Characterization of CORN inbred lines for disease resistance

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PREFACE

The characterization of corn inbred lines for disease resistance represents a fundamental basis for advances in genetic improvement programs. In an agricultural context marked by increasing production demands, intensified cropping systems, and increasingly complex phytosanitary challenges, understanding the mechanisms that confer resistance to the main foliar diseases affecting corn is essential.

This book emerged from the development of a doctoral research project in which the comprehensive evaluation of corn inbred lines yielded not only scientifically relevant findings but also highlighted the necessity to systematize and disseminate information that contributes to the advancement of scientific knowledge. Accordingly, this work was conceived with the objective of expanding the accessibility of scientific evidence, fostering the integration of research, education, and agricultural practice.

The primary objective of this book is to present the conceptual and methodological foundations for the characterization of corn inbred lines with respect to disease resistance, enabling readers to critically interpret phytopathological assessment criteria and the genetic principles that govern the selection of superior inbred lines. The content is structured to integrate concepts from genetic improvement and plant pathology, thereby providing an applied and strategic framework for these disciplines. This book is intended for individuals with an academic or professional interest in corn genetic improvement and plant pathology, including students, researchers, and practitioners involved in the development of disease-resistant cultivars adapted to diverse production environments. The chapter organization supports both sequential reading and targeted consultation, depending on the specific objectives of the reader.

This work is expected to serve as a foundational reference for research in plant pathology applied to corn inbred lines, promoting further investigation, methodological advancement, and scientific contributions that foster the development of sustainable agricultural systems.

CONTENTS

CHAPTER 1

INTRODUCTION

Belisa Cristina Saito

João Antonio da Costa Andrade

The corn (*Zea mays L.*) is a gramineae belonging to the *Poaceae* family, tribe *Maydeae*, diploid species (2n=20), monoecious and allogamous. It is cultivated worldwide between latitudes 58° North and 40° South, distributed in the most diverse altitudes, from locations below sea level until regions with more than 2,500 m of altitude (FANCELLI; DOURADO NETO, 2000). In Brazil, the crop is the second most extensively cultivated, being present in the whole national territory. It is estimated that for the 2016/17 season there will be an increase from 0.8% to 6.4% of the planted area, which in the previous year reached 5,387 thousand hectares. The estimated grain yield for the 2016/17 season will be approximately 83.1 millions tons of grain (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2016).

High losses in grain yield are associated with the incidence of diseases, several disease monitoring studies have been carried out by Brazilian Agricultural Reseach Corporation - Maize & Sorghum (EMBRAPA) and by the private sector. These studies have demonstrated that gray leaf spot, southern rust, tropical rust, common rust and corn stunt are among the main diseases of corn (CASELA et al., 2006).

Due to the peculiar characteristics of the crop, such as the size of the plant, extension of the planting area and the economic yield, the use of genetic resistance is more viable for controlling the disease. Although chemical control is currently well accepted in high tech commercial crops, two decades ago it was viable only in seed production fields (GIANASI; CASTRO; SILVA, 1996). The most efficient strategy for disease control in corn is the identification and introduction of resistance genes, aiming at the production of resistant hybrids to most of the diseases that affect corn crop.

With the development of corn inbred lines in Ilha Solteira – SP, it became interesting to check in greater detail the variation in resistance to disease among them. Thus, the objective of this study was to identify resistant and susceptible corn inbred lines based on the stability and adaptability parameters and Area Under the Disease Progress Curve (AUDPC) for disease symptoms of tropical rust (*Physopella zeae* (Mains) Cummins & Ramachar.), southern rust (*Puccinia polysora* Underw), gray leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels), northern leaf blight (*Exserohilum turcicum* (Pass.) Leonard & Suggs), physoderma brown spot (*Physoderma maydis*) and phaeosphaeria leaf spot (*Phaeosphaeria maydis* in association with *Pantoeae ananas*). These inbred lines are promising to produce resistant synthetics.

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CHAPTER 2

LITERATURE REVIEW

Belisa Cristina Saito

João Antonio da Costa Andrade

2.1 DISEASES IN CORN CROP

There are several diseases that affect the corn crop and they are responsible for reducing the grain yield and reducing the quality of grains and seeds. Losses due to diseases vary from year to year and their occurrence is strongly influenced by the environment. Some diseases may occur generally but do not cause much damage, while others may be potentially more harmful, depending on the disease, season and susceptibility of the genotypes (ROBERTSON et al., 2008).

2.1.1 Rust

According to Pinho et al. (1999), rust is one of the diseases that affect corn crop since these were observed in most producing regions, causing crop limitation, such as direct damage to the plant by reduction of the photosynthesizing area, which may lead to a reduction in grain yield of the crop.

Rust is caused by fungi, and the name of the disease is related to the ferruginous aspect presented by the mass of spores present in the central region of the pustules (REIS; CASA, 1996). In the corn crop, there are common rust, caused by *Puccinia sorghi* Schw, southern rust, caused by *Puccinia polysora* Underw, and tropical rust, caused by *Physopella zeae* (Mains) Cummins & Ramachar.

Rust can cause substantial losses in grain yield in corn crop (CHEN et al., 2004). Costa et al. (2010) reported that in 2009/2010 crop, southern rust was responsible for severe epidemics in many corn producing regions in the states of Paraná, Santa Catarina and Rio Grande do Sul, requiring the application of fungicides for their control. For Reis, Casa and Bresolin (2004) the losses data from common rust have not yet been quantified in isolation. As for tropical rust, there are also no reports in the literature of the economic impact caused by the incidence of this fungus in corn.

Many authors suggest that the use of hybrids or varieties with satisfactory levels of resistance to the pathogen as being the most efficient and least expensive control method to rust (MCGEE, 1988; PINTO; FERNANDES; OLIVEIRA, 1997; SHERF; MACNAB, 1986).

2.1.2 Gray leaf spot

The etiological agent of gray leaf spot is *Cercospora zeae-maydis* Tehon & E.Y. Daniels. This is the most important disease in corn crop (BRITO et al., 2007). In Brazil, the disease was first reported in 1953 (CHUPP, 1953), in São Paulo. It was reported for the first time in the 2000/2001 crop and since then has been occurring generically, causing significant reductions in corn crop (FANTIN, 2004). In other corn-producing states, gray leaf spots have also caused significant damage (FANTIN et al., 2001; PINTO; ANGELIS; HABE, 2004; REIS; SANTOS; BLUM, 2007).The symptoms of gray leaf spot appear first in the lower leaves around 2 or 3 weeks before tasseling. The lesions are rectangular in shape and are delimited in width by the main nerves of the leaf. The lesions present brown coloration and in high humidity conditions (90%), with temperatures ranging from moderate to high (22 a 32 °C) and cold nights with dew, dense sporulation occurs, making the leaves gray, characteristic of this disease (CASELA; FERREIRA, 2003; ROBERTSON et al. 2008).

According to Brito et al. (2007), the pathogen colonizes a large part of the leaf tissue, reducing the photosynthetic area, leading to early senescence and, consequently, to the reduction of grain yield. The same authors evaluated 12 commercial corn hybrids on the incidence of gray leaf spot, evidenced that the level of damage caused by the pathogen varies between planting dates and hybrids, that the reduction in grain yield mainly related to late planting date and that the use of resistant hybrids does not require chemical control of the disease. Munkvold et al. (2001), also suggest that the main strategy of control of gray leaf spot is the use of resistant hybrids.

2.1.3 Northern leaf blight

According to Reis and Casa (1996), three similar diseases are described in corn crop in Brazil: northern leaf blight, southern leaf blight and helminthosporium leaf spot. Northern leaf blights, the most frequent, is caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs (sin. *Helminthosporium turcicum* Pass.). It is distributed in most of crop producing regions of Brazil, constituting one of the main phytosanitary problems of this crop, with losses in grain yield reaching 60% in susceptible genotypes (RAYMUNDO; HOOKER, 1981). Symptoms of the disease appear approximately one week after the onset of the infection, characterized by elliptical lesions of straw staining that measure from 2.5 to 15 cm in length, with well-defined edges that become dark because of the fruiting of the fungus (WORDELL FILHO; CASA, 2012). The development of northern leaf blight is favored by the temperature between 18º and 27ºC, with an optimum temperature of 20ºC and the presence of dew on the surface of the leaves (SABATO et al., 2013).

In Brazil, the disease occurs in greater intensity in the second crop causing the greatest damage when infecting plants during the flowering period. For Fernandes and Oliveira (2000) the development of *E. turcicum* is negatively correlated with photoperiod, light intensity and sugar concentration in corn plants. These conditions are more frequently observed in the second crop, which could explain the greater severity of this pathogen at that time.

Many authors described the mechanisms of genetic inheritance associated with northern leaf blight. The disease is mainly controlled by the use of resistant cultivars through quantitative (non-specific) and qualitative (race-specific) resistance. Qualitative and quantitative resistance sources have been described, however, qualitative resistance is easily breakable in the presence of a virulent lineage (WELZ; GEIGER, 2000). The quantitative resistance confers partial resistance, in the case of northern leaf blight, causing a reduction in the development of the disease and the percentage of affected leaf area that can result in the expression of several components, including the incubation period, latent period, sporulation intensity size, number and rate of lesion growth (CARSON; GOODMAN, 2006; HURNI et al., 2015; PARLEVLIET, 2002).

2.1.4 Physoderma brown spot

Physoderma brown spot is caused by the fungus *Physoderma maydis*, commonly occurring in regions with high temperatures and high precipitations, the first symptoms of the disease usually appear on leaf limbs and nerves with chlorotic spots (LEÓN, 1984). According to Robertson et al. (2008), the pathogen is dormant in infected tissues or soil and produces innumerable zoospores in the presence of water. Leaf infection occurs at whorl when water is present for an extended period of time, occurs in a day cycle and requires a combination of light, free water and temperature between 23.8 and 29.4ºC. According to Fernandes and Balmer (1990), the brown spot is more severe in late plantings, carried out in low areas. There are few reports in the literature regarding the quantification of this disease in corn worldwide.

2.1.5 Phaeosphaeria leaf spot

Phaeosphaeria leaf spot, whose etiological agent is *Phaeosphaeria maydis* (PINTO; FERNANDES; OLIVEIRA, 1997) in association with the bacteria *Pantoeae ananas* (PACCOLA-MEIRELLES et al., 2001), is a disease that affects the major corn producing regions in Brazil and worldwide. Many researchers have argued that the disease is caused only by a fungus (*Phaeosphaeria maydis*), or only by the bacteria (*Pantoeae ananas*). However, evidence suggests that the symptoms are related to the joint action of both the fungus and the bacteria.

The incidence of phaeosphaeria leaf spot increased significantly since 1990, causing damage mainly when planting occurs in rainy periods and mild temperatures. Losses are grain yields associated with this disease may reach 60% (WORDELL FILHO; CASA, 2012). The symptoms of this disease are related to the appearance of irregular green, dark-green leaf spots that appear on the lower leaves, passing to the higher leaves of the plant. Subsequently, the lesions become necrotic of straw coloration being able to coalesce. The symptoms may present in different severities depending on the corn genotype (PACCOLA-MEIRELLES et al., 2002; REIS; CASA; BRESOLIN, 2004). Sawazaki et al. (1997) suggest that under conditions of frequent and well-distributed rains, the pathogen can cause greater severity and drastically affect the grain yield.

Lopes et al. (2007) studied the control of resistance of phaeosphaeria leaf spot from the evaluation of the means of the generations originating from the crossing between two resistant and one susceptible inbred line. These authors concluded that the additive gene effects predominate in the resistance to the phaeosphaeria leaf spot and that the characteristic has high heritability, which facilitates the genetic improvement.

2.2 DISEASES EVALUATION

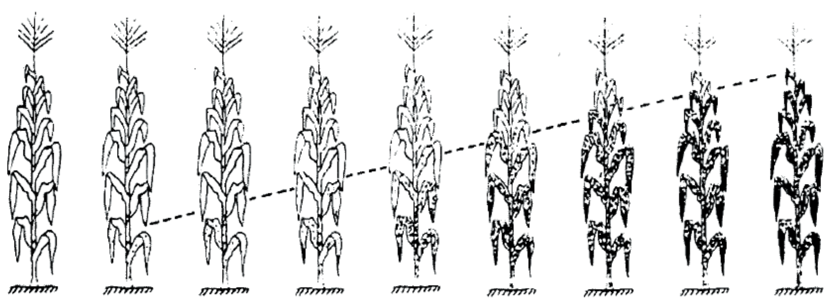
The quantification of plant diseases, known as plant pathophysiology, is necessary for the study of disease control measures, the determination of fungicide efficiency or the characterization of varietal resistance, as well as for epidemiology, in the construction of diseases progress curves and in estimating the damage caused by it (AMORIM, 1995).

The most common terms used in pathophysiology are incidence and severity, terms that are often misused. Incidence refers to the percentage (frequency) of diseased plants or parts of diseased plants in a sample or population. Severity is the percentage of area or volume of tissue covered by symptoms (BERGAMIN FILHO; AMORIM, 1996).

The severity parameter is the most appropriate to quantify foliar diseases in corn, the percentage of tissue area covered by symptoms represents the intensity of the disease better than the incidence. Several methods are described in the literature regarding the quantification of diseases in corn, with more frequent use of diagrammatic scales or scale of notes (AMORIM, 1995). The notes can be attributed to the entire crop, to an experimental plot or to an individualized plant. The best time to determine the resistance should be made at the phenological stages R3 or R4 (SABATO; PINTO; FERNANDES, 2013).

Diagrammatic scales are illustrated representations of a series of plants,parts of plants with symptoms at different levels of severity (Figure 1). These scales are the main tool for assessing the severity of many diseases (BERGAMIN FILHO; AMORIM, 1996).

Figure 1 - Diagrammatic scale for evaluating the incidence of foliar diseases in corn, according to Agroceres (1996).



Affected leaf area (%)								
0	1	10	20	30	40	60	80	> 80
Ratings								
1	2	3	4	5	6	7	8	9
Type of reaction								
HR	R	R	MR	MR/MS	MS	S	S	HS

HR – highly resistant. R – resistant. MR – moderately resistant. MR/MS – moderately resistant/moderately susceptible. S – susceptible. HS – highly susceptible.

Source: Agroceres (1996).

2.2.1 Disease progress curves

The disease progress curve is the best way to represent an epidemy, and it is usually expressed by disease versus time proportion. With the use of disease progress curves, it is possible to characterize the interactions between pathogen, host and environment, to define control strategies and to predict future levels of disease

(BERGAMIN FILHO; AMORIM, 1996). Disease progress curves can be constructed for any pathosystem, with the important parameters being the time of onset of the epidemy, the initial inoculum amount (x_0), the rate of disease increase (r), the shape of the progress curve of the disease, the area under this curve (AUDPC), the maximum (x_{max}) and final (x_f) amounts of disease and the duration of the epidemy (BERGAMIN FILHO, 1995).

The use of mathematical models to analyze disease behavior or progression over time is an important tool for phytopathologists. There are six mathematical models used for this purpose: the exponential model, logistic model, Gompertz model, monomolecular model, Richards model and the time-dependent model (BERGAMIN FILHO, 1995).

2.3 BREEDING FOR DISEASE RESISTANCE

Several studies report that large yield losses of corn are associated with the incidence of diseases (BRANDÃO et al., 2003; JULIATTI et al., 2004; SANTOS et al., 2011). The incidence and severity of the diseases that affect the corn crop have been attributed mainly to the planting of corn in the straw without the adoption of crop rotation and also due to crop overlap. The incidence and severity of the diseases depend on susceptibility of genotypes, the concentration of inoculum, the race or aggressiveness of the pathogen and favorable environmental conditions, provided by the climate, soil, cropping system or inadequate crop management. Under favorable conditions and susceptible genotypes, different diseases can occur in high severity (PINTO; OLIVEIRA; FERNANDES, 2007).

The most efficient control of diseases of the corn crop is the identification and introduction of resistance genes, aiming at the obtention of resistant hybrids and varieties. Many studies have been developed aiming at the identification and control of resistance mechanisms to many diseases (CHUNG et al., 2011; JINES et al., 2007; ZHANG et al., 2012). For Brito et al. (2012), one of the main causes of instability in the use of commercial hybrids in Brazil is the high disease severity, due to variations in the pathogen population, mainly caused by the planting of susceptible hybrids and changes in the production system.

Although the use of resistant cultivars is the most efficient and economical strategy for disease control, Yorinori and Kiihl (2001) consider that, for most diseases, the degree of resistance is insufficient to avoid losses to the level of economic damage and requiring the adoption complementary measures for its control. For diseases in which there is a source of resistance, this may be ephemeral, since the pathogen can develop new races or biotypes, before a new resistance gene. These same authors also suggest that it is fundamental to adopt integrated control strategies, where genetic resistance is one of the elements of the set of measures to be taken for maximum productivity, stability and profitability. In this context, the quantitative resistance, conferred by numerous loci of small effect, is also interesting, since it is more stable.

2.3.1 Interaction Genotypes x Environments

The interaction between genotypes x environments (G x E), can be defined as a change in the relative performance of one trait, of two or more genotypes, measured in two or more environments. This is because the effect of the environment, almost always, is different in each of the genotypes. Interaction may, therefore, cause changes in the order of classification of genotypes in each environment and changes in the absolute and relative magnitude of genetic, environmental and phenotypic variances between environments. (BOWMAN, 1972). This fact demands that the breeding is carried out under the conditions in which the genotype will be used. This interaction is characterized when the behavior of the races, inbred lines or cultivars are not consistent in the different environments, that is, the response of each genotype is specific and different from other genotypes to the changes that occur in the environments (RAMALHO et al., 2012). The effect of the interaction GxE describes the differential behavior of the genotypes in contrasting environments (COIMBRA et al., 2009). It is important to evaluate the magnitudes of the interactions GxE, since this knowledge guides the planning and the strategies of the improvement in the recommendation of cultivars, besides being determined in the phenotypic stability of cultivars, for a determined region (VENCOVSKY; BARRIGA, 1992). The use of phenotypic stability in the selection, in the early stages of breeding, is still rare, but it can be implemented in some cases where it is possible to evaluate a reasonable number of genotypes in several environments, which may even include different planting dates. For resistance to diseases this is still more valid because the potential of inoculum and conditions favorable to the various diseases vary throughout the year.

The analysis of the interaction GxE is important for breeding programs, because it provides the base for selection to broad or specific adaptation, to choose selection environments, to identify the level of stress in the selected environments and to indicate satisfactory levels of resistance (FOX; CROSSA; ROMAGOSA, 1997). Thus, the identification of genotypes with high adaptability and phenotypic stability is the most advantageous way to explore the interaction GxE (PEREIRA et al., 2009).

2.3.2 Adaptability and stability

Many authors describe the concepts of phenotypic adaptation and stability, as well evaluation methods. Mariotii et al. (1976) describe the adaptive term as a potential capacity to develop environmental performance assessment systems, and stability is considered as an ability to generate performance data in environmental assessment systems. Chaves (2001) suggest that most methods of adaptability and stability analysis, using regression techniques, measure the variation of the quantitative character in relation to an environmental index. The methods differ according to the type of regression model used and the way of determining the environmental index.

According to Cruz (2006), many methods have been proposed for the analysis of adaptability and stability, aiming to evaluate genotypes in different environments. These methods are based on G x E interaction and are distinguished from the concepts of stability and adaptability adopted and certain statistical principles selected. Among these are the methods based on analysis of variance (YATES; COCHRAN, 1938; PLAISTED; PETERSON, 1959; WRICKE, 1965; ANNICCHIARICO, 1992), linear regression (FINLAY; WILKINSON, 1963; EBERHART; RUSSELL, 1966; TAI, 1971), bissegmented regression (VERMA; CHAHAL; MURTY, 1978; SILVA; BARRETO, 1985; CRUZ; TORRES; VENCOVSKY, 1989), nonparametric analysis (HUEHN; NASSAR, 1990; LIN; BINNS, 1988), factor analysis (MURAKAMI; CRUZ, 2004) and main components (centroid and AMMI). The choice of the adaptability and stability analysis method depends on the experimental data, mainly related to the number of environments available, the precision required and the type of information desired (CRUZ; REGAZZI; CARNEIRO, 2012).

The analysis of adaptability and stability widely used by corn breeders is the methodology proposed by Eberhart and Russell (1966).

2.3.2.1 Method proposed by Eberhart and Russell

Finlay and Wilkinson (1963) proposed a methodology to evaluate the genotype performance for each genotype, adjusting a simple linear regression of the dependent variable in relation to the environmental index. Eberhart and Russell (1966) expanded the model proposed by Finlay and Wilkinson (1963), in that both the regression coefficients of phenotypic values of each genotype in relation to the environmental index and deviations from that regression would provide parameter estimates of stability and adaptability (CRUZ; REGAZZI; CARNEIRO, 2012).

Eberhart and Russell (1966) proposed a method of adaptability and stability study based on regression analysis. The parameters that express adaptability and stability are the average, the linear response to the environmental variation and the deviation of the regression of each genotype, obtained from the model:

$$Y_{ij} = \beta_{oi} + \beta_{1i} I_j + \delta_{ij} + \varepsilon_{ij} \quad (1)$$

Where:

β_{oi}: Overall average of genotype *i* (*i* = 1, 2, .., g);

β_{1i}: Linear response of genotype *i* to environmental variation;

I_j: Environmental index (*j* = 1, 2, ..., a), being $I_j = \frac{Y_{.j}}{g} - \frac{Y_{..}}{ga}$;

δ_{ij}: Regression deviation;

ε_{ij}: Average experimental error.

According to this method, for disease symptoms analysis an ideal cultivar is one with an overall mean (β_o) around 1, a linear regression coefficient (β_1) lower than 1 and a variance of the regressions deviations (σ^2_{di}) equal to zero. Such a value of $\beta_1<1$ indicates that the genotype did not increase the symptoms of the disease with the improvement of the environment for disease. The variance of the regression deviations should be the smallest possible, close to zero, indicating that the cultivar modifies with the environmental variations in a predictable way, that is, following a perfect forecast line. With σ^2_{di} high, the behavior of the genotype will be unpredictable. If $\beta_1 = 1$ the genotype will be responsive to environmental improvement for disease, but in this case, for disease symptoms this is not interesting. Being $\beta_1 > 1.0$ the cultivar is less responsive and less demanding, being suitable for environments of inferior quality for disease, because the disease decreases quickly in these environments.

There are few reports in the literature using the Eberhart and Russell (1966) methodology for evaluating the resistance to disease (PINHO et al. 2001; BRITO et al., 2011). However, this methodology is widely used to evaluate stability and adaptability of grain yield in corn (CHANGIZI et al., 2014; KHALIL, 2013; BUSANELLO et al., 2015), sugarcane production (BARBOSA et al.,2015; FERRAUDO; PERECIN, 2014); soybean (SILVEIRA et al., 2016), wheat (HUANG et al., 2016) and peanuts (VASCONCELOS et al., 2015).

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CHAPTER 3

RESISTANCE OF CORN INBRED LINES TO FOLIAR DISEASES IN TWO PLANTING DATES

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3.1 INTRODUCTION

High losses in grain yield in corn are associated with the incidence of diseases. Disease monitoring studies have demonstrated that rust, gray leaf spot and Phaeosphaeria leaf spot are among the major diseases that affect the corn crop in Brazil (CARSON, 2005; CASELA et al., 2006).

Due to the characteristics of corn growing in Brazil, such as plant height, length of the planting season and economic yield and, in some cases, continuous planting of corn years, the most viable measure to control disease is use of genetic resistance. For nearly two decades chemical control was practically viable only in seed production fields (GIANASI et al.,1996). Currently crops grown with the highest level of technology, with higher income potential, can often economically use chemical control for these diseases, however, the use of genetic resistance is preferred. For the farmer, the desired resistance is in the hybrid planted, but breeders must also worry about resistance in the parental lines that give rise to these hybrids. In addition, resistant inbred lines can be used for adding resistance to other inbred lines and better performance in future hybrids. There is also the possibility of using synthetics from resistant inbreds as commercial varieties for corn producers with lower technological level.

Many reports in the literature indicate that there is genetic variability in cultivars in disease resistance (NIHEI; FERREIRA, 2012; VIEIRA et al., 2012; ZAMBRANO et al., 2014); however, few papers discuss genetic resistance to diseases in inbred lines. Colombo et al. (2014) reported that the Area Under the Disease Progress Curve (AUDPC) can quantify the progression of disease during a certain period, and it has been frequently used to evaluate the level of resistance in field conditions.

The objectives of this study were to identify inbred lines resistant to tropical rust (*Physopella zeae* (Mains) Cummins & Ramachar.), southern rust (*Puccinia polysora* Underw), gray leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels), northern leaf blight (*Exserohilum turcicum* (Pass.) Leonard & Suggs), physoderma brown spot (*Physoderma maydis*) and phaeosphaeria leaf spot (*Phaeosphaeria maydis* in association with *Pantoeae ananas*), using the area under disease progress curve, in two planting dates. Evaluate resistant inbred lines which may use for obtaining synthetics and determinate the better planting date for disease resistance evaluations.

3.2 MATERIAL AND METHODS

Fifty inbred lines were used, eighteen derived from the Isanão-VF1 population, nine from the Isanão-VD1 population, ten from the Flintisa population, eight from the Dentado population and five from EMPASC 151- Condá. The first two populations are brachytic, the others have normal height. Flintisa and Dentado lines were obtained from the corn breeding program of São Paulo State University – UNESP – Ilha Solteira – SP (Brazil). EMPASC 151- Condá is an old open pollinated variety from the state of Santa Catarina (Brazil).

The experiments were conducted at the Fazenda de Ensino e Pesquisa da UNESP - Ilha Solteira, located in Selvíria – Mato Grosso do Sul (MS) - Brazil (20° 20'S, 51° 23› and the altitude of 335 m). The climate of the region, according to Köppen classification, is Aw, defined as tropical humid with a rainy season in summer and dry in winter. The average annual rainfall is 1,330 mm, with the average annual air temperature of about 25°C and average humidity of 66% (CENTURION, 1982).

The fifty experimental inbred lines were evaluated in a randomized block design with three replications in two seasons (planting on Feb,20,2014 and Apr,17,2014). Each plot was a single row 8 m in length with spacing of 0.45 m between plots and an average of 0.4 m between plants. Planting was with normal tillage, irrigated by center pivot, with twice the number of seeds needed and thinned at six fully developed leaves. Fertilization was done according to soil analysis with 300 kg ha^{-1} of 8-28-16 applied followed by 250 kg ha^{-1} of urea at the 6 leaf stage.

The inbred lines were evaluated for tropical rust (TR), southern rust (SR), gray leaf spot (GLS), northern leaf blight (NLB), physoderma brown spot (PBS) and phaeosphaeria leaf spot (PLS). Evaluations were carried out at 45, 60, 75 and 90 days after planting, determining the severity of disease based on the percentage of symptoms of the plot, according to the diagrammatic scale suggested in Agroceres Guide to Sanity (AGROCERES, 1996). The ratings were assigned values of 1, 2, 3, 4, 5, 6, 7, 8 and 9, corresponding to 0, 1, 10, 20, 30, 40, 60, 80 and> 80% of leaf symptoms, respectively. These inbred lines have already been selected for good yielding in crosses.

The Area Under the Disease Progress Curve (AUDPC) for each disease was calculated as suggested by Campbell and Maddenn (1990):

$$AUDPC = \sum_{i=1}^{n-1} \left(Y_{i+1} + Y_i\right)\left(T_{i+1} - T_i\right) \quad (2)$$

Where:

Y_i: severity of the disease at the stage of evaluation i (i = 1,...n).

Y_{i+1}: severity of the disease at the stage of evaluation i+1.

T_i: evaluation stage i, is the number of days after planting.

T_{i+1}: evaluation stage i+1.

n: total number of evaluations.

For statistical analysis, the scores were transformed by $\sqrt{x+0.5}$, using the Genes software (CRUZ, 2013) for the individual analyses and the combined analyses of variance, Microsoft Excel 2010® was used for calculating the AUDPC.

Temperature and humidity were collected from the weather station located near the experiment (latitude: 20º 25' 24.4" and longitude: 51º 21' 13.1") for the period from February until July 2014 (Figure 2).

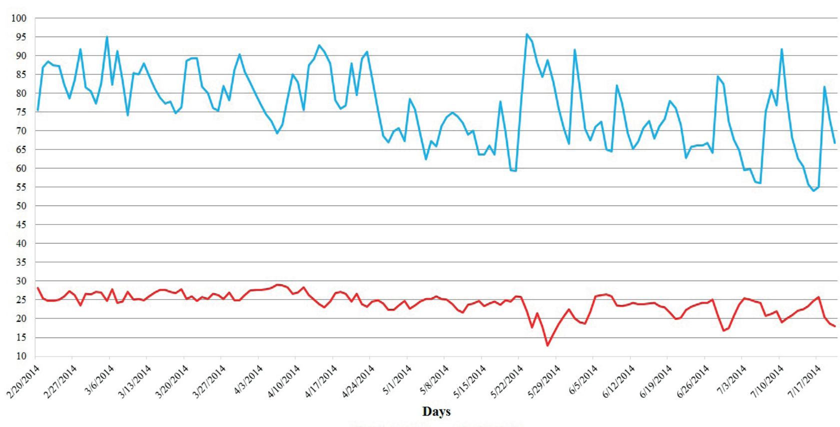


Figure 2 - Temperature and relative humidity in Ilha Solteira – SP, Brazil from February to July 2014.

Source: Canal Clima UNESP (2014).

3.3 RESULTS AND DISCUSSION

In the joint analyses of variance for AUDPC (Table 1), the F test for inbred line variation is significant for tropical rust (TR), southern rust (SR), gray leaf spot (GLS) and phaeosphaeria lead spot (PLS), showing that the inbred lines had different responses to the natural infection of these diseases. However, the discrimination among inbred lines observed in the joint analysis occurred for both planting seasons only for SR. The analyses of individual seasons (Table 2) indicated that the significance of the F test for inbred lines for TR and GLS occurred only for the first season, while for the PLS significance differences occurred only for the second season. Therefore, early planting can be used to select more resistant inbred lines for TR, SR and GLS while a later planting is more appropriate for PLS.

Table 1 - Joint analysis (mean squares) of Area Under the Disease Progress Curve (AUDPC) for tropical rust (TR), southern rust (SR), gray leaf spot (GLS), northern leaf blight (NLB), physoderma brown spot (PBS) and phaeosphaeria leaf spot (PLS). Selvíria - Mato Grosso do Sul (MS), Brazil, 2014.

Source of variation	**DF**	**TR**	**SR**	**GLS**	**NLB**	**PBS**	**PLS**
Inbred lines (L)	49	0.4665**	1.8852**	1.0177**	0.3414	0.0590	0.3511**
Seasons (S)	1	0.1611	22.2522*	0.3628	0.0758	8.2220**	2.0415*
Lx S	49	0.5005*	1.7671**	0.7771	0.2426	0.0545	0.2598
Error	196	0.2450	0.7678	0.5911	0.2618	0.0800	0.1999
Average		103.0	134.4	108.1	95.3	93.5	94.4
CV%		4.87	7.58	7.4	5.24	2.92	4.6

Nota: **. * Significant at 1% and 5% probability level for the F test.

Source: Prepared by author

For northern leaf blight (NLB) and physoderma brown spot (PBS) there was no discrimination among inbred lines in either season (Tables 1, 2, 3 and 4), with AUDPC average of 95.3 and 94.4, respectively, showing moderate resistance, inadequate conditions for the development of diseases or insufficient natural inoculum pressure. The possibility exists that the tested inbred lines are similar in levels of resistance to these two diseases. White (1999) suggests that NLB epidemics are related to temperatures around 20°C and relative humidity above 90%. These conditions of humidity were not observed in the two evaluation periods of this study. For the development of PBS, the optimum temperature is between 23°C and 30°C with constant water accumulation on the leaves and is favored by the presence of free water on the surface of leaves as described by Robertson et al. (2014), which was often observed in this study, showing weather conditions sufficient for the development of the pathogen in the tested inbred lines. As there were no differences between inbred lines, it can be considered that they have equal levels of resistance, although further assessment covering other planting dates are recommended for a more accurate conclusion on the subject. The use of a known susceptible check may be useful for this purpose, but in this work there was no prior information available to select a susceptible check, as this is the first report on inbred lines from São Paulo for resistance to these diseases.

Table 2 - Individual analysis of variance for Area Under the Disease Progress Curve (AUDPC) for both planting dates (season 1: 02.20.2014 and season 2: 04.17.2014) to tropical rust, southern rust, gray leaf spot, northern leaf blight, physoderma brown spot and phaeosphaeria leaf spot. Selvíria - Mato Grosso do Sul (MS), Brazil, 2014.

Source of variation	DF	Season 1	Season 2
		Tropical rust	
Blocks	2	1.1953	1.0820
Inbred lines	49	0.7426**	0.2243
Error	98	0.2610	0.2290
Average	-	102.65	103.4
CV%	-	5.04	4.70
		Southern rust	
Blocks	2	5.3528	0.1828
Inbred lines	49	2.2758**	1.3764*
Error	98	0.6445	0.8912
Average	-	140.85	128.05
CV%	-	6.78	8.36
		Gray leaf spot	
Blocks	2	0.7130	0.0427
Inbred lines	49	1.2002*	0.5946
Error	98	0.7427	0.4396
Average	-	109.05	107.2
CV%	-	8.27	6.40
		Northern leaf blight	
Blocks	2	1.5406	0.9401
Inbred lines	49	0.4039	0.1801
Error	98	0.3689	0.1547
Average	-	95.05	95.45
CV%	-	6.22	4.01

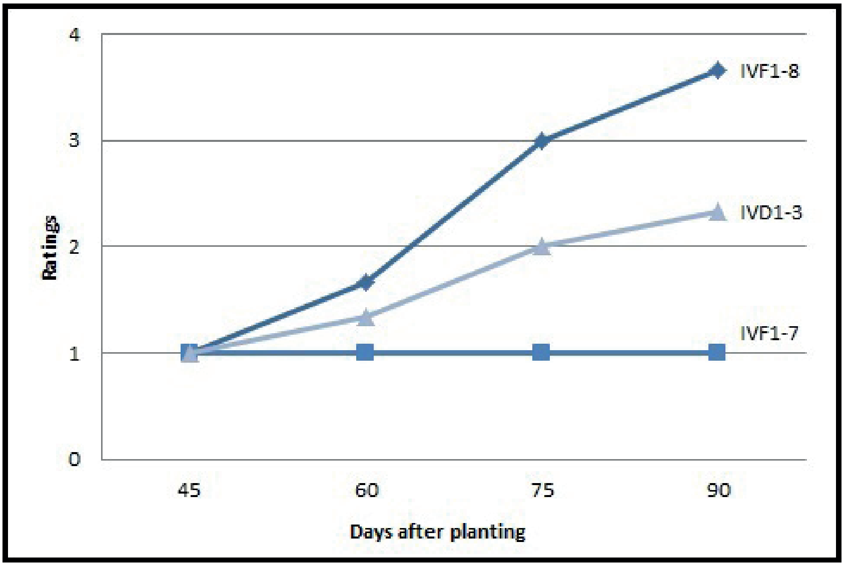
		Physoderma brown spot	
Blocks	2	0.0234	0.2258
Inbred lines	49	0.0119	0.1015
Error	98	0.0119	0.0893
Average	-	90.25	96.75
CV%	-	1.14	3.03
		Phaeosphaeria leaf spot	
Blocks	2	0.1257	0.2984
Inbred lines	49	0.2053	0.4056*
Error	98	0.1445	0.2552
Average	-	92.7	96.06
CV%	-	3.94	5.15

Nota **. * Significant at 1% and 5% probability level for the F test.

Source: Prepared by author

For seasons, significant differences were observed for SR, PBS and PLS (Table 1), with the highest incidence of the first two with planting in February and the highest incidence of PLS with planting in April (Tables 3 and 4). According to the analysis of temperature and humidity (Figure 2), it is possible to verify that in the period from February until May, which includes all the evaluations of the first season, temperature and humidity were higher, while during the second season peaks of higher temperature and humidity occurred on some evaluation dates. These conditions benefited the development of SR and PBS, hindered the development of PLS and were not sufficient to alter the symptoms of other diseases. For selection, only seasons when there is discrimination of genotypes are consequential. Favorable environmental conditions can be sufficient for the development of disease epidemics, provided that sufficient inoculum exists (FERNANDES; OLIVEIRA, 2000; ROLIM et al., 2007).

Figure 3 - Evolution of the scoring of the IVD1-3, IVF1-7 and IVF1-8 inbred lines for gray leaf spot in the first planting season, Selvíria - Mato Grosso do Sul (MS), Brazil, 2014.



Source: Prepared by author

Although it is possible select resistant genotypes when there is statistical discrimination, an issue to be discussed is what is the AUDPC limiting value to consider a genotype resistant to foliar diseases. By the Agroceres Guide to Sanity (AGROCERES, 1996), a genotype is considered resistant with score lowers than or equal to 3 at 30 days after silking. Projecting this for our evaluation dates, it would correspond to a score of 1 for 45 and 60 days, a score of 2 to 75 days and a score of 3 for 90 days. These values correspond to an AUDPC of 120, which can be regarded as a limit for a genotype to be considered resistant. Genotypes do not exhibit these scores exactly, but those with AUDPC less than 120 can be considered resistant. Taking as an example GLS, the IVF1-8 inbred line (Figure 3) is highly resistant, with AUDPC equal to 90, which indicates the absence of disease symptoms. The IVD1-3 inbred line is at the resistance threshold, with AUDPC equal to 120, while the IVF1-7 inbred line is considered susceptible (Figure 3). In commercial hybrids, thinking of the farmer's situation, this threshold could even be increased slightly, but in the selection of inbred lines for production of breeding proposes it is understood that accuracy should be stricter.

For NLB, PBS and PLS there was either not much disease or almost complete resistance. Later planting had slightly increased disease scores, but artificial inoculation may be necessary to discriminate among lines. GLS and both rests had a good range of disease scores, but only the early planting had a fully susceptible line (IVF1-7) for GLS. Both rests have a wide range of scores, but these was a general lock of resistance for SR and for more infection with the earlier planting (mean of 141 vs 128). While the mean scores for TR differed little between planting dates, only one line, 1F, was highly susceptible and that was only for the early planting. Overall, where discrimination among lines was possible, the earlier planting was most useful.

Analysis of the average cluster, the Scott-Knott test (Table 3), showed that there are inbred lines with different resistance levels for various diseases during the first season. In this context, the inbred lines that showed higher levels of resistance to tropical rust, southern rust, gray leaf spot, physoderma brown spot and phaeosphaeria leaf spot were: IVF1-3, IVF1-9, IVF1-10, IVF1-11, IVF1-25 and IVF1-230 from the Isanão-VF1 population; IVD1-2-1 and IVD1-12 from the Isanão-VD1 population; 2F, 3F and 6F from Flintisa population and the inbred line 4C from the Condá population. The inbred lines coming from the Isanão-VF1 had a higher frequency of inbred lines resistant to these diseases. The 1F, 5C and 9D inbred lines were the most susceptible to tropical rust, southern rust and gray leaf spot, respectively, and can be used as checks in future experiments of genotypes for evaluations of resistance to these diseases.

The analysis of the average cluster, the Scott-Knott test for the second season (Table 4) was significant only for phaeosphaeria leaf spot. The inbred lines with higher values of AUDPC for phaeosphaeria were 4C, 10F, 6F, 4F, 8D, 2D, IVD1-10, IVD1-3, IVD1-2, IVF1-12-1, IVF1-11 and IVF1-6-3. Only inbreds IVF1-11, 6F and 4C showed favorable AUDPC for the first season, while in the second season they had higher AUDPC. The analysis of the effect of seasons on phaeosphaeria leaf spot (Table 1) revealed that there were differences between the seasons, which is related to the fact that weather conditions were different in the two seasons. However, in this case, the effect of the seasons was essentially the same for all the inbred lines, as evidenced by no significant interaction of season x inbred lines. The results of this study suggest the need for further assessment, in other months of planting, for the correct evaluation of symptoms of northern leaf blight and physoderma brown spot.

Table 3 - Averages of inbred lines in season 1 (planting date 02.20.2014) for Area Under the Disease Progress Curve (AUDPC, non-transformed). Selvíria - Mato Grosso do Sul (MS), Brazil. 2014.

Inbred line	Tropical rust		Southern rust		Gray leaf spot		Northern leaf blight		Physoderma brown spot		Phaeosphaeria leaf spot	
IVF1-2-1	102.5	a	147.5	b	97.5	a	90	a	90	a	90	a
IVF1-3	97.5	a	132.5	a	135	b	112.5	a	90	a	90	a
IVF1-4	112.5	b	125	a	127.5	b	92.5	a	90	a	90	a
IVF1-5	105	a	125	a	112.5	b	90	a	90	a	90	a
IVF1-6-1	100	a	157.5	b	92.5	a	102.5	a	90	a	90	a
IVF1-6-2	102.5	a	157.5	b	117.5	b	90	a	90	a	90	a
IVF1-6-3	97.5	a	140	b	110	a	102.5	a	90	a	90	a
IVF1-7	92.5	a	100	a	150	b	90	a	90	a	90	a
IVF1-8	102.5	a	145	b	90	a	102.5	a	90	a	90	a
IVF1-9	92.5	a	122.5	a	90	a	90	a	90	a	90	a
IVF1-10	92.5	a	120	a	105	a	115	a	90	a	95	a
IVF1-11	97.5	a	122.5	a	97.5	a	90	a	90	a	90	a
IVF1-12	127.5	c	147.5	b	122.5	b	90	a	90	a	90	a
IVF1-12-1	102.5	a	160	b	97.5	a	90	a	90	a	92.5	a
IVD1-2	117.5	b	167.5	c	95	a	97.5	a	90	a	90	a
IVD1-3	90	a	112.5	a	120	b	90	a	90	a	90	a
IVD1-5	90	a	175	c	127.5	b	90	a	90	a	90	a
IVD1-8	127.5	c	147.5	b	125	b	90	a	90	a	95	a
IVD1-9	100	a	120	a	127.5	b	90	a	90	a	90	a
IVD1-10	105	a	197.5	c	115	b	90	a	90	a	90	a
IVD1-11	120	b	150	b	102.5	a	90	a	90	a	90	a
IVD1-2-1	100	a	132.5	a	102.5	a	107.5	a	90	a	90	a
IVD1-12	105	a	137.5	a	97.5	a	90	a	90	a	92.5	a
1D	110	b	147.5	b	90	a	90	a	90	a	90	a
2D	105	a	127.5	a	115	b	110	a	90	a	95	a
3D	92.5	a	152.5	b	110	a	90	a	90	a	92.5	a
6D	110	b	150	b	97.5	a	97.5	a	90	a	90	a
7D	115	b	145	b	92.5	a	90	a	95	b	112.5	b
8D	95	a	132.5	a	127.5	b	92.5	a	97.5	b	110	b
9D	92.5	a	127.5	a	125	b	90	a	90	a	90	a
10D	105	a	115	a	105	a	90	a	90	a	100	b
1F	140	c	147.5	b	117.5	b	90	a	90	a	90	a
2F	90	a	107.5	a	107.5	a	90	a	90	a	90	a
3F	97.5	a	125	a	100	a	95	a	90	a	90	a
4F	100	a	142.5	b	115	b	92.5	a	90	a	102.5	b

5F	97.5	a	187.5	c	110	a	90	a	90	a	90	a
6F	105	a	135	a	92.5	a	90	a	90	a	90	a
7F	90	a	115	a	125	b	97.5	a	90	a	92.5	a
8F	102.5	a	147.5	b	105	a	90	a	90	a	90	a
9F	97.5	a	142.5	b	97.5	a	105	a	90	a	100	b
10F	107.5	a	175	c	122.5	b	90	a	90	a	90	a
IVF1-5-2	95	a	152.5	b	112.5	a	102.5	a	90	a	110	b
IVF1-247	110	b	130	a	110	a	112.5	a	90	a	95	a
IVF1-25	105	a	122.5	a	105	a	90	a	90	a	92.5	a
IVF1-230	92.5	a	120	a	90	a	90	a	90	a	90	a
1C	107.5	a	165	c	117.5	b	90	a	90	a	90	a
2C	92.5	a	147.5	b	102.5	a	90	a	90	a	90	a
3C	95	a	142.5	b	105	a	102.5	a	90	a	90	a
4C	100	a	117.5	a	100	a	115	a	90	a	92.5	a
5C	102.5	a	180	c	97.5	a	97.5	a	90	a	95	a
Average	102.65		140.85		109.05		95.05		90.25		92.7	

* - Average with the same letter do not differ by the Scott-Knott test at 5% probability.

Source: Prepared by author

Table 5 - Averages of inbred line in season 2 (planting date 04.17.2014) for Area Under the Disease Progress Curve (AUDPC non-transformed). Selvíria - Mato Grosso do Sul (MS), Brazil. 2014.

Inbred line	Tropical rust		Southern rust		Gray leaf spot		Northern leaf blight		Physoderma brown spot		Phaeosphaeria leaf spot	
IVF1-2-1	115	a	127.5	a	97.5	a	107.5	a	95	a	90	a
IVF1-3	102.5	a	130	a	132.5	a	95	a	92.5	a	97.5	a
IVF1-4	107.5	a	142.5	a	102.5	a	97.5	a	95	a	92.5	a
IVF1-5	95	a	117.5	a	112.5	a	92.5	a	97.5	a	92.5	a
IVF1-6-1	97.5	a	105	a	122.5	a	95	a	97.5	a	90	a
IVF1-6-2	105	a	120	a	110	a	95	a	102.5	a	92.5	a
IVF1-6-3	115	a	155	a	102.5	a	90	a	102.5	a	102.5	b
IVF1-7	102.5	a	110	a	105	a	95	a	102.5	a	95	a
IVF1-8	105	a	130	a	97.5	a	97.5	a	95	a	90	a
IVF1-9	102.5	a	127.5	a	107.5	a	92.5	a	95	a	97.5	a
IVF1-10	102.5	a	147.5	a	95	a	90	a	92.5	a	90	a
IVF1-11	95	a	152.5	a	97.5	a	92.5	a	97.5	a	115	b
IVF1-12	97.5	a	112.5	a	105	a	95	a	97.5	a	92.5	a
IVF1-12-1	97.5	a	142.5	a	115	a	90	a	95	a	115	b
IVD1-2	100	a	132.5	a	120	a	92.5	a	102.5	a	105	b
IVD1-3	112.5	a	145	a	140	a	95	a	95	a	105	b

IVD1-5	100	a	107.5	a	115	a	97.5	a	100	a	97.5	a
IVD1-8	110	a	110	a	102.5	a	100	a	97.5	a	92.5	a
IVD1-9	102.5	a	127.5	a	115	a	92.5	a	97.5	a	90	a
IVD1-10	115	a	132.5	a	100	a	92.5	a	92.5	a	107.5	b
IVD1-11	97.5	a	122.5	a	107.5	a	92.5	a	95	a	92.5	a
IVD1-2-1	100	a	132.5	a	125	a	90	a	95	a	90	a
IVD1-12	112.5	a	142.5	a	100	a	97.5	a	95	a	92.5	a
1D	95	a	145	a	107.5	a	97.5	a	95	a	90	a
2D	102.5	a	107.5	a	102.5	a	112.5	a	92.5	a	102.5	b
3D	102.5	a	130	a	97.5	a	90	a	97.5	a	90	a
6D	107.5	a	120	a	102.5	a	90	a	105	a	90	a
7D	102.5	a	120	a	97.5	a	97.5	a	95	a	95	a
8D	97.5	a	147.5	a	100	a	92.5	a	100	a	115	b
9D	107.5	a	112.5	a	97.5	a	90	a	97.5	a	95	a
10D	100	a	107.5	a	105	a	95	a	90	a	92.5	a
1F	102.5	a	145	a	115	a	92.5	a	105	a	90	a
2F	110	a	130	a	100	a	95	a	92.5	a	90	a
3F	95	a	150	a	107.5	a	92.5	a	97.5	a	90	a
4F	107.5	a	112.5	a	125	a	92.5	a	97.5	a	105	b
5F	107.5	a	130	a	100	a	92.5	a	95	a	92.5	a
6F	107.5	a	152.5	a	105	a	107.5	a	95	a	115	b
7F	102.5	a	107.5	a	100	a	90	a	95	a	90	a
8F	102.5	a	120	a	102.5	a	92.5	a	95	a	90	a
9F	110	a	102.5	a	102.5	a	95	a	92.5	a	92.5	a
10F	112.5	a	122.5	a	97.5	a	95	a	97.5	a	102.5	b
IVF1-5-2	105	a	142.5	a	110	a	97.5	a	90	a	92.5	a
IVF1-247	107.5	a	115	a	107.5	a	107.5	a	95	a	97.5	a
IVF1-25	97.5	a	127.5	a	102.5	a	100	a	92.5	a	90	a
IVF1-230	97.5	a	130	a	112.5	a	92.5	a	102.5	a	95	a
1C	102.5	a	125	a	115	a	100	a	97.5	a	90	a
2C	105	a	165	a	102.5	a	97.5	a	100	a	90	a
3C	97.5	a	120	a	112.5	a	92.5	a	100	a	92.5	a
4C	97.5	a	105	a	105	a	102.5	a	95	a	107.5	b
5C	97.5	a	137.5	a	100	a	97.5	a	102.5	a	95	a
Average	103.4		128.05		107.2		95.45		96.75		96.05	

* - Average with the same letter do not differ by the Scott-Knott test at 5% probability

Source: Prepared by author

3.4 CONCLUSION

The resistant inbred lines based on Area Under Disease Progress Curve (AUDPC) for southern rust, tropical rust, gray leaf spot, northern leaf blight, phaeosphaeria leaf spot and physoderma brown spot were IVF1-3, IVF1-9, IVF1-10, IVF1-11, IVF1-25, IVF1-230, IVD1-2-1, IVD1-12, 2F, 3F, 6F and 4C. The inbred lines with dent grains, are the most susceptible, the condá and dentado population have low frequency of alleles of resistance to studied diseases. The inbred lines from flint grains, isanão-VF1 and Flintisa population have higher frequency of alleles for most diseases resistance evaluated when compared to other populations.

The results of this study suggest the need for further assessment, in other months of planting times to determine the best period for disease incidence and discrimination among genotypes for northern leaf blight and physoderma brown spot.

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CHAPTER 4

ADAPTABILITY AND STABILITY OF CORN INBRED LINES FOR RESISTANCE TO GRAY LEAF SPOT AND NORTHERN LEAF BLIGHT

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João Antonio da Costa Andrade

4.1 INTRODUCTION

Gray leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels) and northern leaf blight (*Exserohilum turcicum* (Pass.) Leonard & Suggs) are among the foliar diseases that affect the corn crop in Brazil and worldwide. Susceptible genotypes to these diseases are responsible for causing severe losses in grain yield since they result directly in decreased photosynthetic area due to the destruction of the green tissues. A 50% reduction the catchment of incident radiation caused by the decrease in green tissue 15 days before and after female flowering may represent a reduction of 40% to 50% of grain yield (FISCHER; PALMER, 1984).

Gray leaf spot was first described in the corn crop in Illinois, United States, in 1925. In Brazil, it was described by Chupp (1953), but the disease becomes common in 2000, when epidemy were reported in production fields in the central region of the country, due to the increase of inoculum promoted by the cultural tillage, irrigation pivot and planting the second season (BRITO et al., 2007). The symptoms of gray leaf spot appear first on lower leaves, about two or three weeks before tasseling, leaf lesions are long, with a rectangular shape, and elliptical. Leaf lesions are brown and with high humidity conditions (above 90%), daytime temperatures ranging from moderate to high (22º to 32º C) and cold nights with dew, occurs dense sporulation, rendering the leaves in gray, characteristic of this disease (CASELA; FERREIRA, 2003; ROBERTSON et al., 2008).

Brito et al. (2007) evaluating 12 commercial corn hybrids for the incidence of gray leaf spot, showed that the level of damage caused by the pathogen change between planting dates and hybrids. The reduction in grain yield is mainly related to the late planting date and the use of resistant hybrids dispenses the chemical control. Silva et al. (2012) evaluating two transgenic corn hybrids in two populations (78.000 and 100.000 plants per hectare) concluded that the lower density of plants favored the increase in the severity of disease and contributing to decrease in grain yield.

Northern leaf blight is distributed worldwide and can cause yield losses of more than 60% in susceptible germplasm (RAYMUNDO; HOOKER, 1981). The disease symptoms appear about a week after beginning of infection, characterized by presenting elliptical straw lesions measuring 2.5 to 15 cm in length with well defined edges, which become dark because of fungus fructification (WORDELL FILHO; CASA, 2012). The development of northern leaf blight is favored by a temperature between 18º and 27ºC with an optimum temperature of 20ºC and presence of dew on the leaf surface (SABATO et al., 2013). In Brazil, the disease occurs strongly in the second season due to the most damage when it infects the plants in the female flowering period. According to Fernandes and Oliveira (2000), the development of *E. turcicum* is negatively correlated with the photoperiod, light intensity and the concentration of sugar in corn. These conditions are most often seen in the second season crops, which could explain the higher severity of this pathogen at the time.

Many authors describe the mechanisms of inheritance associated with northern leaf blight. The disease is controlled mainly using resistant cultivars by quantitative resistance (non-race-specific) and qualitative (race-specific). Qualitative and quantitative sources of resistance have been described (WELZ; GEIGER, 2000). The quantitative resistance conferring partial resistance in the northern leaf blight causes reduction in the development of the disease and the percentage of affected leaf area, which may affect of the epidemics, including the incubation period, latent period, intensity of sporulation, the size, number and growth rate of lesions (PARLEVLIET, 2002; CARSON; GOODMAN, 2006, HURNI et al., 2015).

The interaction between the host and pathogen is distinct in different environments; it is often possible to observe a significant interaction between genotype and environment, which may cause variation in disease severity due to the instability of resistance loci in the interaction with the environment or differences in pathogen populations between environments (CARSON et al., 2002). In this context, the objectives of this study were to identify resistant and susceptible inbred lines based on stability and adaptability for disease symptoms to gray leaf spot and northern leaf blight, suggest resistant inbred lines aimed at producing synthetics, as well as identify the planting dates with the higher occurrence of these two diseases to use them for genetic resistance identification.

4.2 MATERIAL AND METHODS

Forty-one inbred lines were used, fourteen derived from the Isanão-VF1 population, nine from the Isanão-VD1 population, ten from the Flintisa population and eight from the Dentado population. The first two populations are brachytic, with flint and dent grains, respectively. The others have normal height, also with flint and dent grains. The inbred lines were obtained from the corn breeding program of São Paulo State University (UNESP) – Campus of Ilha Solteira – SP (Brazil), and have already been selected for general combining ability for yield.

The experiments were conducted at the Fazenda de Ensino e Pesquisa da UNESP – Campus of Ilha Solteira, located in Selvíria – Mato Grosso do Sul (MS) - Brazil (20° 20'S, 51° 23'W and the altitude of 335 m). The climate of the region, according to Köppen classification, is Aw, defined as tropical humid with a rainy season in summer and dry in winter. The average annual rainfall is 1330 mm, with the average annual air temperature of about 25°C and average humidity of 66% (CENTURION, 1982).

Forty-one experimental inbred lines were evaluated in a randomized block design with three replications in eleven planting dates (October and November 2013 and January until September 2014), with each planting being considered as an environment. Each plot was a single row 8 m in length with a spacing of 0.45 m between plots and an average of 0.4 m between plants. Planting was with normal tillage, irrigated by a center pivot, with twice the number of seeds needed and thinned at six fully developed leaves. Fertilization was done according to soil analysis with 300 kg ha^{-1} of 8-28-16 applied followed by 250 kg ha^{-1} of urea sidedress at the six-leaf stage. Temperature and relative humidity were collected from the weather station located near the experiment during all growing seasons (Figure 4).

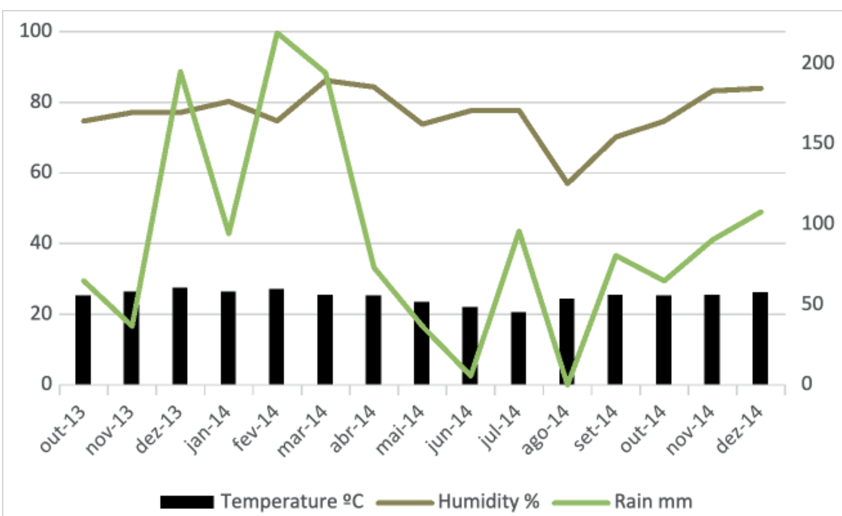

Figure 4 - Temperature, rain and humidity in Selvíria - MS, Brazil from October 2013 until December 2014.

Source: Prepared by author

The inbred lines were evaluated for gray leaf spot (GLS) and northern leaf blight (NLB). Evaluations were carried out at 30 days after silking, determining the severity of disease based on the percentage of symptoms of the plot, according to the diagrammatic scale suggested in the Agroceres Guide to Sanity (AGROCERES, 1996). The ratings were assigned values of 1, 2, 3, 4, 5, 6, 7, 8 and 9, corresponding to 0, 1, 10, 20, 30, 40, 60, 80 and> 80% of leaf symptoms, respectively for each plant plot, using the average plot for statistical analysis. The scores were further classified into the following disease reaction types: 1 – highly resistant; 2-3 – resistant; 4 – moderately resistant; 5 – moderately resistant/moderately susceptible; 6 – moderately susceptible; 7-8 – susceptible and 9- highly susceptible.

The original scores were transformed by $\sqrt{x+0.5}$, and a joint analysis was performed, considering each month as a season of planting and inbred lines with fixed effects and environments as random effects. The Hartley test, which is based on the ratio between the largest and smallest error mean square, was employed, considering the ratio higher than seven as an indication that the error variances were not homogeneous (PIMENTEL GOMES, 2000). To assure the homogeneity of residual variance, the degrees of freedom from residue and inbred lines x environment interaction were adjusted as recommended by Cochran (1954).

For adaptability and stability analysis, the following model, based on regression (Eberhart; Russell, 1966) was used:

$$Y_{ij} = \beta_{oi} + \beta_{1i} I_j + \delta_{ij} + \varepsilon_{ij} \quad (3)$$

Where:

β_{oi} : overall average of genotype *i*;

β_{1i} : linear response of genotype *i* for environmental variation;

I_j : environmental index (j = 1, 2, …, a), being $I_j = \frac{Y_{.j}}{g} - \frac{Y}{ga}$;

δ_{ij} : deviation from regression

ε_{ij} : experimental error.

Data analysis was performed using the Genes Software, version 2015.5.0 (CRUZ, 2013).

4.3 Results and discussion

The F test of joint variance analysis for GLS was significant for inbred lines (L), environments (E) and LxE interactions (P<0.01), while for NLB, the F test, was significant (P<0.01) for environments and LxE interactions (Table 5). As the LxE was significant for each disease studied, were performed the adaptability and stability analysis proposed by Eberhart and Russell (1966).

Table 7 - Summary of the joint variance analysis for Gray leaf spot score (GLS) and Northern leaf blight score (NLB), for 41 corn inbred lines in 11 environments. Selvíria – MS, Brazil, 2014.

Source of variation	DF	GLS	DF	NLB
Inbred lines (L)	40	0.267**	40	0.0723
Environments (E)	10	9.900**	10	2.240**
Lx E	333	0.134**	254	0.084**
Error	720	0.568	538	0.059
Average		2.2		1.7
CV%		14.78		16.67

Note. ** - Significant by the F test ($p \leq 0.01$).

Source: Prepared by author

The severity values for GLS and NLB were different in different months of planting. Averages and environmental indexes (I_j) for each month (Table 6) showed that in the 2013 planting dates and January, February, March and May 2014, there was less GLS pressure and, for NLB the lowest averages were observed in October 2013, January, February and March 2014. In the planting dates of August and September 2014, the highest average scores of inbred lines to the severity of both diseases were found.

According to the averages (β_0), adaptability parameters (β_1) and phenotypic stability () (Table 7) for severity of GLS, 7F, 9F and IVF1-7 presented β_1<1. The inbred lines IVF1-6-1, IVF1-6-2, IVF1-12, IVD1-2-1, IVD1-5, 5F and 6F presented β_1>1, and significantly increased the symptoms of the disease, with increasing of I_j. For the other inbred lines, the regression coefficient (β_1) was equal to 1.

Inbred lines IVD1-8 and 1F responded positively to significantly increase the I_j for NLB. The inbred line IVF1-7 with low average (1.5) for NLB and β_1<1 approached the ideal for a resistant genotype. For the other inbred lines, the average ranged between 0.57 and 1.31 and the regression coefficients were equal to 1.

Table 8 - Environmental indexes (I_j) and environmental averages of 41 corn inbred lines in 11 environments for gray leaf spot (GLS) and northern leaf blight (NLB). Selvíria – MS, Brazil, 2014.

Environment	GLS		NLB	
	Average	I_j	Average	I_j
Oct-13	1.05	-0.3725	1.6	-0.0458
Nov-13	1.25	-0.3011	1.9	0.0692
Jan-14	1.34	-0.2717	1.3	-0.1356
Feb-14	1.44	-0.25	1.1	-0.2073
Mar-14	1.76	-0.1424	1.0	-0.2233
Apr-14	2.66	0.1559	1.8	0.0576
May-14	2.01	-0.0534	1.7	0.0086
Jun-14	3.14	0.262	2.0	0.1067
Jul-14	3.09	0.2593	2.0	0.0821
Aug-14	3.58	0.3791	2.1	0.1211
Sep-14	3.37	0.3348	2.2	0.1668

Source: Prepared by author.

According to the phenotypic stability parameter for severity of GLS, the inbred lines IVF1-2-1, IVF1-3, IVF1-7, IVF1-8, IVF1-11, IVF1-12, IVD1-10, IVD1-11, 6D, 8D, 9D, 10D, 4F, 5F, 6F and 8F were considered unstable ($\square^2_{di}$ non-zero). For the severity of NLB, all variances of the deviations were less than 0.1 and all lines considered stable.

Gray leaf spot (GLS) is more severe and damaging in periods with high humidity, caused by the accumulation of water on the leaf surface and temperatures between 22º and 30ºC (BECKMAN; PAYNE, 1982). The northern leaf blight (NLB) requires temperatures between 18º and 27ºC, with the optimum at 20º C and the presence of dew on the leaf surface (SABATO et al., 2013). Throughout the period that included the first planting date to the final evaluation, the temperature conditions were favorable for the development of GLS and NLB. In the period of July to October 2014, the humidity was close to 70%, which is considered less than ideal for the development of the two diseases. Nevertheless, the severity of the GLS and NLB was largest then because the experiments were conducted under center pivot irrigation, which ensured the presence of free water in the leaves, providing the right conditions for the development of diseases. Therefore, the low averages are due to the good level of resistance of inbred lines with selection using same the standard method. Even so, small environment variations allowed verification of the best times to evaluate the resistance to both diseases are the planting dates between June and September, where I_j were positive and high (Table 6).

Following the methodology proposed by Eberhart and Russell (1966), genotypes with ideal resistance would mean average scale of symptoms around 1, regression coefficient lower than 1, and no significant regression deviations. Ideally, the disease would not consistently increase with the improvement of the environment for the disease (positive I_j), which occurred with inbred lines IVF1-7 and 9F for GLS and IVF1-7 for NLB. However, IVF1-7 it was unstable for GLS, as evidenced by the significance of the variance of the deviations and low coefficient of determination. Inbred lines IVF1-6-1 and IVD1-5 (B_1>1) had strongly increased GLS symptoms with increasing I_j and may be considered the most susceptible group, being interesting to use only in low-pressure conditions disease (negative I_j). For NLB this occurred with IVD1-8 and 1F inbred lines.

In a quantitative approach, the inheritance of resistance and the type of response to I_j depends on the concentration of alleles for disease resistance in each genotype, sensitivity of encoded product of these alleles to environmental changes and the sensitivity of regulatory factors involved in the expression of these alleles. A higher concentration of favorable alleles initially causes the genotype to resist increased disease pressure (positive I_j) and not consistently increasing the scale of symptoms even in the best condition for the disease. This effect will be maximized if the regulation of these alleles and the action mechanisms of their products are also uniformly positive, even with the change of environment. If a genotype has a good concentration of alleles for resistance, but are disabled, in control level or encoded products, their behavior will be unstable with high regression coefficient, as possibly happened with the inbred lines IVF1-6-1 and IVD1-5 for GLS and IVD1-8 and 1F for NLB. For all those unstable GLS inbred lines, one or more such effects may have also occurred.

Another aspect that can give a low response to I_j and good stability is the presence of a locus with major effect, little influenced by the environment. In another case, a locus of small effect, with alleles to increase or decrease the resistance would act as modifiers. Ramalho et al. (2012) say modifiers are smaller effect genes, able to alter the expression of other genes with greater effect. This hypothesis of the occurrence of major effect genes is true because various studies with inbred lines from multiple sources indicate that resistance is qualitatively inherited (locus with major effect) and quantitatively inherited, whereas the additive effect of genes are more important than non-additive (WARD et al., 1999; WELZ; GEIGER, 2000; JULIATTI et al., 2009; VIVEK et al., 2010; BRITO et al., 2012; VIEIRA et al., 2012; ABERA et al., 2016). Modifiers with major effect have not yet been detected in this group of inbred lines.

The results of this study showed that the inbred lines exhibit a high concentration of favorable alleles for resistance to GLS and NLB. Thus, the hybrids between these inbred lines will have good resistance, considering the additive action of these genes. If the intention is to produce a synthetic, the conditions for an inbred line to be included is stability, the lowest coefficient (or equal to one) and the desired type of grain. If the regression coefficient is equal to one, the average should be between the smaller groups.

Table 9 - Adaptability and stability parameters estimated using Eberhart and Russell (1966) method, for gray leaf spot and northern leaf spot for 41 corn inbred lines, in 11 environments. Selvíria – MS, Brazil, 2014.

Inbred lines	Gray leaf spot				Northern leaf blight			
	β_0	β_1	$\square^2_{di}$	R^2 (%)	β_0	β_1	$\square^2_{di}$	R^2 (%)
IVF1-2-1	2.0	0.83	0.0171*	63.13	1.7	1.15	0.0019	54.98
IVF1-3	2.4	1.12	0.0331**	68.17	1.6	0.99	-0.0093	65.23
IVF1-4	2.2	0.95	0.0004	80.60	1.6	0.57	-0.0061	32.28
IVF1-5	2.1	0.99	0.0002	82.18	1.8	0.76	-0.0052	44.59
IVF1-6-1	2.5	1.41^{+}	0.0030	89.05	1.7	0.87	-0.0101	61.14
IVF1-6-2	2.7	1.28^{+++}	0.0005	88.34	1.7	0.93	-0.0033	51.51
IVF1-6-3	2.5	1.24	0.0062	84.62	1.7	1.02	-0.0004	52.23
IVF1-7	3.2	0.61^{++}	0.0754**	25.87	1.5	0.46^{+++}	0.0046	15.01
IVF1-8	1.7	0.82	0.0253*	57.80	1.7	0.66	0.0154	19.99
IVF1-9	1.8	0.90	-0.0103	89.33	1.6	1.11	-0.0036	60.83
IVF1-10	1.9	0.90	-0.0069	85.75	2.1	1.02	-0.0088	65.69
IVF1-11	2.4	1.25	0.0450**	68.49	1.7	1.12	-0.0087	69.69
IVF1-12	2.9	1.25^{+++}	0.0909**	56.04	1.6	0.98	-0.0116	70.27
IVF1-12-1	1.9	0.93	0.0042	76.96	1.7	1.20	-0.0057	67.21
IVD1-2	2.1	0.79	-0.0004	75.06	2.0	0.87	-0.0007	44.20
IVD1-3	2.4	0.92	0.0032	77.31	1.8	1.26	0.0119	50.23
IVD1-5	2.4	1.30^{++}	0.0031	87.34	2.0	1.43	0.0119	56.63
IVD1-8	2.1	1.16	-0.0006	86.68	1.8	1.53^{+++}	-0.0084	80.68
IVD1-9	2.4	0.84	0.0095	68.77	1.8	0.98	-0.0019	52.01
IVD1-10	2.5	1.04	0.0523**	57.56	2.0	1.31	0.0119	52.30
IVD1-11	2.2	1.01	0.0250*	67.61	1.8	1.21	-0.0007	60.58
IVD1-2-1	2.2	1.07^{+++}	0.0015	83.43	1.9	1.23	0.0153	46.39
IVD1-12	2.1	1.10	0.0010	84.51	1.8	0.90	-0.0097	61.61
1D	2.2	1.19	-0.0041	89.50	1.7	0.87	-0.0093	59.42
2D	2.3	1.02	-0.0052	87.16	1.7	0.96	-0.0124	71.51
3D	1.9	1.12	0.0017	84.46	1.5	0.86	0.0107	32.88
6D	1.7	0.85	0.0256*	59.12	1.6	0.89	0.0176	30.12
7D	2.1	0.89	-0.0128	92.14	1.7	0.85	-0.0020	44.96
8D	2.6	0.83	0.0587**	44.45	1.6	0.65	-0.0002	30.38
9D	2.7	0.88	0.0528**	48.94	1.6	1.19	-0.0118	78.06
10D	2.0	0.95	0.0578**	51.28	1.3	0.84	-0.0124	66.04
1F	2.1	0.86	0.0123	68.19	1.9	1.63^{+++}	0.0105	64.08

2F	2.0	0.98	0.0125	73.32	1.6	0.82	-0.0097	57.47
3F	2.1	1.12	0.0042	82.83	1.7	0.73	-0.0078	47.06
4F	2.4	0.76	0.0292**	51.64	1.6	1.19	0.0019	56.91
5F	2.7	1.28+++	0.0209*	78.68	1.7	1.21	-0.0109	76.81
6F	2.4	1.29+++	0.0598**	65.56	1.4	0.81	0.0012	38.84
7F	1.9	0.74+++	-0.0072	80.51	1.7	1.10	-0.0056	63.40
8F	2.0	1.02	0.0224*	69.33	1.6	1.16	0.0031	54.12
9F	2.1	0.51+	0.0166	39.69	1.5	0.73	-0.0078	47.28
10F	2.0	0.99	0.0073	76.80	1.6	0.97	-0.0050	56.34
Average	**2.2**	-	-	-	**1.7**	-	-	-

(β_1) +, ++,+++ Differs from one, by the t test, at $p \leq 0.01$, $p \leq 0.05$ and $p \leq 0.10$, respectively.

($\square^2_{di}$) *, ** Differs from zero, by the F test, at $p \leq 0.05$ and $p \leq 0.01$, respectively.

(β_1): Regression Coefficient; $\square^2_{dii}$: Variance deviation regression; R^2 (%): Determination Coefficient.

Source: Prepared by author.

4.4 CONCLUSION

The planting dates most suitable for evaluation of genotypes for genetic resistance were August and September, as it showed the highest environmental indexes to gray leaf spot and northern leaf blight. The inbred lines IVD1-2, IVD1-3, IVD1-9, 2D and 7D, may be used to form a synthetic with dent grains for resistance to these two diseases. For synthetic flint grains, the inbred lines IVF1-7, IVF1-10, 2F, 9F and 10F can be used for resistance to both diseases.

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CHAPTER 5

ADAPTABILITY AND STABILITY FOR RESISTANCE TO PHYSODERMA BROWN SPOT AND PHAEOSPHAERIA LEAF SPOT IN CORN INBRED LINES

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5.1 INTRODUCTION

Physoderma brown spot is caused by *Physoderma maydis*, occurs commonly in regions that have high temperatures and high precipitation. The first symptoms of the disease usually appear on the leaf limbs and nerves with chlorotic spots (LEÓN, 1984). According to Robertson et al. (2008), the pathogen is dormant in infected tissues or soil and produces innumerable zoospores in the presence of water. Leaf infection occurs at whorl when is present for an extended period. Occurs in a day cycle and requires a combination of light, free water and temperature between 23 and 30ºC. According to Fernandes and Balmer (1990), the brown spot is more severe in late plantings, carried out in low areas. There are a few reports in the literature regarding the quantification of this disease in corn worldwide.

Phaeosphaeria leaf spot whose etiologic agent is the fungus *Phaeosphaeria maydis* (PINTO; FERNANDES; OLIVEIRA, 1997) in association with the bacteria *Pantoeae ananas* (PACCOLA-MEIRELLES et al., 2001), occurs in the main corn producing regions in Brazil and worldwide. Under favorable conditions, it can cause premature leaf drought, reduction of the plant cycle, reduction in grain size and weight, leading to a decrease in grain yield of over 60% (FERNANDES, 2004). Leaves with 10 to 20% disease severity present a reduction in the net photosynthetic rate around 40% in susceptible cultivars evidencing the effect of the disease on photosynthesis (GODOY; AMORIM; BERGAMIN FILHO, 2001). The symptoms of this disease are related to the appearance of irregular shaped leaf stains, dark green color, that appear in the lower leaves passing to the higher leaves of the plant. Subsequently the lesions become necrotic of with straw coloration, being able to coalesce. The symptoms may present in different severities depending on the corn genotype (PACCOLA-MEIRELLES et al., 2002; REIS; CASA; BRESOLIN, 2004). Lopes et al. (2007), evaluating the genetic control of the resistance of the Phaeosphaeria leaf spot, concluded that the additive effects predominated in the control of resistance, and the characteristic has high heritability.

Loss of crops from plant diseases may result in hunger and starvation, especially in developing countries where access to disease-control methods is limited and annual losses of 30 to 50% are common for major crops (ALI; YAN, 2012). Some of these diseases were firstly considered secondary, but from 1990s onward their incidence and severity increased expressively (ARAUJO et al., 2013).

In this context, the objectives of this study were to identify resistant and susceptible inbred lines based on stability and adaptability for disease symptoms to phaeosphaeria leaf spot and physoderma brown spot, suggest resistant inbred lines aimed at producing synthetics, as well as identify the planting dates with the higher occurrence of these two diseases to use them for genetic resistance identification.

5.2 MATERIAL AND METHODS

Forty-one inbred lines were used, fourteen derived from the Isanão-VF1 population, nine from the Isanão-VD1 population, ten from the Flintisa population and eight from the Dentado population. The first two populations are brachytic, with flint and dent grains, respectively. The others have normal height, also with flint and dent grains. The inbred lines were obtained from the corn breeding program of São Paulo State University (UNESP) – Campus of Ilha Solteira – SP (Brazil), and have already been selected for general combining ability for yield.

The experiments were conducted at the Fazenda de Ensino e Pesquisa da UNESP – Campus of Ilha Solteira, located in Selvíria – Mato Grosso do Sul (MS) - Brazil (20° 20'S, 51° 23'W and the altitude of 335 m). The climate of the region, according to Köppen classification, is Aw, defined as tropical humid with a rainy season in summer and dry in winter. The average annual rainfall is 1330 mm, with the average annual air temperature of about 25°C and average humidity of 66% (CENTURION, 1982).

Forty-one experimental inbred lines were evaluated in a randomized block design with three replications in eleven planting dates (October and November 2013 and January until September 2014), with each planting being considered as an environment. Each plot was a single row 8 m in length with a spacing of 0.45 m between plots and an average of 0.4 m between plants. Planting was with normal tillage, irrigated by a center pivot, with twice the number of seeds needed and thinned at six fully developed leaves. Fertilization was done according to soil analysis with 300 kg ha^{-1} of 8-28-16 applied followed by 250 kg ha^{-1} of urea sidedress at the six-leaf stage. Temperature and relative humidity were collected from the weather station located near the experiment during all growing seasons (Figure 5).

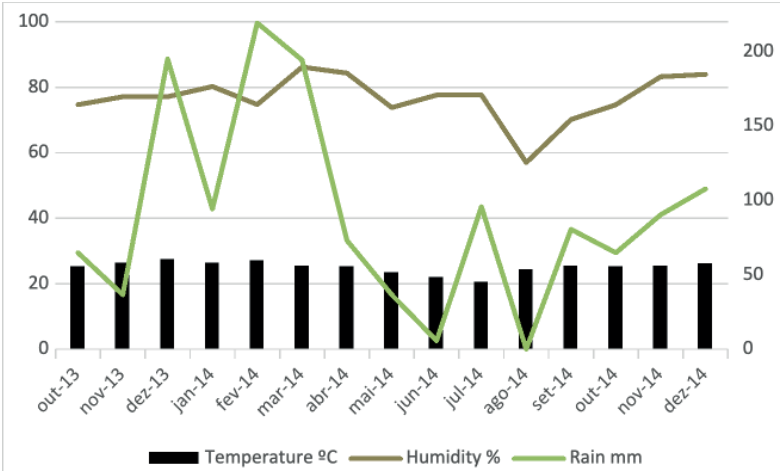


Figure 5 - Temperature, rain and humidity in Selvíria - MS, Brazil from October 2013 until December 2014.

Source: Prepared by author

The inbred lines were evaluated for physoderma brown spot (PBS) and phaeosphaeria leaf spot (PLS). Evaluations were carried out at 30 days after silking, determining the severity of disease based on the percentage of symptoms of the plot, according to the diagrammatic scale suggested in the Agroceres Guide to Sanity (Agroceres 1996). The ratings were assigned values of 1, 2, 3, 4, 5, 6, 7, 8 and 9, corresponding to 0, 1, 10, 20, 30, 40, 60, 80 and> 80% of leaf symptoms, respectively for each plant plot, using the average plot for statistical analysis. The scores were further classified into the following disease reaction types: 1 – highly resistant; 2-3 – resistant; 4 – moderately resistant; 5 – moderately resistant/moderately susceptible; 6 – moderately susceptible; 7-8 – susceptible and 9- highly susceptible.

The original scores were transformed by $\sqrt{x+0.5}$, and a joint analysis was performed, considering each month as a season of planting and inbred lines with fixed effects and environments as random effects. The Hartley test, which is based on the ratio between the largest and smallest error mean square, was employed, considering the ratio higher than seven as an indication that the error variances were not homogeneous (PIMENTEL GOMES, 2000). To assure the homogeneity of residual variance, the degrees of freedom from residue and inbred lines x environment interaction were adjusted as recommended by Cochran (1954).

For adaptability and stability analysis, the following model, based on regression (EBERHART; RUSSELL, 1966) was used:

$$Y_{ij} = \beta_{oi} + \beta_{1i} I_j + \delta_{ij} + \varepsilon_{ij} \quad (4)$$

Where:

β_{oi} : overall average of genotype *i*;

β_{1i} : linear response of genotype *i* for environmental variation;

I_j : environmental index (j = 1, 2, …, a), being $I_j = \frac{Y_{.j}}{g} - \frac{Y}{ga}$;

δ_{ij} : deviation from regression

ε_{ij} : experimental error.

Data analysis was performed using the Genes Software, version 2015.5.0 (CRUZ, 2013).

5.3 RESULTS AND DISCUSSION

In joint analysis of variance for PBS and PLS, the F test were significant (P<0.01) for inbred lines (L), environments (E) and for inbred lines x environments (L x E) (Table 8). The significant effect of inbred lines indicates the presence of variability for the selection, the effect of environments indicates variability between the months of planting, an important fact to make the process of indicating inbred lines for developing resistant hybrids more efficiently.

Since the interaction inbred lines x environments (L x E) was significant, the analysis of adaptability and stability proposed by Eberhart and Russell (1966) was performed. The L x E interaction is fundamental for the breeding programs, since it is possible to reduce this effect from genotypes with wide adaptability or to recommend genotypes specific to certain environments (ENGELSING et al., 2012). When genotypes are compared over a series of environments, the relative rankings usually differ. This causes difficulty in demonstrating the significant superiority of any genotype. This interaction is usually present whether the genotype are pure lines, single-cross or double-cross hybrids, top crosses or any material with which the breeder may be working (EBERHART; RUSSELL, 1966). Brito et al. (2011), also found significance in the interaction L x E when evaluating 12 commercial hybrids, in three locations, for resistance to gray leaf spot and phaeosphaeria leaf spot.

The environmental indexes (I_j) and the averages non-transformed for each month of planting are shown in Table 9. The data of averages and environmental indexes showed that for PBS, in the planting date of July and August 2014, there was a higher pressure of the disease, expressing an average of 2.97 and 4.09. Despite being highest average found for PBS, inbred lines with these notes are considered resistant and moderately resistant, with 10 and 20% of the leaf area affected. For PLS the highest disease pressure occurred in the planting date of April and June, with averages of 1.88 and 2.24 respectively, notes that classify inbred lines as resistant.

Table 11 - Summary of the joint variance analysis for physoderma brown spot (PBS) and phaeosphaeria leaf spot score (PLS), for 41 corn inbred lines in 11 environments. Selvíria – MS, Brazil, 2014.

Source of variation	DF	PBS	DF	PLS
Inbred lines (L)	40	0.184**	40	0.205**
Environments (E)	10	11.365**	10	2.801**
Lx E	275	0.093**	400	0.070**
Error	585	0.054	880	0.033
Average	-	2.2	-	1.5
CV%	-	14.63	-	12.72

Note. ** - Significant by the F test ($p \leq 0.01$).
Source: Prepared by author.

Table 12 - Environmental indexes (I_j) and environmental averages of 41 corn inbred lines in 11 environments for physoderma brown spot (PBS) and phaeosphaeria leaf spot score (PLS). Selvíria – MS, Brazil, 2014.

Environment	PBS		PLS	
	Average	I_j	Average	I_j
Oct-13	2.40	-0.3538	1.36	0.2646
Nov-13	2.21	0.0342	1.07	-0.175
Jan-14	1.23	-0.2835	1.07	-0.1739
Feb-14	1.04	-0.3485	1.24	-0.1237
Mar-14	1.04	-0.3471	1.22	-0.1289
Apr-14	2.96	0.261	1.88	0.1029
May-14	2.01	-0.0257	1.82	0.0743
Jun-14	2.20	0.049	2.24	0.2182
Jul-14	2.97	0.2521	1.56	-0.0166
Aug-14	4.09	0.5393	1.54	-0.0136
Sep-14	2.88	0.2231	1.52	-0.0285

Source: Prepared by author.

For the development of PBS, the optimal temperature must be between 23 and 30ºC, while for PLS the nighttime temperature must be between 14 and 20ºC, being that for both diseases, the humidity above 60% and free water on the leaf surface is required. In this study the presence of favorable environmental conditions for the development of PBS and PLS was observed, being the months of April, June, July and August the most suitable for planting inbred lines to be evaluated for both diseases.

For the diseases analysis, the interesting thing is to find genotypes with a low average (β_0), in this case, around 1 and adaptability parameters (β_1) less than 1, because in these cases the genotypes did not increase the symptoms of the disease with the improvement of the environment. In this context, for PBS the inbred lines IVD1-9, IVD1-10, 7D, 10D and 2F can be indicated for planting in all environments (Table 10). For PLS the inbred lines 10D, 2F, 4F and 9F showed β_1 <1, indicating that these inbred lines did not increase the symptoms of the disease according to the improvement of the environment. However, these inbred lines were not stable, since the regression deviation were significant, indicating that the behavior is unpredictable, thus not being suitable for any environment. Although the inbred lines IVD1-9 and IVD1-10 presented β_1 = 1, for PLS, it can be indicated for planting in all environments.

The inbred lines IVF1-3, IVF1-6-2 and IVD1-2 may be indicated for environments with less favorable for PLS symptoms, since the disease decreases more rapidly in these environments. For PBS the inbred lines IVF1-6-1, IVF1-6-2, IVF1-6-3, IVD1-2 and 4F, may be indicated for environments with low disease pressure.

The adaptability and stability parameters is an important tool in the process of the breeder to indicate genotypes more appropriate to the studied environments. In the selection of inbred lines for the reaction to diseases in the corn crop, few or any study was developed in this context. In Figures 6 and 7 are selected inbred lines for the resistance to PBS and PLS respectively.

Reports in the literature suggest that for the reaction of corn to PLS and PBS are quantitatively inherited characteristics with the predominance of additive effects in the control of disease resistance, being more important than the effects of dominance and the epistatic effects (CARSON; STUBER; SENIOR, 2005; MOLL; THOMPSON; HARVEY, 1963; MOREIRA et al., 2009). In such cases, the inheritance of resistance and the type of response of the environment depends on the concentration of alleles for disease resistance in each genotype, the sensitivity of the encoded product by those alleles to the environmental changes and the sensitivity of the factors involved in the expression of these alleles. The results of this study demonstrated that the inbred lines presented high concentration of favorable alleles for the resistance of PBS and PLS, being favorable for the selection of inbred lines for making synthetics and hybrids. According to Brito et al. (2012), the most recommended breeding strategy would be to obtain hybrids from resistant inbred lines, taking advantage of the additive effects of the large-effect loci of the parents and the dominance that will be expressed in divergent loci of smaller effect.

Table 13 - Adaptability and stability parameters estimated, using Eberhart and Russell (1966) method, for physoderma brown spot (PBS) and phaeosphaeria leaf spot (PLS) score for 41 corn inbred lines, in 11 environments. Selvíria – MS, Brazil, 2014.

Inbred lines	Physoderma brown spot				Phaeosphaeria leaf spot			
	β_0	β_1	$\square^2_{di}$	R^2 (%)	β_0	β_1	$\square^2_{di}$	R^2 (%)
IVF1-2-1	2.6	1.15	-0.008	93.21	1.3	1.03	-0.0049	81.78
IVF1-3	2.0	0.97	-0.009	91.54	1.3	1.39^{+++}	0.0010	80.53
IVF1-4	2.3	1.02	0.0008	84.96	1.7	1.35	0.0147*	64.21
IVF1-5	2.2	1.02	-0.005	89.16	1.6	1.17	0.0053	67.99
IVF1-6-1	2.9	1.49^{+}	-0.0108	96.96	1.2	1.28	0.0085	68.17
IVF1-6-2	2.6	1.31^{++}	-0.0009	91.23	1.4	1.48^{++}	0.0008	82.54
IVF1-6-3	2.8	1.44^{+}	0.0153	86.48	1.3	1.32	-0.0025	84.06
IVF1-7	2.0	0.78	-0.0019	79.73	1.3	0.95	0.0005	66.64
IVF1-8	2.1	1.12	0.0222*	76.21	1.2	0.66	0.0069	38.48
IVF1-9	2.3	1.02	-0.0059	89.76	1.2	0.86	-0.0050	75.58
IVF1-10	2.5	0.92	0.0051	79.17	1.5	1.02	0.0003	69.91
IVF1-11	2.0	0.85	-0.0094	89.72	1.5	1.05	0.0209**	46.83
IVF1-12	2.1	0.91	-0.0065	88.14	1.8	1.49^{++}	0.0169**	66.94
IVF1-12-1	2.3	0.90	0.0241*	66.22	1.5	0.75	0.0268**	27.39
IVD1-2	2.7	1.23^{+++}	-0.0003	89.81	1.5	1.70^{+}	0.0026	84.42
IVD1-3	2.2	0.89	-0.0081	89.27	1.9	1.24	0.0070	68.63
IVD1-5	2.2	0.97	-0.0104	92.71	1.2	1.20	-0.0030	82.13
IVD1-8	2.4	1.05	-0.007	91.08	1.5	1.18	0.0051	68.88
IVD1-9	1.8	0.72^{++}	-0.0079	84.06	1.5	0.81	0.0041	52.16
IVD1-10	1.8	0.72^{++}	-0.0134	92.25	1.3	0.97	-0.0034	75.81
IVD1-11	2.5	1.12	0.0179*	78.17	1.4	1.03	-0.0006	72.22
IVD1-2-1	2.1	0.88	-0.0081	89.04	1.4	0.83	0.0078	48.09
IVD1-12	2.4	1.12	-0.0069	92.08	1.6	1.15	0.0355**	41.83
1D	2.1	0.92	-0.0117	93.25	1.7	0.86	0.0285**	32.00
2D	2.1	0.90	0.0135	72.39	1.9	0.65	0.0034	42.97
3D	1.9	0.80	-0.0053	83.98	1.6	1.02	0.0114*	54.37
6D	2.1	1.11	0.0167*	78.57	1.1	0.67	0.0203**	26.55
7D	2.9	0.76^{+++}	0.0147	64.76	2.0	0.87	0.0319**	30.80
8D	2.4	0.88	0.0505**	53.58	1.2	0.96	0.0250**	39.09
9D	2.2	1.04	0.0106	79.42	1.3	0.87	-0.0035	71.89
10D	1.8	0.73^{+++}	-0.004	79.78	1.5	0.63^{+++}	0.0157**	27.57
1F	2.4	1.08	-0.0072	91.74	1.4	1.25	0.0011	76.72
2F	1.7	0.68^{++}	-0.0081	83.00	1.3	0.21^{+}	0.0360**	2.35
3F	2.8	1.18	-0.0043	91.31	1.5	1.13	0.0021	71.36

4F	2.7	1.35++	0.0111	86.54	1.9	0.37+	0.0510**	5.24
5F	2.1	0.94	-0.0078	89.91	1.8	0.97	0.0063	58.14
6F	2.1	0.93	0.0143	73.43	1.4	0.98	0.0049	60.43
7F	2.1	0.96	-0.0105	92.77	1.4	1.06	-0.0039	80.26
8F	2.4	1.13	-0.0024	89.38	1.3	1.05	-0.0046	81.60
9F	2.1	0.90	0.0202*	68.57	2.0	0.60+++	0.0207**	22.51
10F	2.5	1.11	0.0011	86.83	2.5	0.92	0.0959**	16.67
Average	**2.2**	**-**	**-**	**-**	**1.5**	**-**	**-**	**-**

(β_1) +, ++,+++ Differs from one, by the t test, at p ≤ 0.01, p ≤ 0.05 and p ≤ 0.10, respectively.

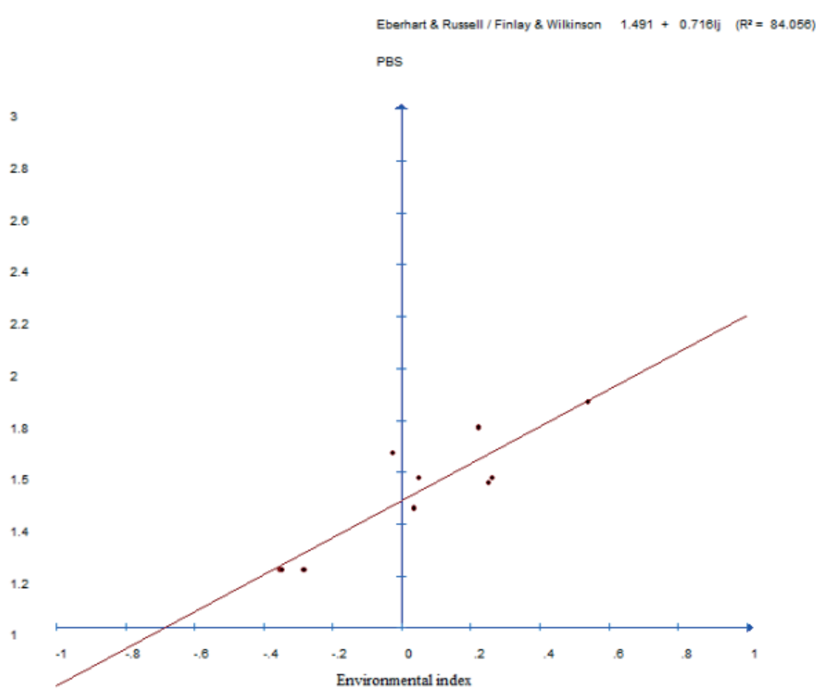
($\square^2_{di}$) *, ** Differs from zero, by the F test, at p ≤ 0.05 and p ≤ 0.01, respectively.

(β_1): Regression Coefficient; S^2_{di}: Variance deviation regression; R^2 (%): Determination Coefficient.

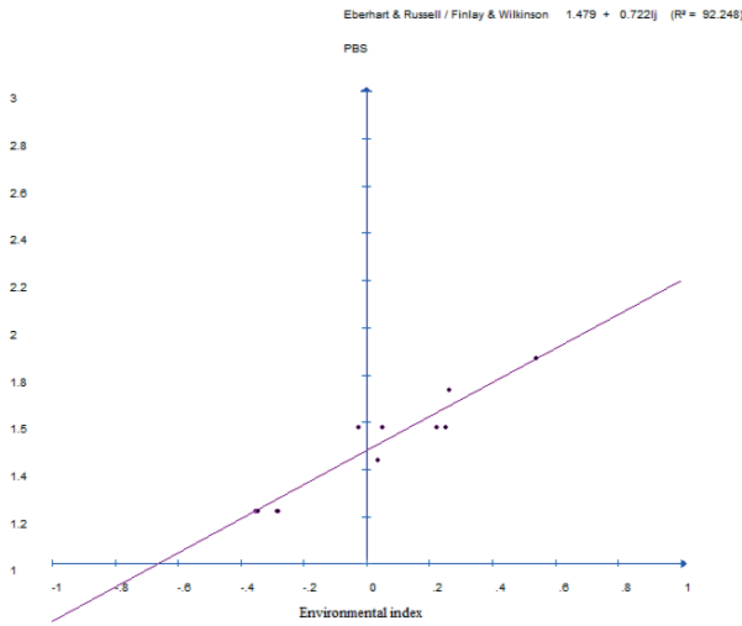
Source: Prepared by author.

Figure 6 – Regression of severity for physoderma brown spot (PBS) symptoms as a function of environmental indices for the five corn inbred lines considered more resistant, evaluate in 11 environments (October and November 2013 and January until September 2014). Selvíria - MS, Brazil, 2014.

IVD1-9

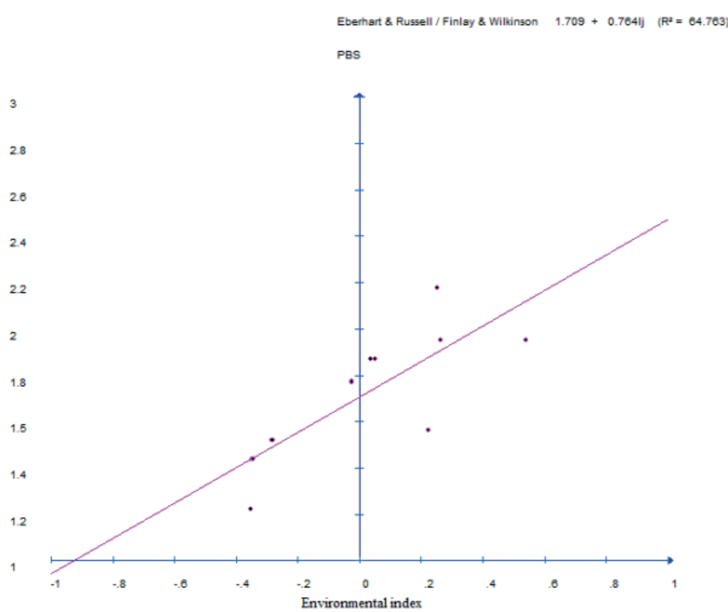


IVD1-10



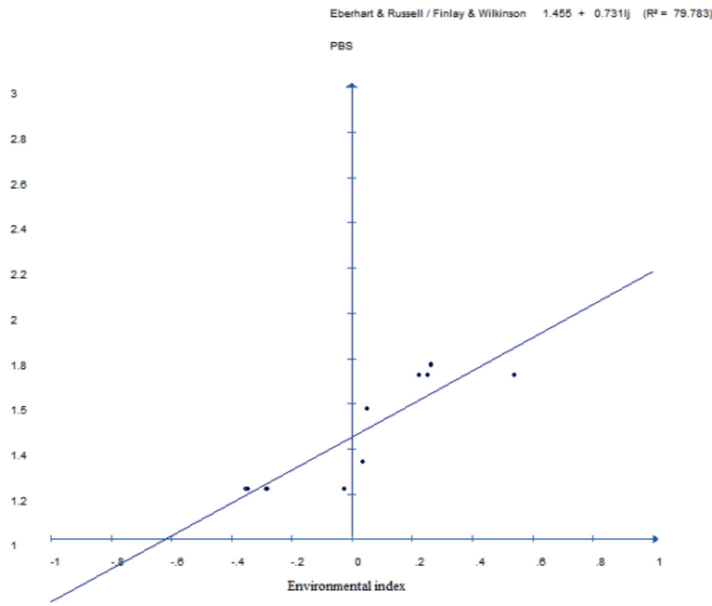
Eberhart & Russell / Finlay & Wilkinson 1.479 + 0.722Ij (R² = 92.248)
PBS
Environmental index

7D



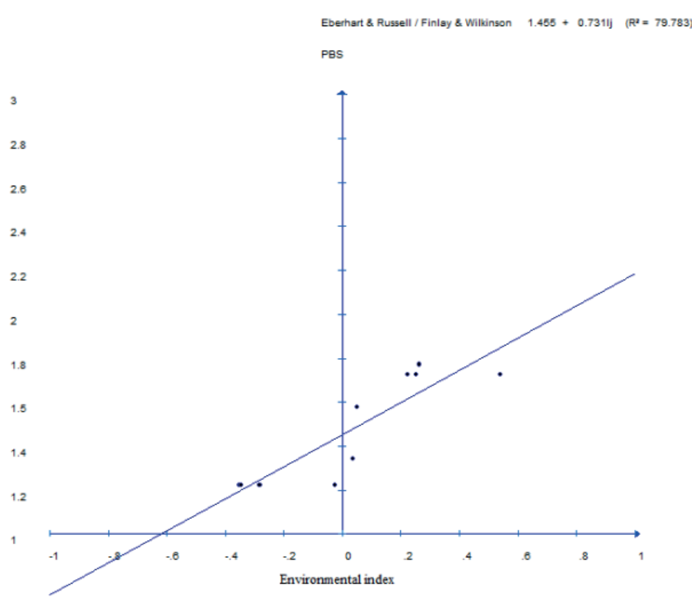
Eberhart & Russell / Finlay & Wilkinson 1.709 + 0.764Ij (R² = 64.763)
PBS
Environmental index

10 D



Eberhart & Russell / Finlay & Wilkinson 1.455 + 0.731Ij (R² = 79.783)
PBS
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
-1
-.8
-.6
-.4
-.2
0
.2
.4
.6
.8
1
Environmental index

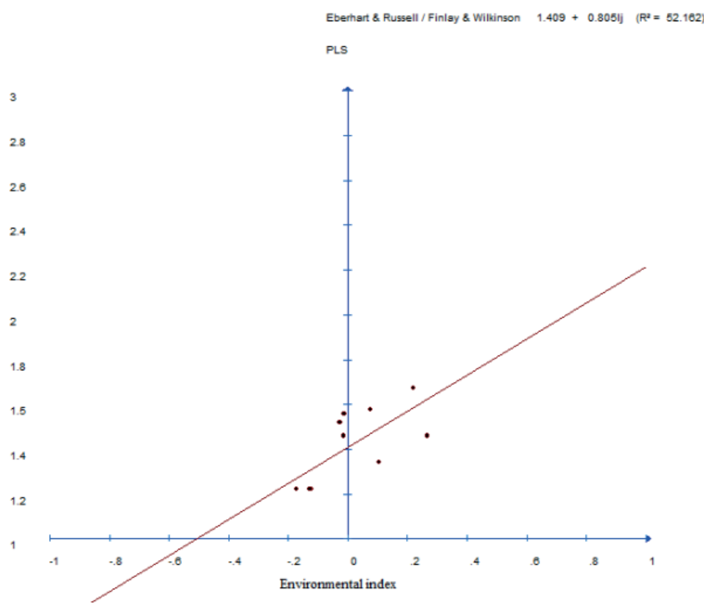
2F



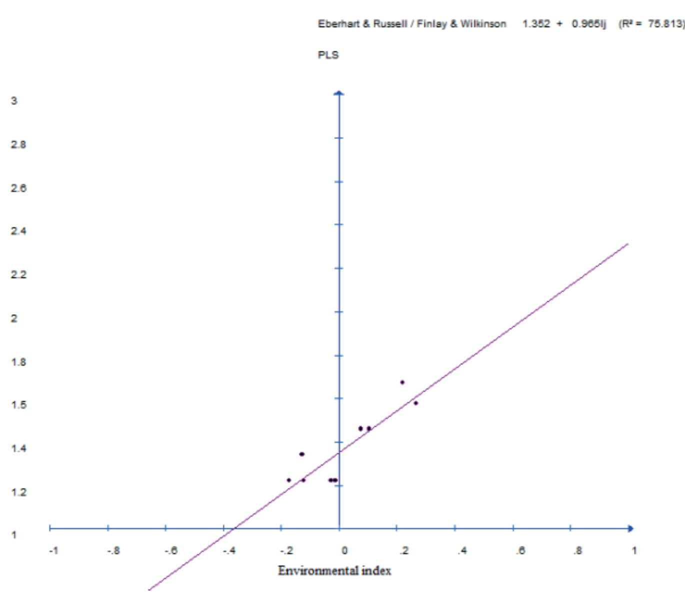
Eberhart & Russell / Finlay & Wilkinson 1.455 + 0.731Ij (R² = 79.783)
PBS
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
-1
-.8
-.6
-.4
-.2
0
.2
.4
.6
.8
1
Environmental index

Figure 7 - Regression of severity for phaeosphaeria leaf spot (PLS) symptoms as a function of environmental indices for the two corn inbred lines considered more resistant, evaluate in 11 environments (October and November 2013 and January until September 2014). Selvíria - MS, Brazil, 2014.

IVD1-9



IVD1-10



5.4 CONCLUSION

The planting dates most suitable for evaluation of genotypes for genetic resistance to physoderma brown spot and phaeosphaeria leaf spot were April, June, July and August, once that showed the best information for selection. The inbred lines IVD1-9, IVD1-10, 7D, 10D and 2F may be used to form a synthetic resistant to physoderma brown spot and phaeosphaeria leaf spot.

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CONCLUSION

The use of the Area Under Disease Progress Curve (AUDPC) and an analysis of stability and adaptability proved to be efficient in the identification of resistance corn inbred lines for the following diseases: southern rust, tropical rust, gray leaf spot, northern leaf blight, phaeosphaeria leaf spot and physoderma brown spot.

The months with the highest disease severity occur between April and September, due to favorable climatic conditions for disease development.

The corn inbred lines IVF1-3, IVF1-7, IVF1 -9, IVF1-10, IVF1 -11, IVF1 -25, IVF1-230, IVD1-2, IVD1 -2-1, IVD1-3, IVD1-9, IVD1-10, IVD1 -12, 2F, 3F, 6F, 9F, 10F, 4C, 2D, 7D and 10D showed to be resistant to studied diseases and may be indicated to produce synthetic corn.

FINAL CONSIDERATION

The initial objective of this study was to characterize corn inbred lines for disease resistance, planting each month during a year, using adaptability and stability parameters and area under the disease progress curve. The planting of December we lose due to very severe weather conditions. Another difficulty we found was the lack of a previously recognized check as susceptible. We decided did not use hybrids as a check to avoid competition between genotypes and the possible influence on disease symptoms. The use of adaptability and stability parameters proved to be efficient for the analysis of disease resistance in corn inbred lines because it is easy to explain and facilitates the interpretation of data, thus, we indicate the use of this methodology. The area under disease progress curve is widely used by phytopathologists and has proved to be efficient for corn resistance evaluation for genetic improvement.

The evaluation of diseases occurred by the same evaluators during the whole experiment, being indispensable the presence of at least 2 evaluators for the correct analysis of the percentage of diseased plants. In this study, we used the same scale to evaluate the following diseases: tropical rust, Southern rust, gray leaf spot, northern leaf spot, phaeosphaeria leaf spot, physoderma brown spot and corn stunt (data not were shown). However, the diseases hinder the development of plants differently and at different times requiring a different scale to evaluate the disease. Another relevant aspect was the lack of reports in the literature of studies such this, considering the evaluation of corn diseases in inbred lines.

Given the above, we hope that other studies evaluating disease resistance in corn inbred lines can be developed, using the information of this study.

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