.059	-0.055	-.316**	1	
$CaCO_3$	-0.003	-0.068	-0.063	-0.072	.338**	0.046	.229*	-0.006	-0.066	.275*	-0.033	1
** – Correlation is significant at the 0.01 level; * – Correlation is significant at the 0.05 level.												

Table 6. Correlation of viable sclerotia number (VS) with soil elements concentration of 94 soil samples analyzed in 2019

Indicator	VS	Na_2O	Fe	Mn	Zn	Cu	B	MgO	K_2O	P_2O_5	SOM	NO_3N	NH_4N	CaO
VS	1													
Na_2O	-0.063	1												
Fe	.803**	-0.067	1											
Mn	.234*	0.179	.489**	1										
Zn	-0.108	-0.062	-0.113	-0.019	1									
Cu	.634**	0.031	.602**	.310**	-0.067	1								
B	-0.073	.241*	-0.041	.262*	0.114	-0.005	1							
MgO	0.003	.498**	0.036	0.169	-0.087	-0.037	0.121	1						
K_2O	.352**	0.094	.212*	0.065	0.135	0.188	0.097	.370**	1					
P_2O_5	0.092	-0.053	.341**	.474**	.434**	0.201	0.116	0.064	.239*	1				
SOM	-.734**	-0.031	-.624**	-.232*	0.168	-.518**	0.042	0.017	-.243*	-0.103	1			
NO_3N	-0.043	.667**	-0.003	.222*	0.023	0.044	.289**	0.078	0.032	0.050	-0.065	1		
NH_4N	-.880**	0.078	-.688**	-0.186	0.015	-.570**	0.061	0.048	-.287**	-0.027	.646**	0.037	1	
CaO	0.215	0.133	0.029	-.305**	-.358**	0.070	-0.122	.365**	.252*	-.271*	-0.157	0.070	-0.149	1
** – Correlation is significant at the 0.01 level; * – Correlation is significant at the 0.05 level														

Table 7. Correlations between the number of VS and the physicochemical properties of the soil

Variable	Per variable	*r*	LCI 95%	LCS 95%	p-value
VS	Clay	0.27	0.07	0.45	0.0089*
	C. Silt	0.17	-0.03	0.36	0.0921
	F. Silt	0.11	-0.09	0.31	0.2926
	C. Sand	-0.35	-0.51	-0.15	0.0006*
	F. Sand	-0.21	-0.39	-0.01	0.0436*
	pH	0.05	-0.16	0.25	0.6454
	SOM	-0.74	-0.81	-0.62	<.0001*
	EC	-0.05	-0.25	0.15	0.6258
	$CaCO_3$	-0.002	-0.23	0.22	0.982

Note: * Statistically significant value.

Effect of soil texture

The Pearson correlation of VS with soil texture shows positive and statistically significant correlations with clays (r = 0.27; P = 0.0089), and negative and statistically significant correlations with coarse sands and fine sands with (r = -0.35; P = 0.0005) and (r = -0.21; P = 0.0436), in agreement with those found by (Bushby and Marshall 1977) on the protective effect of clays which is exerted in various forms. In fact, clay can improve resistance to desiccation by ensuring a more regular dehydration of cellular content and decreases the diffusion and inhibitory effects of toxins and antibiotics by adsorbing them (Campbell and Ephgrave 1983). The presence of clay also leads to the creation of a large number of micro-habitats that are difficult to penetrate by predators (Heijnen and van Veen 1991). The positive correlation with silts may be due to their relatively higher drainage than clays which ensures better aeration.

Effect of pH

The weak positive correlation (r = 0.05; P = 0.64) of pH with the number of viable sclerotia in the soil shows that the germinative power of sclerotia does not seem to be affected by the mild alkalinity of the medium, since the average pH is 8.08. This result goes in agreement with that of (Raghavendra et al. 2018) who showed that the germination of sclerotia was not affected and reached 100% at pH 7.5 and 100% with the native soil control at pH 7.2. This clearly indicates that a slightly basic pH is very agreeable for sclerotia survival. The same authors also found

a slight reduction in germination percentage of 3.34% at neutral pH without statistical significance. On the other hand, a reduction in the survival of sclerotia was observed with a reduction in pH, i.e., 90% at pH 6.5 and 86.60% at pH 6, and the difference between them was 3.40%. The highest reduction in the percentage of sclerotia germination that has reached 23.34% was recorded at pH 9.0 and the difference in sclerotia germination at 8.5 and 9.0 pH is not significant. Regarding mycelial growth, Gour et al. (2010) reported that *S.rolfsii* grew over a wide range of pH from 4 to 9 but the maximum growth of the fungus was recorded on the medium having a pH 6.0 and the lowest mycelial growth was obtained at pH 9.0 and pH 8.0.

Effectof soil organic matter (SOM)

The negative correlation (r = -0.73; P<0.0001) of the number of viable sclerotia in the analyzed samples is in agreement with previous studies (Noble 2011) that reiterate the beneficial effects of SOM on root health. Generally, suppressive effects of soils are attributed to physical, chemical (Lazarovits 2001) or biochemical (Morra and Kirkegaard 2002), or microbiological or enzymatic factors (Rasmussen et al. 2002). Lazarovits (2001) suggests that the decomposition processes of organic amendments and their by-products play a major role in the suppression phenomena. A priori, composting on the ground would therefore be preferable to the application of compost at various degrees of maturity for obvious reasons of convenience and economy.

Asirifi et al. (1994) indicate that organic amendments significantly reduce sclerotinia of lettuce and survival of *S. sclerotiorum* sclerotia compared to the control treatment without organic amendments. The in-vitro resultsfound by Kokalisburelle and Rodriguezkabana (1994) show that inhibition of mycelial growth of *S. sclerotiorum* by pine bark powder (*Pinus elliottii*, *Pinus taeda*) incorporated fresh or composted into the growing medium. These results support the hypothesis that the absence of *Sclerotinia* in biologically active soils is related to the induced competition of saprophytic microorganisms with *S. sclerotiorum*, removal of surface crust, increase in aggregate size and better drainage (Bueno et al. 2007). The general improvement of these properties would affect the survival of sclerotia.

Specifically, in *Sclerotium rolfsii*, Khattabi et al. (2004) showed in-vitro that the fungus can use the horse compost amendment at low doses – equivalent to 2 kg.m^{-2}– as a source of nitrogen and carbon for its growth, but beyond 4 kg.m^{2} the mycelial growth is inhibited. They showed in parallel that the maximum inhibitory action, of the antagonist *Trichderma harzianum*, is observed with the highest dose of horse manure compost. The bivariate adjustment of viable sclerotia with SOM in the studied fields gave statistically significant equations in this sense (Fig. 5).

Effect of electrical conductivity (EC)

Salinity can affect the ability of plant pathogenic fungi to produce cellulolytic enzymes in-vitro differently (El-Abyad et al. 1994). For

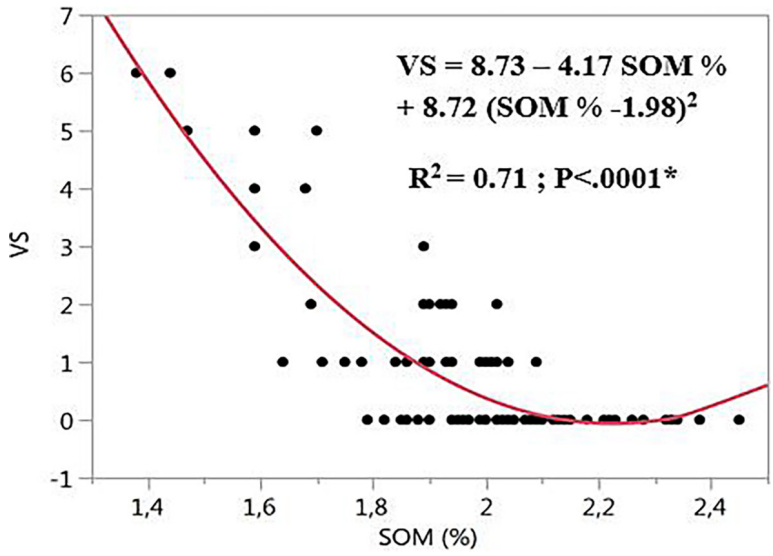

Figure 5. Bivariate fit between of VS by SOM

example, *Sclerotium rolfsii* and *Rhizoctonia solani*, two soil-borne fungi pathogenic to beet, have their ability to solubilize their host cell walls disrupted by salt in vitro.

The negative correlation found with (r = -0.05; P = 0.62) related to the number of viable sclerotia in soil with EC (ms.cm^{-1}), goes in agreement with those found by Regragui et al. (2003)who showed that the maximum mycelial growth was observed in the medium without salt amendment, while the number of sclerotia produced decreased with increasing salt concentration in the medium while testing in-vitro the effect of different concentrations of NaCl on the production of sclerotia of *Sclerotinia sclerotiorum* that causes cabbage crown rot. Regragui et al. (2003) reported that mycelial germination of *S. sclerotiorum* sclerotia decreased when grown on medium amended with salts. Sclerotia production, following mycelial germination, is decreased by more than 50 % with increasing salt concentration.

Correlations between the number of viable sclerotia and nutrients in the soil

Positive correlations

For the 94 fields, the multivariate correlation shows that the number of viable sclerotia in the soil is positively correlated, with statistical significance, with Iron (Fe) Copper (Cu), Potassium (K), Manganese (Mn) with r = 0.80; r = 0.63; r = 0.35 and r = 0.23, respectively, and without statistical significance with Calcium (Ca), Phosphorus (P) and Magnesium (Mg) with r = 0.21, r = 0.09 and r = 0.01 respectively (Table 8).

Iron (Fe) and Copper (Cu)

The results are consistent with the findings that sclerotia biogenesis in *Sclerotium rolfsii* is associated with lipid peroxidation. Sclerotial initials show a 100-fold increase in lipid peroxides in their total lipids compared to young mycelia grown under reducing conditions in the dark and without Fe^{2+}. There was a direct relationship between the number of sclerotia formed and the levels of lipid peroxidation in the mycelial colonies. Lipid peroxides of possible membranous and cytoplasmic origin were found in the sclerotial exudate. Thus, a new approach is advanced for the understanding of the mechanism of sclerotial formation in *Sclerotium rolfsii* and in other fungi. The data on lipid peroxidation, as well as the data from past experiments, strongly suggest that this phenomenon may be associated with the oxidative stress caused by growth conditions (Georgiou 1997). Thus, the bivariate adjustment of the number of viable sclerotia with the Fe and Cu contents in the soil gave statistically significant equations (Fig. 6).

Sideri and Georgiou (2000) have reported that hydrogen peroxide is produced by *Sclerotium rolfsii* during sclerotial differentiation in response to oxidative growth conditions generated by light and iron and that the auto-oxidation of Fe^{2+} and Cu^{+} ions is a source of superoxide and hydrogen peroxide. Thus, complexing iron and copper ions in a form that blocks their redox activity is a mechanism of antioxidant action.

Since of the humification of sugar beet wastes, there may be a drop in the soil Fe and Cu concentrations because these ions bind to humic compounds. Due to low level of humification of sugar beet residues, this slight drop can be explained (Rerhou et al. 2022) who found an increase in SOM content of DIP between 2012 and 2019 accompanied by a slight decrease in the soil content of Fe and Cu. This finding supports laboratory research by Vizier (1978) that demonstrated the ability of humic and fulvic acids to form complexes with iron in amounts ranging

Table 8. Positive correlations between the number of viable sclerotia and nutrients in soil

Variable	Per variable	*r*	LCI 95%	LCS 95%	p-value
VS	Fe	0.80	0.72	0.86	<.0001*
	Cu	0.63	0.49	0.74	<.0001*
	K_2O	0.35	0.16	0.52	0.0005*
	Mn	0.23	0.03	0.42	0.0231*
	CaO	0.21	-0.01	0.42	0.0622
	P_2O_5	0.09	-0.11	0.29	0.378
	MgO	0.003	-0.2	0.21	0.9765

Note: * Statistically significant value.

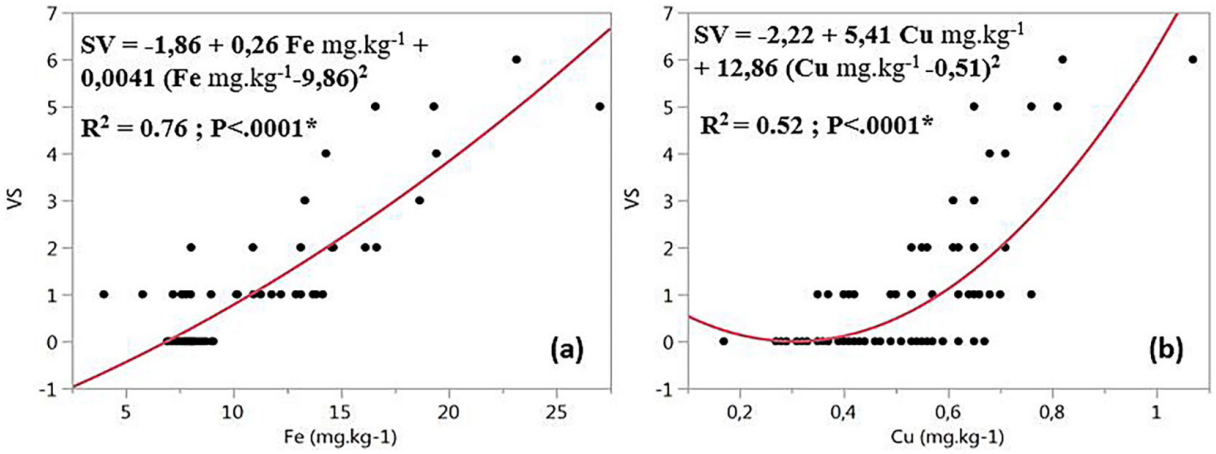

Figure 6. Bivariate fit between Fe (a) and Cu (b) content and the number of viable sclerotia (VS) in soil

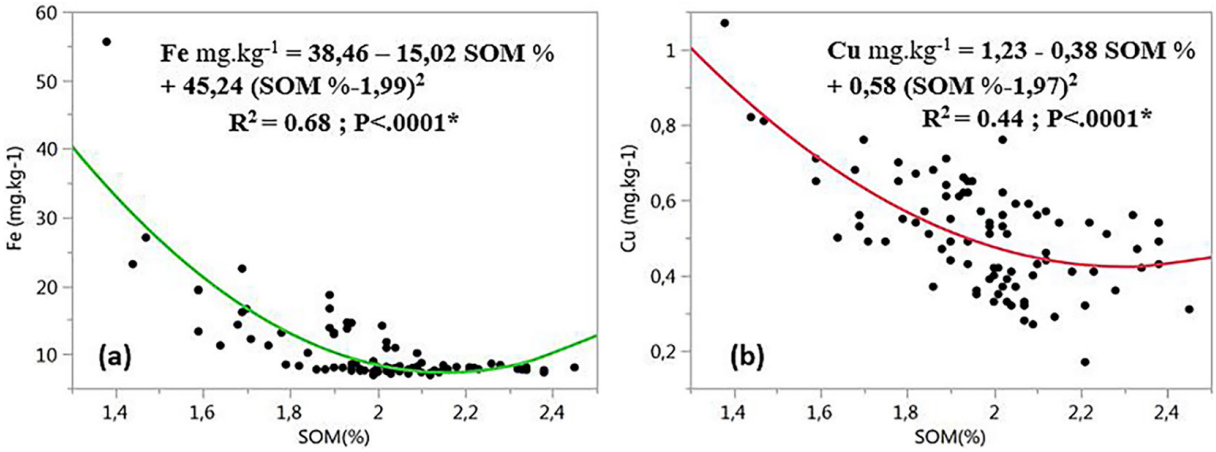

Figure 7. Bivariate fit between Fe (a) and Cu (b) and SOM in soil

from 15 to 35 mg Fe per 100 mg C, or from 0.15 to 0.35. Similarly, Zanin et al. (2019) demonstrated a strong correlation between total carbon losses and, to a lesser extent, iron losses, and the rate at which humified material is extracted.It appears that these losses are all the more important as the organic matter is more humified. It is thus remarkable that it is not the most organic soils that present the most important reduction phenomena, nor the reduction phenomena that develop the most rapidly, but that on the other hand the degree of evolution of the organic matter and consequently its quality seem to affect the importance of these phenomena.

From this result it would be of great interest to study the effect of composting beet residues as a way to have more humification enabling to chelate Fe and Cu in the soil and thus prevent their auto-oxidation that promotes sclerogenesis. The bivariate adjustment of Fe and Cu with SOM in the studied fields gave statistically significant equations that confirm this hypothesis (Fig. 7).

Calcium (Ca)

The negative correlation is in agreement with previous results found in-vitro by (Horner et al. 1985) who found that the crystals formed along the hyphal infection were due to the ability of the fungus to produce oxalic acid which in turn sequesters calcium from the host to form said crystals. Thus, calcium in the soil is much more involved in the infection process than in the differentiation of sclerotia.

Potassium (K), Manganese (Mn), Phosphorus (P) and Magnesium (Mg)

Potassium

The positive and statistically significant correlation found confirms the results found by (Wheeler and Sharan 1965) by testing in-vitro the effect of different concentrations of potassium in the culture medium on the production of sclerotia by *S. rolfsii* and showed that with very low

concentrations of potassium the initiation of sclerotia stops even if the mycelial growth continues. Abbas et al. (2021) exposed *Macrophomina phaseolina* to various concentrations of simple phosphorus or potassium fertilizers in Potato dextrose agar (PDA) medium and found that simple super phosphate increased the number of sclerotia by up to 300% and under the influence of potassium fertilizers, the number of sclerotia increased by 200–400%, maximum with potassium nitrate followed by muriate of potash and sulfate of potash.

Manganese

The positive and statistically significant correlation found in this study is consistent with the results found by (Gupta et al. 2005) which showed that manganese is necessary for mycelial growth and sclerotia formation in *Sclerotium rolfsii* which justifies the high content of this element in the soil in fields where sclerotia numbers are elevated. Dutton et al. (1996) reported that the secretion of oxalate by *Sclerotium rolfsii* allows the chelation of manganese by enabling the dissolution of Mn^{3+} from the manganese-enzyme complex, thus stimulating the extracellular activity of manganese peroxidase.

Phosphoru sand magnesium

Wheeler and Sharan (1965) showed in-vitro that the presence of phosphorus in the hyphal phase of *Risoctonia solani* leads to a low formation of sclerotia compared to a culture transferred from a medium without phosphorus to a medium containing phosphorus.Moromizato et al. (1991a) showed in-vitro that sclerotia formation for *Rhizoctonia solani* was proportional to the concentration of phosphorus in the form of KH_2PO_4 up to a concentration of 100 ppm; above this concentration, the effect of phosphorus was negative on sclerotia formation which is in agreement with the weak positive correlation found in this study. On the other hand, a recent study conducted by (Mendes et al. 2022) showed that the oxalic acid secreted by *Sclerotium rolfsii* contributes to the solubilization of phosphate rock in the soil, resulting in the relatively high phosphorus content found in the fields with high sclerotia numbers.

For Magnesium, the weak positive correlation found confirms the results obtained by (Survase et al. 2006) while testing in-vitro the response of scleroglucan production involved in *Rhizoctonia solani* sclerotia formation under increasing doses of $MgSO_4$. They have shown that when magnesium was added, the number and amount of sclerotia increased in proportion to the concentration. On the basis of these facts, the authors considered that magnesium ion has a favorable effect on sclerotial induction (Moromizato et al. 1991b).

The significant correlation with potassium comparing it to the weak one of phosphorus goes in agreement with the results of previous researches found in-vitro by testing different concentrations of the culture medium in phosphorus and potassium and that showed that a considerable number of sclerotial initials and mature sclerotia that were formed in a medium without P was ten times higher in a medium without K (Moromizato et al. 1991) from where the determining character of potassium compared to phosphorus comes.

Negative correlations

For the 94 fields, the multivariate correlation shows that the number of viable sclerotia in the soil is negatively correlated, with statistical significance, with nitrogen in the form of NH_4^+ with r = -0.88, and without statistical significance with Zinc (Zn), Boron (B), Sodium (Na) and Nitrate (NO_3^-) with r = -0.11, r = -0.07 and r = -0.04 respectively (Table 9).

Ammonium (NH_4^+)

The statistically significant negative correlation with NH_4^+ confirms the results found by (Matti and Sen 1985) by testing the effect of urea

Table 9. Negative correlations between the number of viable sclerotia in the soil and nutrients

Variable	Per variable	*r*	LCI 95%	LCS 95%	p-value
VS	$NH_{4\_}N$	-0.88	-0.92	-0.82	<.0001*
	Zn	-0.11	-0.30	0.09	0.2999
	B	-0.07	-0.27	0.13	0.4867
	Na_2O	-0.06	-0.26	0.14	0.5453
	$NO_{3\_}N$	-0.04	-0.24	0.16	0.6807

Note: * statistically significant value.

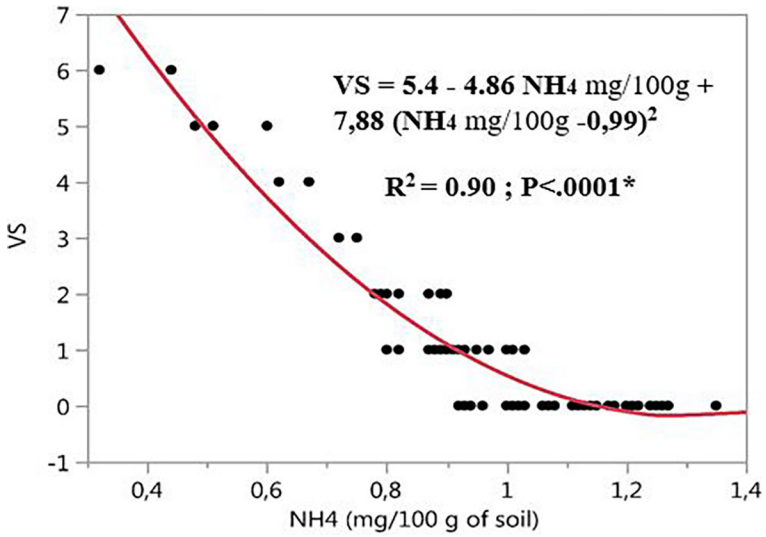

Figure 8. Bivariate fit between NH_4^+ content and the number of VS in soil

on sclerotia viability in the laboratory and found that at a rate equivalent to 40 kg N·ha^{-1} sclerotia viability was significantly reduced.Hoynes et al. (1999) found that ammonium sulfate, ammonium nitrate, diammonium phosphate or urea, applied to the soil at a field rate of 135 kg N·ha^{-1} did not reduce the viability of *S. rolfsii* sclerotia. Ayed et al. (2020a) showed that ammonium acetate used in the laboratory as a nitrogen source completely inhibited sclerotia formation in *Sclerotium rolfsii*. It seems that the effect of nitrogen fertilizers in ammonia form, negatively affects mycelial growth and sclerotia viability at a high rate. The bivariate adjustment of the number of viable sclerotia with the NH_4^+ content in the soil gave the statistically significant equation (Fig. 8) which affirms the inhibiting effect of this form of nitrogen.

Zinc (Zn)

The negative correlation, without statistical significance, with Zn content is in agreement with the results found in-vitro on *Sclerotinia sclerotiorum* (Vega and Tourneau 1974) which showed that high concentrations of Zn in the medium gave very low sclerotia production compared to low concentrations. They found no reduction in the growth of the fungus in liquid media not containing a given combination of Fe, Cu, Zn and Mn. This may be due to the interactions of Zn with other microelements.

Nitrate (NO_3^-)

The negative and non-significant correlations with soil NO_3^- content are in agreement with the results of previous studies (Wheeler and Sharan 1965) which showed that an appreciable number of sclerotia were produced in a NO_3^- free medium, the same study showed that the effect of NO_3^- varied with the source of carbon present in the medium. In studieson the effect of combinations of different sources of nitrogen and carbon, in-vitro, on the production and viability of sclerotia, Ayed et al. (2020b) showed that the effect of NO_3^- is dependent on the source of carbon in the medium. This leads to suggest that the inhibitory effect of these anions is indirect on the production and viability of sclerotia.

Boron (B)

The negative and statistically insignificant correlations with boron in soil are in agreement with previous results (Sharma and Shukla 2020) on the effect of boron on *Pyricularia oryzae* and showed that as the ppm concentration of boron increased, hyphal growth and sporulation decreased. Godara et al. (2007) showed that the radial growth of pathogenic fungi *Pyricularia oryzae*, *Fusarium oxysporum* and *Aspergillus niger* was reduced by Boron activity.Ni and Punja (2020) reported that boron has an indirect effect on fungal diseases through the stimulation of defense in plants.

Prediction model of VS

Using the above data by selecting only the soil properties and nutrients that showed a strong correlation with the number of viable sclerotia in the soil, has made it possible to identify two prediction models of the number of viable sclerotia in

Table 10. Prediction models of the number of viable sclerotia in soil

MLR equations	R^2	R^2 adj	RMSE	F calculated	p-value**
Equation 1: Using data from all fields					
VS = 6.35 + 0.07Fe - 1.23SOM - 3.7NH_4_N	0.87	0.86	0.52	195.78	<0.0001*
p-value*: β0<0.0001*, β1<0.0001*, β2=0.0007*, β3<0.0001*					
Equation 2: Using only data from infested fields					
VS = -31.87 + 0.025Fe - 1.24SOM - 6.44NH_4_N + 0.42Clay + 0.4Sand + 0.39Silt	0.95	0.94	0.39	92.27	<0.0001*
p-value*: β0=0.0493*, β1=0.0312*, β2=0.0235*, β3<0.0001*, β4=0.02*, β5=0.0258, β6=0.0297					

Note: VS – Viable sclerotia in 250grams of soil, β0 (constant), β1 β2 β3 β4 β5 and β6 (Regression coefficient), *p*-value* (statistical significance level), *p*-value** (model significance).

the soil by multiple linear regression (Table 10), The first model was derived from the treatment of data for all fields in the study as a function of Fe, SOM and NH_4^+ and the second was derived from the treatment of infested fields only as a function ofFe, SOM, NH_4^+,clay, sandand silt.

The choice between equation 1 and equation 2 will be made on the basis of the history of the field, so for a field where the root rot has never occurred equation 1 can be used, while for a field with a history of disease occurrence equation 2 can comfortably be used, with the aim of predicting the importance of the inoculum of the fungus in the soil based on the analyses made for the reasoning of the fertilization.

The knowledge of the density of the inoculum is of interest for the estimation of the risk of the expression of the fungal diseases as proved by (Singh et al. 2010) by testing five inoculum densities, i.e. 0.20, 5.5, 6, 7 and 8 mg of *Rhisoctonia solani* sclerotial inoculum, which were inoculated at the maximum tillering stage of rice plants, the highest sheath burn severity and minimum incubation period were observed with 8 mg of sclerotial inoculum.

CONCLUSIONS

A stabilization of the infestation frequency and the number of viable sclerotia of *S. rolfsii* in the soil was observed, with a more marked downward trend for the viable sclerotia. The analysis of the data during 4 beet growing seasons allowed observing the alternating character of these two parameters which evolve inversely to each other. The bivariate adjustment gave a statistically significant prediction model at the perimeter level.

The correlations of the number of viable sclerotia in the soil with the physicochemical parameters of the soil largely confirm the results found in-vitro by studying, separately, the effect of each parameter on the number of viable sclerotia.

For the group of 94 fields studied, positive correlations were found between the number of viable sclerotia and the content of clay and silt, soil pH, Mg, K, Fe, Cu and Mn and Ca, and negative correlations with the content of SOM, sands, EC, $CaCO_3$, Zn, B, Ca, NH_4^+ and NO_3^-.

The fine soil fractions provide protection of sclerotia against irregular desiccation conditions and temperature change while sands expose them to all these conditions that affect their viability, hence the interest to increase vigilance in fields with dominant fine texture.

Contrary to the mycelial growth favored by acidic pH values in previous studies, the results found in this study confirm the work done in-vitro which reported that sclerotia production and viability are favored by slightly alkaline pH values.

For EC, the results found, confirm those found in-vitro which report that sclerotia production and viability are disturbed by increasing salinity reflected by high EC values.

The observed decrease of viable sclerotia in soil between 2016 and 2019 can be attributed among others to a decrease of Fe and Cu in the soil which is due to the chelation of these ions with humified SOM. The results found show that the action of SOM on the production and viability of *S. rolfsii* sclerotia is not direct. It is possible that SOM intervenes, besides the promotion of antagonists competing with *Sclerotium rolfsii* in the soil, with its humified fraction in the neutralization of the redox potential of Fe and Cu involved in the production of sclerotia under oxidative stress conditions. For the elements K, P, Mg and Mn, the positive correlations found are in agreement with previous results found in-vitro.

NH_4^+ showed a negative effect on the number of viable sclerotia, which is in agreement with previous studies and will help to orientate the

choice towards organic amendments that release more nitrogen in this ammoniac form. On the contrary, nitrates gave a negative correlation contradicting previous studies which showed in-vitro that NO_3^- can be used by *S. rolfsii* as a nitrogen source for mycelial growth.

The prediction model found could be of great interest to assess the risk of occurrence of *Sclerotium rolfsii* rot symptoms based on soil tests that beet growers are starting to apply to rationalize the fertilization of their crops.

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