J. Agric. Sci. Mansoura Univ., 26 (11): 6825 - 6833, 2001.

INOCULUM LEVELS of *PERONOSCLEROSPORA SORGHI* in RELATION TO MAIZE DOWNY MILDEW DISESE OUTBREAK.

Rasmy, M. R.; M. M. Saleh; A. M. Abdel - Monem and Mona M. S. Nour El- Din
Plant Pathology Res.Inst.,ARC,Giza, Egypt.

ABSTRACT

Recently, downy mildew of maize caused by *Peronosclerospora sorghi* causes serious losses in maize seed production in Egypt. The downy mildew incitant was detected in seed samples in a various range of infection levels. The obtained results suggest that approxemitely 60% of the infected maize seeds will give rise to infected plants when mature. The correlation of seed infection level /disease development was eight / five. Seed dressing decreased disease seed transmission by about 75%. Fungicidal seed treatment when applied by the solvent infusion method resulted in satisfactory control and reduced disease to about 10% .

INTRODUCTION

Maize (*Zea mays* L.) suffers from serious pathogens, many of which are seed-borne (Richardson 1979, Malcotm,1980). Twenty fungal diseases are seed-borne and seed transmitted, among which sorghum downy mildew (SDM) of maize (Bain, 1969; McGee,1988 and Shetty,*et al.,* 1978). In Egypt, maize is affected by ,black bundle disease incited by the fungus *Cephalosprium acremonium*; black kernel rot caused by *Botryodiplodia theobromae*; ear rot produced by *Aspergillus niger*; fusarium stalk and ear rot induced by *Fusarium moniliforme*; nigrospora ear rot originated from *Nigrospora oryzae* infection; sorghum downy mildew caused by *Peronosclrrospora sorghi*; and other root rot diseases (Melchers, 1931; Assawah and Elarosi,1961; Farag,1964; Kamara, 1965; Fathi, 1966; Samra *et al.,* 1966;Sabet *et al.,*1966). *Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw. the causal pathogen of sorghum downy mildew (SDM) of maize, is a serious and destructive pathogen in tropical and sub-tropical areas(Adenele and Cardwell,1996, Bock,*et al*., 1998; Jeger,*et al.,* 1998 ,Payak,1975 and Show,1980). The SDM disease was not common in Egypt and was observed in the Experimental Station at Sakha , Kafr-El-Sheikh Governorate. A sudden and serious out-break of the disease was occurred in the Nile Delta 1989. THE disease was mostly observed on maize plants grown close to forage sorghum . the disease was also detected at Gimiza , Gharbia Governorate.

Systemically infected sorghum plants can produce oospores within the glumes. Oospores have also been detected in seed pericarp and mycelial fragments have been observed in the endosperm of sorghum seeds (Safeeulla,1976)., seed-borne inoculum is in the form of mycelium in the pericarp, endosperm and embryonic tissues (Jones *et al*.,1972;Safeeulla & Shetty 1977), oospores have also been detected in the pericarp, endosperm and embryo (Muralidhararao, 1982). The downy mildews are managed by the use of host resistance, and systemic fungicides such as Metalaxyl (Anahosur

and Patil,1980; Williams, 1984; Anoso,et al. 1989; Shishupala,*et al.*, 1990; Singh *et al.,* 1993 and Bock,1995). However, either durable host reaistance or effective chemicals are not available to check the entrance of downy mildews. One of the most significant programmes recommended for disease management is production and supply of healthy seeds through effective seed certification programme (Singh and Shetty, 1990).

The damage caused to the subsequent developing crop, depends on the downy mildew inoculum in maize seed lots.The tolerance level set by for downy mildew in pearl millet for field inspection has been fixed at 0.05% of infected plants in foundation seed and as 0.1% in certified seed. However, no standards have been established for seed infection/ contamination (Reddy,*et al*., 1991 and Shetty and Shetty 1993). Tolerance limits have not been set for SDM of maize or sorghum. Seed production agencies in Egypt requested information on level of disease found in maize seed and recommendation for tolerance level for SDM seed infection as weel as for field inspection. Hence, this report was carried out to determine the range of seed infection of SDM commonly found in maize seed lots and the relation of these levels to development of the disease in the field.

MATERIALS AND METHODS

Thirty one seed samples of eleven maize cultivars were collected in summer 1997 from many seed production and research areas used in this study (Table 1). seed of each sample was tested for the presense of *Peronosclerospora sorghi*. , examined visually under the binocular microscope for the presence of oospores masses. Two techniques were used to detect external and internal inoculum of the pathogen :

Table (1): Maize seed samples harvested at 1992 and their collection sites

Sample No.	Cultivar	Total Number	Location
1	SC maize 9	1	Seed Production Unit (Kafr-EL-Sheikh)
2	SC maize 103	1	Seed Production Unit (Kafr-EL-Sheikh)
3	SC maize 104	1	Research & Extension (Gimiza, Gharbia)
4	SC maize 107	1	Seed Production Unit (Kafr-EL-Sheikh)
5	SC maize 310	1	Seed Production Unit (Kafr-EL-Sheikh)
6	3-WC maize 197	1	Nobacid (AboEL-Matameer, Behera)
7	3-WC maize 310	1	Seed Production Unit (Kafr-EL-Sheikh)
8	Strain A	1	Seed Production Unit (Kafr-EL-Sheikh)
9.10	Khahera I	2	Seed Production Unit (Kafr-EL-Sheikh)
11-18	Giza 1	8	Research & Extension (Gimiza, Gharbia)
19-26	Giza 2 (A)	8	Research & Extension (Gimiza, Gharbia)
27-31	Giza 2 (B)	5	Seed Production Unit (Kafr-EL-Sheikh)
Total		31	

1) Washing test :

Five replicates of one hundred seed each were shaken vigorously for 10 min. in 25 ml. distilled water. The suspensions were centrifuged at 2500 rpm for 10 min. The supernatant liquid was discarded and the sediment was resuspended in 2 ml. of distilled water. Eight drops of the suspension were examined separately for each sample by means of compound microscope.

2) Maceration and staining :

The procedure was carried out as described by Mularlidhararao *et al*. 1985. Five hundred seeds were macerated in 5% NaOH with 0.015% trypan blue for 36 hr. at room temperatuare. The seeds were then separated into components in a water stream by hand picking. The seed components are then heated to boiling in lactic acid/ glycerol mixture (1: 2) and examined microscopically. The endosperm dissolved in the alkali solution and could not be collected as a separate component. Infected embryos and pericarps with stained mycelium and oospores of the fungus were counted and percentage of infection was calculated for each sample.

Determination of seed infection levels :

Maize samples showing different infection levels of *Peronosclerospora sorghi* were carried for field trials to assess their potentiality for creating the downy mildew symptoms in developing plants at Abo-El-Matameer, Behera Governorate , seeds were sown during 1998 growing season. Five hundred seed were used for each level. Untreated seed of the resulting range of infection as well as treated ones with Metalaxyl 35% (methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alaninate) at a rate of 3g/1kg seeds applied by two techniques either by direct fungicide application (DFA) or by the organic solvent infusion technique (OSIT) described by Papavizas and Lewis 1976 , Abdel-Monem and abo-Neama 1984; Phookau and Thakur 1990; Shishupola *et al*., 1990 and Reddy *et al.,* 1991). The range of seed infection levels were screened after 45 days and recorded . The ratio of seed infection / disease development was also calculated.

RESULTS

Detection of fungal inocula:

Dry seed examination indicated that none of the seed samples comprised shrivelled seed or seed-borne oospores. The washing test showed the lacking of external oospores in all tested seed samples. Examination of seed components showed that *P. sorghi* mycelia and oospore infection ranged from 0% to 24% (Table 2). Data proved that five cultivars were free from infection. These were SC-9,SC-310,3-WC-197,3-WC-310 and Strain A.Data Table 3 showed that among the fifteen infected seed samples, oospores were observed in seven i.e., samples No. 4 (SC-107),11,14,16(Giza1),20 28,30,(Giza2),while,the mycelium was detected in pericarp of the fifteen tested samples but not observed in the embryo of 4 tested seed samples. Mycelium and oospores found in the pericarp and embryo of the tested samples ranged from 0.2% to 24% and 0.2% to 12% respectively (Table 3).

2

Data shown in the Table 4 shed light upon seed infection levels derived from seed health testing of the 31 seed samples.The results showed that persentages of seed infection ranged from 0.2% to 24%.Samples No.16,14 (Giza1) and 20 (Giza2) showed the higher infection levels , 24%,11% and 15% respectively. Table 5 may bring into view the role of seed-borne infection levels of *P. sorghi* incidence of DSM. The downy mildew pathogen carried through the seeds initiated field infection of 0.6% when seed infection standard was 1.6% i.e., 8:3. The seed-borne inoculum of act as effective for disease development in the field whenever seed infection level was increased. SDM seed infection of 15% and 24% produced 10.8% and 15.6% of diseaesed seedling in the field trials respectively i.e.,,25:18 and 20:13 when calculated as ratio figures. In general, 49.1% of field disease infection came out of 78.6% of infected seeds,that is 62% $\frac{49.1 X 100}{78.6}$. The correlation of seed infection / disease establishment in the field was recorded to be 78.6 : 49.1 (8:5) approx.) i.e., 5/8 of the seed-borne infection were transmitted through seeds to the seedlings . These infected seedlings subsequently act as effecting means for secondary spread of the disease through sporangial inoculum .

Fungicidal seed treatment using Metalaxyl 35% applied by DFA decreased seed transmission of the disease when compared with disease seed to about $\frac{22.2}{49.1}$. Metalaxyl when applied by OSIT mutated the starting point of seed transmission of the disease to 3% seed infection of SDM inocula . Establishment of 8.4% of disease plants developed from 8.6% of *P. sorghi* infected seeds i.e., 11% $\frac{8.4 X 100}{78.6}$.The rate of connection was 78.6 : 8.4 (10 : 1 approx) namely reducing disease transmission to 17% when compared with untreated seeds $\frac{8.4}{49.1}$

Table (3): Percentage of maize seed infection with *Peronosclerospora sorghi* obtained from seed tissues ofthe 11 tested cultivars.

Sample No.	Perecntage of infected seed tissues with				
	Oospores		Mycelium		Total Seed infection
	Pericarp	Embryo	Pericarp	Enbryo	
2	0	0	0.4	0.2	0.4
3	0	0	0.8	0.4	0.8
4	1.0	0	2.0	0.8	2.0
10	0	0	4.0	1.0	4.0
11	2.0	0	6.0	2.0	6.0
14	1.0	1.0	11.0	3.0	11.0
15	0	0	1.0	0	1.0
16	4.0	3.0	24.0	12.0	24.0
18	0	0	3.0	1.0	3.0
19	0	0	1.6	0.6	1.6
20	2.5	2.5	15	5.0	15.0
23	0	0	0.2	0	0.2
26	0	0	2.4	0	2.4
28	1.0	0	5.0	0	5.0
30	1.0	0	2.0	1.0	2.0

Number of samples=31

Table (4): Iinfection levels of maize with *Peronosclerospora sorghi* obtained from 31 seed samples.

Sample No	Cultivar	% Seed infection level
23	Giza 2 (A)	0.2
2	SC- 103	0.4
3	SC-104	0.8
15	Giza 1	1.0
19	Giza 2 (A)	1.6
30	Giza 2 (B)	2.0
4	SC-107	2.2
26	Giza 2 (A)	2.4
18	Giza 1	3.0
10	Khahera 1	4.0
Giza 2 (B)28	Giza2 (B)	5.0
11	Giza 1	6.0
14	Giza 1	11.0
20	Giza 2 (A)	15.0
16	Giza 1	24.0

Table (5): Correlation between maize seed infection level with *Peronosclerospora sorghi* and development of the disease in the field.

%Seed infection Levels		Untreated seeds		Fungicidal treated seeds using DFA		Fungicidal treated seeds using OSIT	
		% infection dev.	Ratio S/F	% infection dev.	Ratio S/F	% infection dev.	Ratio S/F
0.2		0	-	0	-	0	-
0.4		0	-	0	-	0	-
0.8		0	-	0	-	0	-
1.0		0	-	0	-	0	-
1.6		0.6	8:3	0	-	0	-
2.0		0.6	10:3	0	-	0	-
2.2		1.1	2:1	0	-	0	-
2.4		1.6	3:2	0.6	4:1	0	-
3.0		1.8	5:3	1.0	3:1	0.2	15:1
4.0		2.8	10:7	1.6	5:2	0.4	10:1
5.0		3.2	25:16	2.0	5:2	0.6	25:3
6.0		3.4	30:17	1.8	10:3	0.2	30:1
11.0		7.6	55:38	2.6	55:18	2.0	11:2
15.0		10.8	25:18	4.8	25:8	1.8	25:3
24.0		15.6	20:13	7.8	40:13	3.2	15:2
% of Trans			%62		%28		%11
Total	78.6	49.1		22.2		8.4	
Mean Ratio	5.2	3.3	8:5	1.5	4:1	0.6	10:1

DFA=Direct fungicide application .
OSIT=Organic solvent infusion technique.

DISCUSSION

Sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* is important which reduces the crop of maize up to 40% (Safeeulla Shetty, 1978,. Payak, 1975) . Recently, the SDM disease cause serios damage to some maize cultivars in some Governorates of the Nile Delta .

In the present investigation an attempt has been made to assess criteria of seed infection with SDM in maize as correlated with their effective potential to create field disease development. Visual inspection and examination of suspensions of seed washings were unsuccessful in obseving oospore contamination of the SDM in maiz seed samples according to Muralidhararao *et al*., 1985. Using maceration and staining technique the SDM seed-borne inocula in the form of mycelium and oospores in pericarps and embryo has been detected in the 15 seed samples. This is in the line with (*Jones et al*., 1972; Safeeulla and Shetty, 1977; Muralidhararao *et al.*, 1985). The perecentage of seeds having mycelial infection was comparatively more than those showing oospore infection. These results harmonized with those reported by Muralidarao *et al*., 1985).

The SDM inoculum carried through maize seeds transferred the disease to developing seedlings .Seed-borne infection level when gradually increased act as effective agency demonstrating more and more SDM field disease infection. Inoculum threshold of seed-borne DM pathogens is in the amount of inoculum availabe with the seed that results in the development of disease under favourable environment conditions leading to losses. Pathogen-free seed, or seed infected with a DM pathogen, that can not cause loss to the crop when sown is referred to as clean seed. Hence, the inoculum threshold is an important factor in seed health testing and DM disease mangement by the use of clean seed (shetty and Shetty, 1993).

The obtained results suggested that about sixty percent of the seed-borne SDM imocula were transmitted through seeds to the subsequent maize plants. The correlation between seed infection and field disease development may be expressed as ratio eigeht/five. It generally is expected, generally, that one infected seed will give rise to one infected plant. However, such relation will not be observed under field condition (Shetty and Shgetty 1993).

Metalaxyl seed dressing fungicide when applied by DFA minimized SDM disease trnsmission to about one half, whereas aplication of the fungicide by OSIT scaled down the rate of seed transmission to approximately 0.0 %. obligate parasites need living tissues for their survival, and mycelium in the embryo may, therefore, be more important than in the pericarp and endosperm (Shetty and Shetty 1993). This may explain the success of the OSIT in disappointing the fungal inocula in the seed tissues by the ability of the solvent-fungicide mixture to gain deep and to accumulate within the seed and probably translocate to infection sites in the embryonic tissues as the seedling grow (Papavizas and Lewis, 1976; Abdel-Monem and Abou-Neama, 1984).

Further investigations will be carried out to assess actual losses resulting from inoculum load to establish seed tolerances for SDM in seed lots considering nature of resistance or susceptibility of the maize cultivars, chemical seed treatment, health testing methods, secondary spread potentiality of the pathogen and other infection sources.

REFERENCES

Abdel-Monem, A. M. and M. A. Abou-Neama (1984). Comparative study of two seed treatment methods for control of root rot of broadbean. Agric. Res. Rev., Egypt, 62:211-218.

Adenele V. and K.F. Cardwell (1996). Downy mildew diseases of maize in Nigeria, Downy Mildew Newsletter9.1

Assawah, M. W. and H. EL- Arosi (1961). Fungi associated with wheat, barley and maize grains. J. Bot. U.A. R., 4: 153-164.

Farag, Samia A. A. (1964). A survey study of the seed borne microorganisms on some cereal crops. M. Sc., Agric, Alex. Univ., 81 pp.

Fathi, S.M. (1966). Some studies on seed and seedlings diseases of corn. M.Sc., Agric., Ain Shams Univ., 116 pp.

Jones, B. L.; J. C. Leeper and R. A. Frederiksen (1972). *Sclerospora sorghi* in corn: Its location in carpellate flowers and mature seeds. Phytopathology, 62: 817-819.

Kamara, A. M. (1965). A survey on the fungi associating with stalk-rot disease of maize in U. A. R. M. Sc., Agric., Alex. Univ., 90 pp.

Mc Gee, D. C. (1988). Maize diseases: A reference source for seed technologists. The American Phytopathological Society. St. Paul, Minnesota.

Melchers, L. E. (1931). A check list on plant diseases and fungi occurring in Egypt. Trans. Kansas Academy of Sci., 34.

Muralidhararao, B. (1982). Investigations on the establishment and detection of *Peronosclerospora sorghi* in maize seeds. M. Phil dissertation, Downy Mildew Research Laboratory. Mysory University.

Muralidhararao, B.; S. H. Prakash; H. S. Shetty and K. M. Safeeulla (1985). Downy mildew inoculum in maize seeds: Technigues to detect seed-brone inoculum of *Peronosclerospora sorghi* in maize. Seed Science and Technology, 13: 593-600.

Papavizas, G. C. and J. A.Lewis (1976). Acetone infusion of Pyroxychlor into soybean seed for control of *Phytophthora megasperma* var. Sohae. Pl. Dis. Reptr., 60 (6): 484-488.

Payak, M. M. (1975). Epidemiology of maize downy mildew with special reference to those occurring in Asia. Trop. Agric. Res. Ser., 8: 81- 91.

Richardson, M. J. (1979). An Annotated list of seed borne diseases.ISTA, Zürich, Switz. Commonwealth Mycol. Inst. Kew, Surrey England.

Sabet, K. A.; Samra A.S. and F. A. Fadl (1966).Cephalosporium acremonium Corda, the cause of black-bundle disease- Investigations on stalk- root disease of maize in U. A. R. Bull. of Plant Prot. Dept., Min of Agric., 165-169.

Safeeulla, K. M. (1976). Biology and control of the downy midews of pearl millet, Sorghum and finger millet, Wesley Press, Mysore.

Safeeulla, K. M. and H. S. Shetty (1977). Seed transmission of sorghum downy mildew on corn. Seeds an Farms, 3: 21-23.

Safeeulla, K. M. and H. S. Shetty (1978). Sorghum downy mildew in Asia: assessment of present knowledge and futur research needs. In Sorghum Disease: A world Review Proceedings of the International

Workshop on Sorghum Disease (eds. R. J. Williams, R. A. Frederiksen and L.K. Mughogp), pp. 173-183.

Samra A. S.; K. A. Sabet and M. F. Abdel Rahim (1966). Seed transmission of Stalk -rot fungi and effect of seed treatment. Investigations on stalk-root diseases of maize in U.A. R. Bull. of Plant Prot. Dept., Min of Agric., 94-116.

Shetty, H.S. (1985). Movement of sorghum, maize and pearl millet germplasm free from downy mildew inoculum. In Proceedings: Regional Conferences in Plant Qurantine Support for Agricultural Development (eds. K.G. Singh and P.L. Manalo), pp. 235-239, ICRISAT, India.

Shetty, H. S. and S.A. Shetty (1993). Seed certification for the management of cereal downy mildews. Proceedings of the 1st ISTA plant Disease Committee, Symposium on Seed Health Testing,Ottawa, Canada, August, 9-12(1993): 92-98.

Singh, S.D.; H.S. Shetty (1990). Efficacy of systemic fungicide metalaxyl for control of downy mildew (*Sclerospora graminicola*) of pearl millet (*Pennisetum glaucum*). Indian Journal of Agricultural Sciences, 60: 575-581.

Singh, S.D.; S. B. King and J. Werder (1993). Downy mildew disease of pearl millet, Information Bulletin No. 37 ICRISAT India 30 pp.

Williams, R. J. (1984). Downy mildews of tropical cereals-Advances in Plant Pathology,2:1-103.

تـــأثير مســـتويات إصـــابة بـــذور الـــذرة الشـــامية بفطـــر البيـــاض الزغبـــي بيرونوسكليروسبورا سورجاى على حدوث الإصابة في الحقل

محمد رفعت رسمي , محسن محمد السيد صالح , عبد الله محمود عبد المنعم , منى محمد سعيد نور الدين

معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة - مصر

احدث مرض البياض الزغبي المتسبب عن الفطر " بيرونوسكليروسبورا سورجاى" إلخسائر فادحة في إنتاج بذور الذرة الشامية حديثا في مصر .

وتهدف هذه الدراسة إلى التعرف عن مستويات إصابة البذورلمعرفة المسنويات الحرجة منها والتى عندها تحتاج البذور للمعاملة الكيماوية . أجريت عدة اختبارات لفحص بذور الذرة للامراض المصاحبة لها في عدد من العينات لأصناف مختلفة وقد أمكن الكشف عن فطر البياض الزغبي في بعض العينات وكانت درجات الأصابة متفاوتة وبحساب النسبة بين البذور المصابة ودرجة إحداثها للإصابة للنباتات النامية منها لإيجاد العلاقة بين اللقاح الفطري بالبذرة والإصابة الفعلية للنباتات الناتجة منها وكذلك تم تقدير المستويات الحرجة للإصابة الفطرية. وأظهرت النتائج أن حوالي ٦٠% من لقاحات الفطر المصاحبة للبذور قد انتقلت خلال البذور إلى نباتات الذرة الناتجة عنها . وكانت النسبة بين مستويات إصابة البذرة وتطور المرض هي ٨ إلى ٥ .وأدت معاملة البذور بالمبيد الفطري ميتالوكسيل ٣٥%إلى خفض حدوث المرض الى ٧٥% تقريبا . وزادت كفاءة المبيد فى مقاومة المرض عند حقنها بالمذيبات العضوية الأمر الذي أدى إلى خفض ظهور المرض فى الحقل إلى ما يقرب من العشر.

Table (2): Percentage of seed infection with sorghum downy mildew to some maize tested cultivar using macerating and staining methods.

Cultivar	SC-9	SC-103	SC-104	SC-107	SC-310	3-WC-197	3-WC-310	StrainA	Khahera1	Giza1	Giza2 (A)	Giza2(B)	Total
Number of samples	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(2)	(8)	(8)	(5)	31
Seed infection range (Ospores and mycelium)	0	0.4	0.8	2.2	0	0	0	0	(0-4)	(0-24)	(0-15)	(0-5)	
Mean Seed infection	0	0.4	0.8	2.2	0	0	0	0	2.0	5.6	2.4	1.4	

J. Agric. Sci. Mansoura Univ., 26 (11): 6825 - 6833, 2001.