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Vertical distribution of sclerotia of *Macrophomina phaseolina* in a natural infested soil in Portuguesa state.

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Abstract

The vertical distribution of *M. phaseolina* sclerotia among the influence of the temperature and humidity were evaluated on a soil infested naturally. The essay was carried out at the experimental field Turén, which is part of INIA-Portuguesa, in a plot of 285 m^2 with a totally randomized blocks design, where treatments were depths (0-5 cm, 5-10 cm, 10-20 cm) and the blocks were the collection dates of the samples. The plot was sowed in succession with the corn-sesame crops, doing the evaluations in the seasons of scarce precipitations, 30 days after had emerged the sesame seedlings and ended on the terminal phase of flowering; for this, soil samples were taken with a borehole of 20 cm of height and 10 cm of diameter. On each depth, 5 sub-samples were taken to create one of 250 g, these were air-dried for 24 h in a lab and sclerotia were extracted using the flotation method. The lineal regression analysis for the three depths showed a highly significant negative association ($P<0.01$) among variables humidity and quantity of sclerotia, and a highly positive association ($P<0.01$) among temperature and sclerotia content variables. The lineal regression analysis showed a highly significant association ($P<0.01$) among variables temperature, humidity and sclerotia content. It was determine that at a higher depth, the quantity of sclerotia reduces the humidity content increases and the differences between the maximum and minimum temperatures reduce.

Key words: Charcoal root, temperature, humidity, distribution

Introduction

Macrophomina phaseolina (Tassi) Goid is a fungus that lives in the soil, and causes the disease known as charcoal rot. This fungus has been reported affecting more than 300 species of sowed plants (12). There are lots of proofs that *M. phaseolina* survives in the soil as sclerotia, which are produced in the tissues of the infected plants (3, 5) which once incorporated in the soil increase and disseminate the primary inoculum (3, 6, 8).

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Sclerotia can survive in the soil from 3 months to 3 years (4). *M. phaseolina* is a pathogen considered able to disseminate through seeds being this an efficient way to disseminate and an inoculum source (1).

Temperature and humidity contents of the soil are two main abiotic factors in the epidemiology of the disease that affect the ecology and the growth of *M. phaseolina* as pathogen and saprophyte (2, 7).

In the soils sowed in Portuguesa state, *M. phaseolina* presents very frequently affecting lots of crops in the region, among these is sesame (*Sesamun indicum* L.), reporting incidences from 10 to 25%, which may cause losses of even of 10% in the crop's yield (11).

Few researches have been done about the vertical distribution in *M. phaseolina* soil and the factors that affects it. The aim of this research was to evaluate the vertical distribution of the fungus in a soil infested naturally and the influence of abiotic factors (temperature and humidity contents in the soil) on the *M. phaseolina* growth.

Materials and methods

A plot of 19 m x 15 m (285 m^2) was selected in the experimental field Turén, located at the agriculture field Turén that belongs to the National Institute of Agriculture Research, Portuguesa State (INIA-Portuguesa). The plot has a loamy texture soils, pH of 8.1 and is naturally infested with *M. phaseolina*.

During the rainy season, the plot was sowed with corn (*Zea mays* L.) using the traditional system of the area. Meanwhile, for the season with few precipitations sesame was sowed of the Píritu variety at a distance between rows of 0.6 m. The handle and cultural work were done according to the traditional system that farmers of the area do, and evaluations were done during the crop cycle of sesame.

The essay was established on a randomized block design with three treatments and seven replications, where treatments were depths (P): 0-5 cm, 5-10 cm and 10-20 cm, and blocks were constituted by the dates when samples were taken.

The evaluation to determine the number of soil g/sclerotia (egs) of *M. phaseolina* in different depths started 30 days after emerged sesame seedlings (dde) and ended once flowering was over. The sample work was done with a borehole of 20 cm of height and 10 cm of diameter.

For each depth 5 sub-samples were taken at random in order to form only one sample of 250 g, which was taken to the Phytopathology laboratory of INIA-Portuguesa, these were let to dry in open space for 24 hours and were macerated in a sterile mortar to be sifted through a sieve N° 60, 5 sub-samples of 0.5 g each were taken from each sifted material, extracting the sclerotia through the floating method of Watanabe *et al.* (15). At the same time, samples of the soil were taken on each evaluated depth to determine the humidity percentage (h %) based on the dry weight, drying the soil in a stove at 110ºC for 24 h. Also, temperature registers of the soil (TS) were obtained for each depth at the weather station located at CET, at 200 m from the location of the essay.

The information obtained were processes through the variance analysis mean test and simple and multiple lineal regression, using as independent variables TS and h % (14).

Results and discussion

Number of sclerotia: Sclerotia population of *M. phaseolina* fluctuated in the time at the depth of 0-5 cm (P1) from 94.4 to 252.0 egs, in the one of 5-10 cm (P2) from 49.2 to 227.2, and in the one of 10-20 cm (P3) from 47.6 to 172.0 egs. The statistical analysis showed highly significant differences among treatments (depths) (P<0.01; CV=16.48%), with the highest average number of sclerotia in P1 (80.34 egs), followed by P2 (60.31 egss) and P3 (53.63 egs) (table 1).

Soil humidity: h % for each depth fluctuated like this, P1 from 35.8% to 8.1%, P2 from 37.7% to 12.0% and P3 from 34.41% to 15.23%. The statistical analysis showed significant differences among the humidity content of treatments (P<0.01, CV=10.56%), with the highest h average percentage in P3 (23.64%), then P2 (23.13%) and the lowest h average percentage in P1 (21.16%) (table 2).

Soil temperature: Soil temperatures fluctuated for each depth as mentioned next: P1 from 26.2ºC to 31.7ºC, P2 from 27.0ºC to 30.7ºC and P3 from 27.8ºC to 30.6ºC. The statistical analysis did not show significant differences among the average temperatures in treatments, but analyzing the variation of the soil temperature registered in the sample dates, the statistical analysis showed highly significant differences (P<0.01, CV=3.74%) and mean test evidences that the highest variation (maximum TS – minimum TS) occurred in P1 (5ºC) followed by P2 (1.82ºC) and finally by P3 (0.51ºC) (table 3).

Table 1. Number of g sclerotia of *M. phaseolina* soil^{-1} in three depths of soil sowed with sesame.

Depth	G sclerotia of soil^{-1}
0–5 cm	80.34^{a2}
5–10 cm	60.31^{b}
10–20 cm	53.63^{b}

1=Average calculated in based to seven replications
2=Values with the same letter are statistically equal according to the Duncan's multiple range test (P<0.01) CV = 16.5%

Table 2. Humidity content of the soil in three depths of soil sowed with sesame.

Depth	Humidity percentage[1]
0–5 cm	21.16[b2]
5–10 cm	23.31[ab]
10–20 cm	23.64 a

1=Average calculated in based to seven replications
2=Values with the same letter are statistically equal according to the Duncan's multiple range test (P<0.01) CV =10,6%

Regression and lineal correlation: Variables h % and egs showed a highly significant negative lineal regression:

P1 (P<0.01); r= -0.923; R^2= 0.8519; Y= 276.914 – 5.493 X

P2 (P<0.01); r= -0.935; R^2= 0.8740; Y= 279.180 – 6.856 X

P3 (P<0.01); r= -0.936; R^2= 0.8760; Y= 284.516 – 7.412 X

The analysis for the differences among the lineal regression for h % and egs variables showed significant differences compare to the sclerotia g content of soils^{-1}, but it did not show statistical differences among the straight lines angle for each depth, which indicated that these had the same gradient.

TS and egs variables showed a highly significant positive lineal regression:

P1 (P<0.01); r= 0.949; R^2= 0.9010; Y = 529.745 + 23.926X

P2 (P<0.01); r= 0.954; R^2= 0.9110; Y = 1083.473 + 41.892X

P3 (P<0.01); r= 0.943; R^2= 0.8892; Y = 1356.180 + 50.290X

The analysis to compare the lineal regressions for TS and egs variables among depths showed highly significant differences (P<0.01) for both the position as for the straight line angle, which means that straight lines are different, proving that the temperatures fluctuations in the soil

Table 3. Soil temperature variation in three depths of the soil sowed with sesame.

Depth	Temperature variation (ºC)[1]
0–5 cm	5.00[a2]
5–10 cm	1.82[b]
10–20 cm	0.51[c]

1=Average calculated in based to seven replications
2=Values with the same letter are statistically equal according to the Duncan's multiple range test (P<0.01) CV =3,74%

affect significantly the populations of *M. phaseolina* in the soil.

Multiple regressions: The multiple regression analysis showed a highly significant association among h% and egs variables:

P1 ($P<0.01$); $r= 0.9778$; $R^2= 0.950$; $Y= -292.8361 – 1.7563 X_1 + 1.7004 X_2$

P2 ($P<0.01$); $r= 0.9788$; $R^2= 0.958$; $Y= -670.3426 – 2.2176 X_1 + 2.9303 X_2$

P3 ($P<0.01$); $r= 0.9883$; $R^2= 0.966$; $Y= -627.2843 – 3.6772 X_1 + 2.8260 X_2$

The obtained results showed the same tendency than those reported for that location (CET) (2), referent to the influence of the independent variables humidity and temperature of the soil on the dependent variable number of g sclerotia of the soil^{-1}. For each evaluated depth was observed a highly positive correlation among the variable number of sclerotia and soil temperature while for variables number of sclerotia and humidity percentage of the soil showed a highly negative correlation.

It was observed that at the time that it is getting deeper in the soil, the number of *M. phaseolina* sclerotia reduces, meanwhile, the humidity percentage of the soil increases and differences between the maximum and minimum temperature reduces.

Differences among the straight line angles were not observed, that is, these had the same gradient but without significant statistical differences between the sclerotia quantity, comparing each of the regression lines between them, when the independent variable was humidity percentage and the independent variable was sclerotia number; this shows that once the humidity content of the soil increased, the number of sclerotia reduced.

Meanwhile, for the regression lines in relation to the variables soil temperatures and number of sclerotia, there were highly significant statistical differences between the sclerotia contents and the straight lines gradient. Thus, observing that when the difference between maximum and minimum increased there was a higher growth of the fungus reflected in the increment on the number of sclerotia.

The temperature variable has been mentioned as an important factor in the development of *M. phaseolina*, observing a higher development of the fungus when it was submitted to temperature fluctuations compare to conditions of steady temperatures (5).

M. Phaseolina is a fungus that lives in the soil and has the capacity of growing vegetatively and to produce a huge quantity of sclerotia on vegetal tissue in conditions of low water potentials (–1.880 J kg^{-1}) (10) and to maintain in 100% viable its sclerotia in soil dried in opened air, pasteurized as well as natural (9), which proves the great survivor capacity of *M. phaseolina* in dried soils.

Likewise, it has been reported in relation to the depth in the soil the existence of a higher number of sclerotia from 0 to 7.5 cm than from 7.5 to 15 cm (12), and lower fluctuation of the number of sclerotia at depths from 10-20 cm than depths from 0-5 cm, finding an inversely proportional relation from depth and rapidity to which was developed the maximum number of sclerotia (13). In relation to the incidence of the damage caused by *M. phaseolina* in the plant, this was higher in depths from 0 to 5 cm, and with temperatures that oscillated from 28 to 30ºC (8), which occurs in some areas of the soil where water is exhausted more rapidly due to the high evapotranspiration, also because there is the most favorable temperature for the growth of *M. phaseolina* (5). All these observations agree to the results obtained in this research, where it is shown that the highest contents of sclerotia, the highest fluctuation of the temperature and the lowest humidity content occur in the soil at depths from 0 to 5 cm.

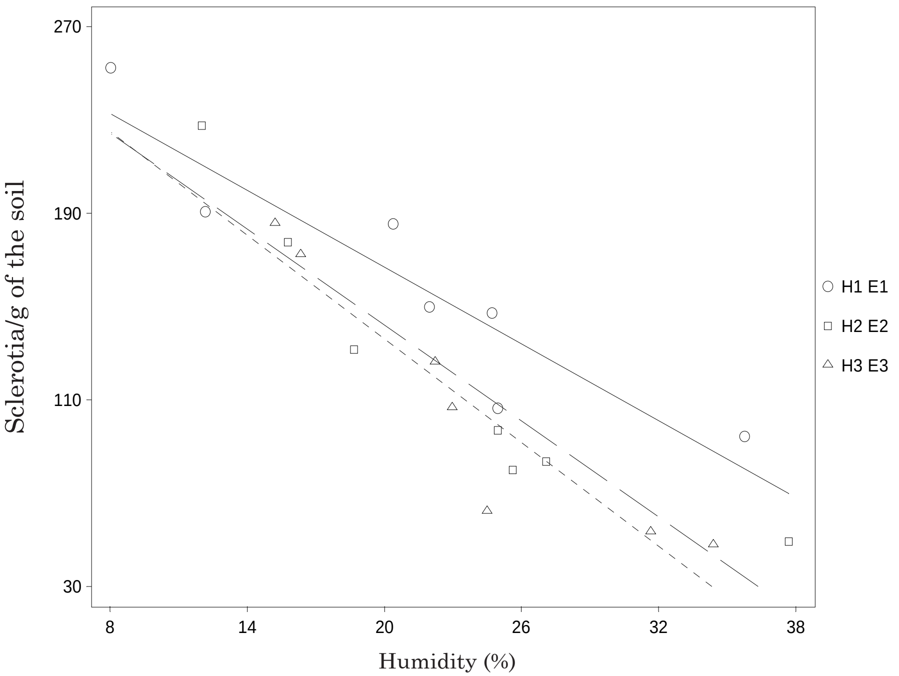

Figure 1. Lineal regression between the humidity percentage of the soil (%) and the number of g/sclerotia of the soil for the three evaluated depths at the experimental field Turén. E1: Sclerotia from 0 to 5 cm of depth. E2: sclerotia from 5-10 cm of depth. E3: Sclerotia from 10-20 cm of depth. H1: Temperature from 0 to 5 cm of depth. H2: Temperature from 5 to 10 cm of depth. H3: Temperature from 10 to 20 cm of depth.

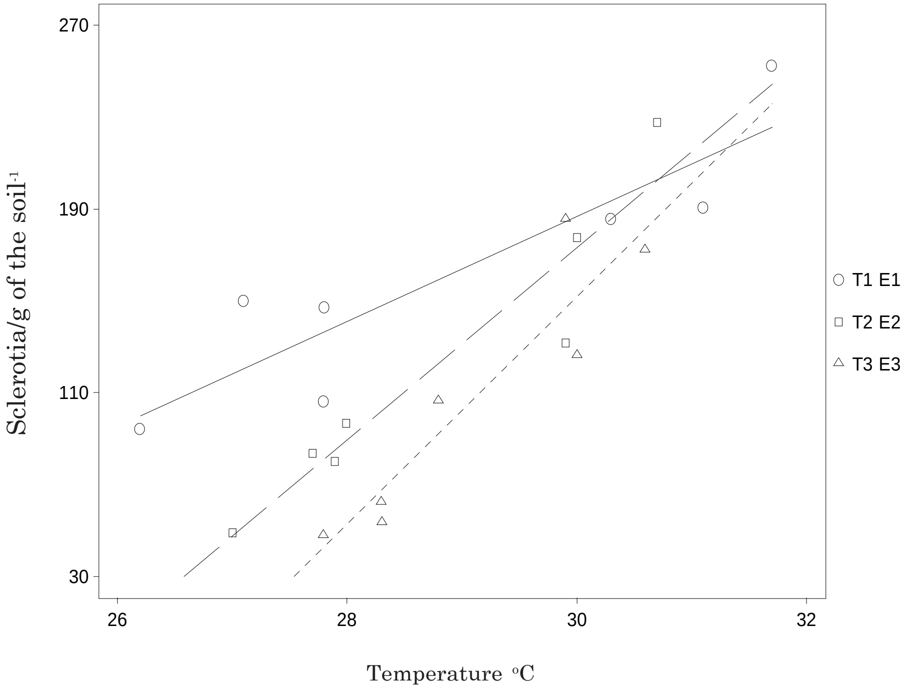

Figure 2. Lineal regression from the soil's temperature and the number of g sclerotia in the soil for the three evaluated depths at the experimental field Turén. E1: Sclerotia from 0 to 5 cm of depth. E2: sclerotia from 5 to 10 cm of depth. E3: Sclerotia from 10 to 20 cm of depth. T1: Temperature from 0 to 5 cm. T2: Temperature from 5 to 10 of depth. T3: temperature from 10 to 20 cm of depth.

Conclusion

M. phaseolina under the research conditions presented a higher growth in low humidity percentage conditions (8%) and temperatures from 28 to 32ºC, finding sclerotia on even 20 cm of depth in the soil. It was proved that there was a high correlation among the factors temperature, humidity content and the number of g sclerotia of soil^{-1}. These two abiotic factors might explain the epiphyte of the fungus in areas characterized by high temperatures and drought conditions.

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