

EFFECT OF DIFFERENT SOIL MOISTURE REGIMES ON THE DEVELOPMENT OF THE CHARCOAL ROT DISEASE OF SOYBEAN BY *MACROPHOMINA PHASEOLINA*

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(Received 21 June 1995; Revision accepted 12 October, 1999)

ABSTRACT

Disease index (DI) was significantly low (2.0; $P = 0.05$) when 14-day old soybean seedlings, inoculated with *M. phaseolina* were watered regularly to maintain a high (60-70%) soil moisture level. But DI was high (5.0) when inoculated seedlings were water-stressed and grown at low (10-20%) soil moisture levels. In inoculated, watered seedlings, the mycelium of the pathogen was re-isolated on potato dextrose agar (PDA) plates from tissues 1-2cm from the points of inoculation. Water-stressed seedlings yielded the fungus from tissues 5-7cm from the points of inoculation. The study showed that high soil moisture levels were unfavourable for the growth and pathogenicity of *M. phaseolina*, while low soil moisture levels favoured the growth and pathogenicity of the fungus in the humid tropics of SouthWestern Nigeria.

Key Words: Charcoal rot, *Macrophomina phaseolina*, disease escape, soil moisture, SouthWestern Nigeria.

Running title: Charcoal rot disease and soil moisture levels.

INTRODUCTION

The charcoal rot disease of soybean (*Glycine max* L. Merr.) incited by *Macrophomina phaseolina* (Tassi) Goid is becoming a major constraint on soybean production in Nigeria (Wokocha, 1976; IITA, 1996). This is just as the crop has become increasingly more acceptable and important in the Nigerian diet due to its high (34-38%) protein content.

Several forms of environmental and physiological stresses are associated with the incidence and severity of the charcoal rot disease on different crops. Hoffmater *et al.*, (1943) observed that the invasion of host plants by *M. phaseolina* was favoured when such plants were subjected to environmental stresses or wounds. Edmunds (1964), Ilyas and Sinclair (1974) reported increased severity of the disease on soybean with increased plant age.

Jooste (1969) noted that adequately watered, inoculated maize plants were more resistant to charcoal rot infection than those growing on dry soil.

Several other workers have reported the favourable effect of low water potentials in plants infected by the pathogen on rapid disease progression (Vasudeva, 1937; Hsi, 1961; Ghaffar and Erwin, 1969). Soil moisture content, therefore appeared to be critical to infection and disease development in crops attacked by *M. phaseolina*.

The aim of the present investigation was to study the effect of high and low soil moisture regimes on the charcoal rot disease of soybean. Information gained could be useful in the selection of suitable planting date for soybean in the rain forest zone of South-Western Nigeria.

MATERIALS AND METHODS

Soybean seeds (cultivar Samsoy 1) obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, were surface disinfected for 5 minutes in commercial bleach, washed in several changes of sterile distilled water, and sown in heat-sterilized soil in 37 x 28 x 13cm wooden trays.

INOCULUM PREPARATION AND SEEDLING INOCULATION

Hyphal mat from ten, 3-day old potato dextrose agar (PDA) cultures of *M. phaseolina*, incubated at $30 \pm 1^\circ\text{C}$, was scraped aseptically onto a membrane filter and washed in several changes of sterile distilled water with the help of an electric vacuum pump to remove traces of stalling materials. The mat was then transferred aseptically into 200ml of sterile distilled water containing 5% glucose solution in waring blender and homogenized for one minute at low speed. The concentration of inoculum suspension was adjusted to 2×10^7 sclerotial and hypha fragments/ml, using a haemocytometer. This concentration was used for the inoculation of seedlings. Two-week old soybean seedlings were carefully lifted from soil in the wooden tray, the roots washed free of soil and seedling stem bases wounded. Wounds were made gently as vertical stokes, using flame sterilized, cool inoculating needle. Seedlings were then inoculated by dipping wounded stem bases for 3 minutes into 25ml suspension of the inoculum in 50ml flasks. Inoculated seedlings were transplanted into sterilized soil in 15 x 13cm plastic pots at the rate of two seedlings per pot. Non-inoculated control seedlings were similarly treated except that they were dipped into sterile distilled water. Inoculated and non-inoculated seedlings were each separated into two lots comprising two hundred seedlings each. All seedlings were watered immediately after transplanting into pots and placed in the green house at temperatures of 29-31°C.

INDUCING DIFFERENT SOIL MOISTURE REGIMES

After the initial watering, half of the

inoculated seedlings were subjected to a low soil moisture regime (10-20%) according to the method of Ghaffar and Erwin (1969). The pots were left unwatered for several days during which soil samples were taken for soil moisture determination. Seedlings were allowed to remain at the desired soil moisture level until symptoms of wilt appeared. Soil in the pots was again watered to relieve the stress. The seedlings were thus subjected to alternate periods of soil moisture stress throughout the 21-day period of observation. The other half of inoculated seedlings were regularly watered to maintain the seedlings at a high soil moisture regime (60-70%), regularly checked. Control seedlings were similarly treated.

To determine soil moisture levels, soil samples taken daily from each lot were composited separately and used to determine the moisture holding capacity (MHC) of soil in each treatment. The percentage soil moisture (based on the weight of oven-dried soil) was calculated according to the formula of Johnson and Curl (1972):

$$\frac{W \times 100}{Y}$$

Y

Where W is the weight of water lost after oven-drying a known quantity of soil at 80°C to constant weight and Y, the weight of the oven-dried soil.

In order to determine how far the mycelium of *M. phaseolina* progressed within inoculated soybean seedlings under different moisture regimes, root, stem and leaf samples were taken from randomly selected inoculated plants from both high and low soil moisture regimes and from control seedlings at the end of the observation period and plated on PDA plates. Disease severity on inoculated seedlings of both treatments was evaluated 21 days after inoculation on a scale of 1-5 as follows:

- 1 = No visible disease symptom
- 2 = Less than 15% of seedling hypocotyl infected
- 3 = 15% - 29% of seedling hypocotyl infected
- 4 = 30% - 49% of seedling hypocotyl infected
- 5 = more than 50% of seedling hypocotyl infected

RESULTS AND DISCUSSION

Results in Table 1 show that disease index was significantly low (2.0) ($P = 0.05$) when soybean seedlings inoculated with *M. phaseolina* were regularly watered and maintained at a high (60-70%) soil moisture level. When the seedlings were water stressed and grown at low (10-20%) soil moisture level, a disease index of 5.0 was recorded. Counts of yellowing cotyledons (a symptom of the charcoal rot disease) made four days after inoculation also showed that only 10% of cotyledons of seedlings grown at high soil moisture levels yellowed, while 50% of cotyledons of seedlings subjected to soil moisture stress yellowed during the same period. No disease symptoms were observed in control seedlings at both soil moisture regimes. In inoculated plants grown at low soil moisture level, the mycelium of *M. phaseolina* was readily re-isolated from tissues 5-7cm from the point of inoculation at the base of the stem. In regularly watered seedlings grown at high soil moisture level, only tissues 1-2cm from the point of inoculation yielded the pathogen on PDA plates.

Results obtained in this investigation are similar to observation made on the disease and the pathogen by Vasudeva (1937), Hsi (1961) and Ghaffar and Erwin (1969). While high soil moisture level of 60-100% have been found to cause almost total decline in sclerotial populations of *M. phaseolina* in soil (Dhingra and Sinclair, 1975), dry soil was reported to have a favourable effect on the development of the charcoal rot disease (Jooste, 1969). Cook (1973) therefore, related pathogenicity in this fungus to the existence in plants of a host-pathogen water relationships. Such a relationship, seems unique in the case of *M. phaseolina* infection. High host-pathogen water relationships which usually increase disease caused by most pathogens in the tropics, depressed it in the presence of *M. phaseolina* (Jooste, 1969). These observations support findings made in this study that high soil moisture levels are unfavourable to and limit the growth and

Table 1: Effect of High and Low Soil Moisture Regimes on the Severity of the Charcoal Rot Disease of Soybean incited by *M. phaseolina* in Southwestern Nigeria.

SOIL MOISTURE REGIME (%)	DISEASE	YELLOWING
	INDICES	COTYLEDONS (%)
60-70 (Watered regularly)	2.0 ^a	10 ^a
10-20 (Water stressed)	5.0 ^b	50 ^b
Control (Watered regularly)	0 ^c	0 ^c
Control (Water Stressed)	0 ^c	0 ^c

Values within columns followed by the same letter are not significantly different ($P=0.05$) according to Duncan's Multiple Range Test.

pathogenicity of *M. phaseolina*, while low soil moisture levels greatly increase the severity of the charcoal rot disease and the growth of the pathogen in infected plants. At high soil moisture levels, most inoculated plants did not manifest disease symptoms throughout the period of observation and therefore may have been symptomless carriers of the disease. It is therefore plausible that soybean crop grown during the wetter parts of the year, particularly April - August, in the South-Western rain forest zone of Nigeria is more likely to escape damage due to the charcoal rot disease than that cropped during the drier months (October - March).

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