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CEREAL IMPROVEMENT PATHOLOGY

STUDIES ON SORGHUM DOWNY MILDEW

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FORWARD

Narong Singburaudom, a Thai national, is a Masters graduate working for the Rockefeller Foundation Corn Program centered at Bangkok, Thailand. He has undertaken a Research Fellowship at ICRISAT, in the Cereal Pathology Program from August 8 to November 16, 1977. During his time at ICRISAT he has concentrated his activities on the study of sorghum downy mildew (SDM) with particular emphasis on the development of an effective field screening technique. In this document we present the results of his research studies on SDM. We hope that the information contained in this report will assist those working with SDM to perform resistance screening work more efficiently.

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INTRODUCTION

Sorghum downy mildew (SDM), caused by the fungus *Sclerospora sorghi* Weston & Uppal, is a disease with great destructive potential on sorghum and maize. It occurs on these crops in several countries in Asia, Africa and North and South America. In India, which has an annual sorghum production area of 18 mn ha, SDM has been reported to be common in Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh and Uttar Pradesh (Ramakrishnan, 1963). Because of the destructive potential and wide-spread occurrence of SDM it is one of the diseases on the priority list of ICRISAT's Cereal Pathology Program. The basic aim of the program with respect to SDM is to locate sources of stable SDM resistance and to assist the breeders in incorporating this resistance into elite sorghum cultivars so that these will not be vulnerable to SDM epidemics. In order to meet these aims an efficient field screening technique is vital and the studies presented below were conducted to provide information that would allow efficient SDM resistance screening to be conducted at ICRISAT's Hyderabad headquarters.

Traditionally soil borne oospores are used to screen for resistance to SDM in plots developed as 'sick plots'. However such screening has several deficiencies including uneven inoculum distribution within the plot, time taken to develop the 'sick' plot, inflexibility in size and location of the screening plot, and reliance upon particular soil moisture conditions following planting for infection to take place. More efficient and reliable screening can be done using conidia which are abundantly produced on young infected leaves. The first successful inoculations with conidia were reported by Safeeulla (1955). In their study conidia were

produced in the laboratory in detached leaf cultures by floating pieces of freshly infected leaves on water surface in Petri dishes, and incubating them at 20°C and below. A thick felt of conidiophores bearing conidia, produced on the leaf surface by this method, was used to inoculate sorghum and maize seedling by brushing them on young leaves previously wetted with a water-spray containing 0.05% sodium recinoleate. They obtained 10-30 percent infection.

Jones (1970) devised a method of conidial inoculation in which leaf sections were taken from infected plants with sorghum downy mildew and placed on the leaves of sorghum seedlings. He covered the seedlings with polythene sheets to create a high relative humidity. When cold night temperature occurred, the excised leaf sections produced conidia which were deposited on the leaves of the seedlings. He reported that levels of infection were high and sorghum plants 1 to 5 days old were more susceptible to infection than older plants.

Inoculation by spraying with conidia of *S.sorghi* has been successfully used for identification of downy mildew resistance of corn selections in Thailand.

Schmitt and Freytag (1974) recently reported a technique for artificial inoculation of sorghum and corn by spraying with a water suspension of conidia.

Craig (1975) examined the efficiency of inoculation methods in the identification of downy mildew resistance with five commercial sorghum hybrids in the green-house and in the field. He reported that the correlation between reactions of the resistance of hybrids to conidial inoculation in the green house and field were good, but the less resistant

hybrid exhibited much higher level of infection in response to artifical inoculation than those observed in the field. Some varieties resistant in the field were highly susceptible to artificial inoculation. This indicates that certain types of resistance to downy mildew may be circumvented by the artificial inoculation technique.

These and other reports led us to believe that we should utilise the conidia for our SDM resistance screening. In preliminary attempts to use conidia at our Hyderabad farm several difficulties were encountered and the levels of infection produced were disappointingly low. The major difficulty was the nocturnal production of the conidia on infected leaves and their germination long before day-light hours. In view of these difficulties and disappointing results the following questions were set out:

i. how do we maintain a source of conidial inoculum at all times

ii. how do we produce enough conidia at any one time to inoculate several hundred plants

iii. how do we get over the difficulty of sporangial production and germination in the early morning hours

iv. what is the best inoculation method, and at what age are seedlings best inoculated, and at what time of day

v. what is the relationship between resistance to conidial infection and resistance to oospore infection

vi. what is the relationship between local lesion production and resistance to systemic infection from conidia and oospores.

In this study attempts have been made to find answers to questions ii, iii, part of iv and part of vi. In some cases answers are incomplete and new questions have been generated. The work is written up at this point, even while there are still several gaps in our knowledge, because it represents the efforts of one Research Fellow who is shortly leaving ICRISAT for his

home country. The various experiments are reported independently and a final overall discussion is made.

I. STUDY OF CONIDIAL PRODUCTION ON INCUBATED INFECTED LEAVES

INTRODUCTION

The three main factors affecting conidial production in SDM infected sorghum leaves are likely to be temperature, humidity, and light energy received prior to the initiation of conidial production. In the case of SDM of maize in Thailand, a minimum of 3 hr sunlight is necessary between successive crops of conidia. In practice these leaves were harvested from the field between 11.00 and 13.00 hr and were subsequently incubated at the optimum humidity and temperature. It was found that the same leaves could be used two or three times for conidial production provided certain critical light requirements were satisfied following each conidial crop.

This experiment was undertaken to determine the effect on conidial production of different light and incubation treatments and to examine the possibility of utilizing one leaf for several conidial crops.

MATERIALS AND METHODS

Treatment 1. SDM infected sorghum leaves were collected from the field at 15.00 hr and were washed with tap water to remove old conidiophores, conidia and dust. After washing they were cut into 1 cm^2 pieces and placed in Petri dishes containing moist blotting paper. The Petri dishes were incubated at 20°C from 17.00 hr to mid-night after which the incubator temperature was changed to 5°C which was maintained until the conidia were harvested the next morning.

Treatment 2. The same as Treatment 1, but then the leaf pieces were rewashed

to remove the first crop of conidia and their conidiophores. After washing the Petri plates were maintained under a combination of incandescent and flourescent light (280 ft-candle) from 08.00 hr to 15.00 hr. From 17.00 hr they were incubated in the same way as in Treatment 1.

Treatment 3. Following the first washing after collection, infected leaves were kept under a combination of incandescent and flourescent lights from 17.00 hr to 08.00 hr after which they were cut into 1 cm^2 pieces and placed in Petri dishes containing moist blotting paper. The Petri dishes were incubated at 20°C for 8 hr after which conidia were harvested.

Measurement of conidial production.

After incubation the conidia were harvested from each leaf piece and suspended in 5 ml of water. From each leaf piece the number of conidia in 10 microscope fields was determined using a haemacytometer.

RESULTS AND DISCUSSION

The data in Table I 1 indicate that Treatment 1 was the best of the three for conidial production, but double incubation is possible if sources of conidia are limited.

Treatment 1 is useful for inoculation in the morning at the beginning of the normal working day. The viability and infectivity of the conidia produced in this way must be evaluated. If injection inoculation is made there should be no problem with day-time inoculation. However injection inoculation is slow and laborious and if large numbers of plants are to be inoculated; e.g.. by spray, then evening inoculation may be

more appropriate. If so the efficiency of Treatment 3 will need to be improved, perhaps by increasing the overnight light treatment.

Table I 1. The number of conidia produce per cm^2 infected leaf under three incubation treatments.

Treatment*	Conidin/cm^2**
Single, immediate	11.2×10^4
Double	2.5×10^4***
Single, delayed	1.3×10^4

* Single immediate: leaves harvested at 15.00 hr and incubated from 17.00-24.00 hr at 20°C followed by 8 hr at 5°C.

Double: leaves first incubated as in single, followed by maintenance under light source for 7 hr and then incubated in the same way a second time.

Single delayed: after harvest at 15.00 leaves maintained under lights 17.00-08.00 hr and then incubated at 20°C until 16.00 hr.

** Mean of 100 leaf segments each 1 cm^2.

*** Data from the second incubation.

II. MAINTENANCE OF MATURE CONIDIA OF *SCLEROSPORA SORGHI* AT LOW TEMPERATURE FOR INOCULATION.

For the graminicolous downy mildews, no *in vitro* culture technique is available. Therefore, screening for resistance has been practiced only by field test, by natural infection or by inoculation of conidia directly collected from naturally diseased plant. In the latter case, it is quite difficult to obtain conidia of an uniform stage, because immature conidia as well as germinated ones are collected together.

At the present time, the problem of immature conidia and germinated conidia are solved by artificial incubation. In this case, the infected leaves are collected from the field in the day time and are incubated at the optimum condition for sporulating. After incubation, we can collect an uniform stage conidia for use in inoculation. However, we cannot keep the mature conidia for a long time for inoculation any time, especially inoculation in the day time.

Recently, Kimigafukuro and Lou (1973) found that agar media containing some neutral salts were effective in keeping mature conidia from germination without causing loss of pathogenocity with *S. sacchari*. For *S. philippinensis* they found that medium containing 1/8 M KNO_3 is able to delay the germination of conidia without causing the loss of pathogenicity but this medium was not useful because of difficulty in obtaining exact density of conidial suspensions (Kimigafukuro, 1976).

In this study, we examined an artificial incubation technique which can be used effectively to keep conidia at the right stage for inoculation in the day time. The purpose of this study was to find out how many hours

we can keep the mature conidia at 5°C without germination and loss of pathogenecity.

MATERIALS AND METHODS

DMS-652 seeds were planted in large clay pots (approximate 20 cm diameter) and were kept outside in the open air. The experiment was designed in randomized complete blocks with 4 replications, 7 treatments and 8 pots per treatment. There was an average of 30 plants per pot after emergence.

Inoculum preparation.- Infected leaves were collected from the field at 15.00 hr and were washed in tap water to remove old conidiophores, conidia and dust from the leaves. Then the leaves were put in a moist chamber in an incubator set at 20°C from 17.00 hr until midnight. After midnight the temperature was set at 5°C until the time of inoculation. There were seven times of inoculation, (6.00, 7.00, 8.00, 9.00, 10.00, 11.00 and 12.00 hr).

Inoculation.- Seedlings, 7 days after emergence, were inoculated by injection with a syringe. The conidial suspension was prepared in distilled water with a concentration of 21 conidia per low power field (10x) of a microscope.

Observation.- Germination of conidia was observed after inoculation (approximately 20 minutes at room temperature).

Infectivity of conidia was tested by inoculation of seedlings 7 days after emergence every hour from 06.00 to 12.00 hr. Local lesions were observed 7 days after inoculation and systemic symptoms were observed 14-21 days after inoculation.

RESULTS AND DISCUSSION

Every hour after preparing the conidial suspension the conidia began to germinate within half an hour. Examination of the remaining conidial suspension after inoculation revealed some conidia germinated but the percentage of conidial germination was not calculated.

After inoculation, the seedlings showed local lesions at the position of inoculum placement and they were more severe 7 days after inoculation. At the early stage, two or three days after inoculation, small pale green spots appeared on inoculated leaves and they turned reddish brown with a yellow halo later. Finally between seven days to ten days after inoculation, yellow-green streaks appeared on inoculated leaves and there was sporulation on these local lesion at high humidity.

The percentage of local lesion is shown in Table II.1 and the analysis of variance is shown in Table II.2. The differences in percent local lesion among the times of inoculation were significant at 0.001 level of probability. There were no significant differences between inoculations at 6.00, 7.00, 8.00 and 9.00 hr. The results of the inoculation at 10.00 hr were not significantly different from those at 06.00 hr.

The systemic symptoms were observed 14-21 days after inoculation in the form of pale yellow streaks or chlorosis. The percent systemic symptoms shown in Table II.3 and the analysis of variance is shown in Table II.4. These indicate that the percent systemic symptom was highest in plants inoculated at 06.00 hr. However the level of infection was disappointingly low.

CONCLUSION

The conidia were viable and infective after keeping at 5°C for at least 10 hr.

The percentage of systemic symptom was very low at each time of inoculation. One possible reason for this is that the inoculum may not have been placed deep enough in the leaf whorl so that it did not reach the growing points of seedlings.

Table II.1. The percent local lesion 7 days after inoculation.

Time of inoculation	Replication				Average
	I	II	III	IV	
6.00	68	77	69	75	72
7.00	68	65	81	61	69
8.00	60	92	79	72	76
9.00	78	98	86	70	81
10.00	64	58	65	53	60
11.00	71	51	47	23	48
12.00	46	10	1	3	15

L.S.D. (P=0.05) 13

Table II.2. The analysis of variance of the percent local lesion at 7 days after inoculation.

Source of varintion	Degree of freedom	Sum of square	Mean of square	F.test
Replication	3	790	263	
Time of inoculation	6	12240	2040	12.98***
Error	18	2829	157	
Total	27	15859		

*** significant (P = 0.001)

C.V. = 21%

L.S.D. .05 = 12.8

Table II.3. The percent systemic symptom at 14-21 day after inoculation.

Time of inoculation	Replication				Average
	I	II	III	IV	
6.00	11	14	13	6	11
7.00	11	9	5	2	7
8.00	7	3	5	2	4
9.00	3	0	6	2	5
10.00	3	3	0	2	2
11.00	5	0	0	2	2
12.00	2	1	0	2	1
L.S.D. (P = 0.05)		4			

Table II.4. The analysis of variance of the percent systemic symptom at 14-21 days after inoculation.

Source of variation	df	S.S	M.S.	F
Replication	3	66	22	
Time of inoculation	6	268	45	7.6***
Error	18	106	6	
Total	27	440		

*** Significant (P = 0.001)

C.V. = 55%

L.S.D. .05 = 3.6

III. EFFECT OF PLANT AGE ON SYMPTOM DEVELOPMENT

There are reports that plant age affects the success of conidial infection of sorghum. Jones (1971) reported high infection levels in 1 to 5 day old sorghum plants which were more susceptible to infection than older plants. Kenneth and Shahor (1975) showed that in sorghum plants at the three leaf stage, conidial inoculation of the coleoptile area gave higher levels of infection than inoculation of leaves above the coleoptile. On downy mildew of maize in Thailand, we found that the best plant age for inoculation with conidia was 5 days after emergence.

Butler (1918) described three distinct types of symptom development of SDM infected sorghum plants. The first type was seedling infection where the leaves became pale yellow and supported abundant production of conidia and conidiophores on both leaf surfaces. After five to six weeks of growth, white streaks appeared on the upper leaves. These later turned brown numerous oospores were formed, and leaf shredding occurred. Infected plants did not head. In the second type, symptoms appeared in the upper leaves and bases of the lower leaves of two-month-old plants. These leaves also turned brown and abundant oospores were produced. On the lower leaves, yellow patches with conidial stage appeared. The ear-heads were either absent or contained only a few grains. In the third type, symptoms appeared on the leaves in the form of long narrow streaks, or patches, which turned orange and finally dark brown in colour. These patches produced only conidia and mainly on the lower side. Thus, no shredding of the leaves occurred.

For downy mildew of maize, Kajiyara (1975) described the stage of symptoms development as follows:

Day after inoculation	Symptoms
2	Small (1-2 mm) pale green spots appear on inoculated leaf
5	Yellowish green streaks appear on inoculated leaf
6	The streaks become clear, and some inoculated leaves turn yellow
7	Yellowing of inoculated leaves is conspicious, some of them begin to wilt
9	1st - 3rd leaves die, streaks on 4th leaves elongate and are clear
14-25	Typical systemic symptoms appears on newly developed leaf.

Early work in India, Israel (Kenneth, 1966) and elsewhere separate symptoms into the systemic and the local lesion phases. The objective of this experiment was to determine the effect of plant age on symptoms induced by conidia and the pattern of development.

MATERIALS AND METHODS

The susceptible cultivar, DMS-652, was planted at six dates at 5 day intervals to prepare plant ages after emergence of 5, 10, 15, 20, 25 and 30 days. The experiment was designed in randomized complete blocks, four replications and six treatments. Eight pots were included in each treatment, containing 10 plants per pot after thinning. At each planting date two check pots were included. All pots were kept in the screenhouse.

Infected leaves were collected from the field at 11.00-12.00 hr and were washed in tap water to remove old conidia, conidiophores and dust

from the leaves. After washing, the leaves were placed in a moist chamber and incubated at 20°C for 8 hr after which the mature conidia were harvested. The conidia were suspended in distilled water and contained 29 x 10^4 conidia per ml as determined by hemocytometer counts.

Seedlings of all ages were inoculated at the same time by injection of a conidial suspension into the whorl. Inoculation was done during night (22.00 to 01.00 hr) and fresh inoculum was used in each replication.

Observations were made on the occurrence of three types of symptom, viz

Local lesions The direct effect from the insertion of the needle and conidia which were deposited at the injection point. Lesions appeared as pale green spots which turned yellowish to reddish brown. The number of leaves with local lesion were counted 2 and 5 days after inoculation (DAI).

Local colonization. We term the condition in which the pathogen spreads locally within the tissue from the injection point, producing evident chlorosis, as local colonization. In these studies local colonization appeared both at the center and the tip of the leaves; i.e., any tissue inoculated. The local colonization can extend for several cm from the inoculation point. The data were recorded 10 DAI.

Systemic infection: The plants systemically infected were recorded from 14 until 28 DAI. Plants were rated systemically infected when systemic chlorosis was present from the base to the entire leaf of those leaves which unfolded after inoculation.

Pattern of symptom development: Symptom development was examined 2, 5, 10, 14, 21 and 28 DAI. All symptoms which appeared were recorded and described separately for each plant age.

RESULTS

Effect of plant age on local lesion production. Local lesion development was more extensive on young than older plants 2 DAI (Table III.1), but by 5 DAI there was no difference among ages. However, the nature of local lesions varied among ages. The younger plants showed more chlorosis around the infection point and the local lesions supported asexual sporulation. The local lesions on the older plants were in the form of reddish spots with yellow halos.

Effect of plant age on local colonization. The greatest incidence of local colonization developed on seedlings inoculated 5 days after emergence (Table III.1). Local colonization was more in seedlings inoculated 5-10 days than 15 days after emergence. Both local lesion and local colonization development were greatest in seedlings inoculated 5 days after emergence. The analysis of variance of local colonization data indicates no statistically significant difference between seedlings inoculated 5 and 10 days after emergence, but these were significantly different to seedlings inoculated at other ages.

Effect of plant age on systemic infection. The greatest amount of systemic infection occurred in seedlings inoculated 5 days after emergence (37%). The next greatest occurrence was on the seedlings inoculated 10 days after emergence (24 %). The difference between 5 and 10 day seedlings was not statistically different (P = 0.05), however, the cv was very high.

Symptom development description. The observations on symptom development are described in Table III.2.

DISCUSSION

The percent local lesions 2 DAI was lower on the older plants than 5 DAI. An explanation is that, on the older plants, injection was deeper into the whorl than with the younger plants and thus the infection points appeared later. On the younger plants, the whorl inoculation was not so deep so the local lesions appeared at an early stage after inoculation.

The local lesions on the older plants did not develop to the local colonization stage, but on the younger plants the pathogen continued development from the local lesions and a high percentage of local colonization resulted.

With the seedlings inoculated 5 and 10 days after emergence, there was good correlation between the local colonization and systemic infection; i.e., the younger plants which gave a high percent local colonization gave the highest percent systemic infection also.

The percentage systemic infection at 28 DAI was higher than at 21 DAI for all plant ages. However, the percent increase from 21-28 days was very low for the younger plants and was higher on the older plants. On the oldest plants no systemic infection was detected at 21 DAI but was seen 28 DAI. This result indicates that the percent systemic infection should be observed about 28 DAI. The younger plants developed the three types of symptoms - local lesion, local colonization and systemic symptoms. The older the plants the less was the development of local colonization and systemic symptoms.

The low figure for systemic infection, even on the youngest plants, is disappointing and is likely to be related to depth of inoculum placement

in the whorl, for unless the inoculum gets to the growing point systemic infection will not occur.

CONCLUSIONS

In the study three basic types of infection were detected:

i. Local lesions. Discrete lesions formed in response to infection at the site of inoculation. These local lesions are seen in the middle of leaves partially emerged at the time of whorl inoculation.

ii. Local colonization. Symptoms appear throughout the tissue around the infection points of inoculated leaves and at the tips of leaves which were deep in the whorl at the time of inoculation. Colonization of the tissue occurs so that the infection is not seen as discrete lesions. Symptoms appear identical to those of true systemic infection but they occur at the centers and tips of the inoculated leaves and not the bases, and do not necessarily indicate that subsequent leaves will be infected.

iii. Systemic infection. Infection that occurs through the infection of the growing point and which manifests itself first on the lower portions of emerging leaves and increases in severity progressively on subsequent leaves until the leaf laminae are 100% infected.

For all these symptoms the greatest incidence occurred on plants inoculated 5 days after emergence. We should examine whether even younger plants will give a greater proportion of systemic infection. Local colonization can be recorded 10 DAI. The best time to record systemic symptoms was found to be about 28 DAI and even later recordings should be made for detection of further infection.

Table III.1. The proportion of plants with local lesions, local colonization and systemic infection various days after inoculation (DAI) sorghum plants of six ages with a conidial suspension of *Scleraospora sorghi*.

Plant age (days) at inoculation	% local lesions		% local colonization	% systemic infection	
	2 DAI	5 DAI	15 DAI	21 DAI	28 DAI
30	35	96	0	0	11
25	53	84	3	6	11
20	62	83	2	7	10
15	89	85	18	4	13
10	87	83	82	22	24
5	95	94	94	33	37
L.S.D. (0.05)	20	15	10	12	16
C.V. (%)	19	11	21	64	57

Table III.2. Process of symptom development after whorl injection inoculation with conidial suspension.

Plant age at inoculation	Days after inoculation	Symptoms
30 days	2	Small pale green spots appear at the infection points
	5	Pale green spots become clear and develop reddish color with yellow halo
	10	Infection point appears as, reddish spot with yellow halo, and no yellow streaks
	14	" -- "
	21	No symptoms appear on the newly developed leaves
	28	White streaks present on the newly developed leaves at the top.

Plant age at inoculation	Days after inoculation	Symptoms
25 days	2	Small pale green spots appear at the infection points
	5	Pale green spots become clear and reddish-brown color with yellow halo
	10	Infection points appear only as red spots with yellowish halo, no yellow streaks
	14	Infection points appear only as red spots with yellowish halo, and white streaks present on the newly developed leaves
	21	White streaks appear on the upper newly developed leaves, and turn brown
	28	White streaks turn brown and reddish color
20 days	2	Small pale green spots appear on the infection points
	5	Pale green spots become clear and reddish brown with yellowish halo and no yellow streaks
	10	Infection points appear as red spots with yellowish halo, no yellow streaks
	14	Infection points appear as red spots with yellowish halo, and white streaks present at the base on newly developed leaves.
	21	White streaks appear on the upper newly developed leaves and turn brown
	28	White streaks turn brown and reddish brown color
15 days	2	Small pale green spots appear on the infection points
	5	Pale green spots become reddish brown and yellowish green streaks appear clearly
	10	Infection points appear reddish brown and yellowish green streaks appear clearly

Plant age at inoculation	Days after inoculation	Symptoms
	14	Inoculated leaves begin to wilt and die, systemic symptoms in the form of chlorosis and white streaks appear on some of the newly developed leaves
	21	White streaks appear on the upper newly developed leaves and turn brown
	28	White streaks turn brown and reddish-brown color
10 days	2	Small pale green spots appear on the infection points
	5	Pale green spots become reddish brown and bigger, yellowish green streaks present at the tip of leaf
	10	Infection points appear reddish-brown and yellowish green streaks become clear
	14	Inoculated leaves begin to wilt and die, typical systemic symptom of chlorosis and white streaks appear on the newly developed leaves
	21	White streaks appear on the upper newly developed leaves
	28	White streaks turn brown and reddish-brown color
5 days	2	Small pale green spots appear on the infection points
	5	Pale green spots become reddish brown and bigger, yellowish green streaks present at the tip of leaf
	10	Infection points appear reddish-brown and yellowish green streaks become clear

Plant age at inoculation	Days after inoculation	Symptoms
	14	Inoculated leaves begin to wilt and die, typical systemic symptom of chlorosis and white streaks appear on the newly developed leaves
	21	White streaks appear on the upper newly developed leaves
	28	White streaks turn brown and reddish brown color

IV. EFFECTS OF CONIDIAL INOCULATION AT DIFFERENT TIMES OF THE DAY IN THE FIELD

One of the major limitations to the SDM screening work has been the need to inoculate in the early hours of the morning (shortly after midnight). In an earlier experiment we found that conidia could be obtained at various times of the day by manipulating incubation parameters. The object of this experiment was to see whether these conidia could be successfully used for resistance screening.

MATERIALS AND METHODS

DMS-652 seeds were planted in two row plots (20 plants per row after thinning). Inoculations were done three times, as the treatments, at 1700 hr, 2400 hr and 0600 hr. There were four replications per treatment and at each inoculation time a check plot was maintained non-inoculated.

For the 1700 hr inoculation, infected leaves harvested at 1500 hr were maintained for 1700 hr under fluorescent lights and then were incubated

at 20°C in a moist chamber for 7 hr after which conidia were harvested in water to make a suspension containing 16 x 10^4 conidia per ml.

For the 2400 hr inoculation, infected leaves were harvested from the field at 1500 hr washed in tap water and were incubated in a moist chamber at 20°C for 7 hr, after which a conidial suspension was prepared containing 16 x 10^4 conidial per ml.

For the 0600 hr inoculation, infected leaves were collected from the field at 1500 hr, were maintained under fluorescent light until 2100 hr and suspension containing 18 x 10^4 conidia per ml was prepared.

The conidial suspension was injected into the whorls of the seedlings (5 days after emergence) and the incidence of systemic infection was recorded 14 days after inoculation.

RESULTS

As some plants were attacked by shootfly they did not have the opportunity to express systemic symptoms, therefore they were not included in the record and analysis.

The data on percent systemic infection 14 days after inoculation (Table IV.1) indicated that the 2400 hr inoculation gave the greatest level of infection which was not statistically significant from the 1700 hr inoculation. The 1700 hr inoculation gave significantly greater infection than the 0600 hr inoculation which was not significantly greater than the check. However, the 1700 hr inoculation gave a reasonably high level of infection - high enough to designate the cultivar as highly susceptible, and had recording been made at 21 days after inoculation or even later than the level of infection could well have been higher.

The background level of about 25 percent infection in the check presumably come from oospores in the soil and air borne conidia from nearby plots.

CONCLUSION

This result indicated that inoculation can be made at 1700 hr with a good level of success as same as the 2400 hr inoculation. One possible reason that the inoculation method used in this study was the syringe injection, so the conidial suspension was placed deep into the whorls where the moisture can be maintained for long time.

Table IV.1. Percent systemic infection 14 days after inoculating DMS-652 seedling at three times of the days.

Treatment	Time of Inoculation		
	1700 hr	2400 hr	0600 hr
Inoculated	65	78	38
Control	20	20	20

L.S.D. (0.05) = 24

V. STUDY OF THE TIME OF OCCURRENCE AND DURATION OF THE ASEXUAL AND SEXUAL REPRODUCTIVE PHASE OF SORGHUM DOWNY MILDEW *SCLEROSPORA SORGHI* ON INFECTED SORGHUM PLANTS

Sclerospora sorghi, the causal agent of sorghum downy mildew (SDM), produces both conidia and oospores on infected sorghum plants. The conidial production stage can occur at the seedling stage and appears in the form of pale yellow leaves covered with downy growth of conidia mainly on the under surface but which can appear on both surfaces of the leaf. The oospore production stage appears after five to six weeks and

the leaves in which the numerous oospores are formed finally shred releasing the oospores to the environment. In the case of SDM on sorghum, asexual sporulation diminishes as the sexual phase increases and this may be a limiting factor for using the asexual spores in a disease nursery situation to screen for resistance.

In this study the periods of conidial production and oospore production were examined to obtain information on the best time for planting the infector material in relation to the test material. This information is requisite to obtaining a high level of infection from asexual inoculum.

MATERIALS AND METHODS

The susceptible cultivar, DMS-652, was planted in the field and was syringe inoculated with conidia 5 days after emergence. The plants developing systemic symptoms were used for this study.

The total number of leaves, number of leaves with systemic chlorosis, number of leaves with only conidia, number of leaves with conidia and oospores and number of leaves with only oospores were observed and recorded on individual plants 2, 3, 4 and 5 weeks after inoculation (WAI). The average number of leaves per plant were calculated by using the following formula, where "x" is the total number of leaves with systemic chlorosis per plant, number of leaves with conidia only per plant, number of leaves with conidia and oospores per plant and number of leaves with oospores only per plant.

$$\text{Average number of x per plant} = \frac{\text{Total number of x}}{\text{Total number of observed plants}}$$

The percentage of infected leaves with systemic chlorosis, percentage of leaves producing only conidia, percentage of leaves producing both conidia and oospores and percentage of leaves producing oospores only were calculated using the following formula

$$\text{Percent of } x = \frac{(\text{Total number of leaves with } x) \times 100}{\text{total number of leaves}}$$

Percent infected leaves was calculated by using the formula

$$\text{Percent infected leaves} = \frac{\text{Total number of all infected leaves}}{\text{total number of leaves}} \times 100$$

RESULTS

Systemic symptoms were first observed on inoculated plants two weeks after inoculation. The average numbers of leaves per plant (Table V.2) were 4, 8, 10 and 7 at 2, 3, 4 and 5 WAI respectively. At 5 WAI the number of leaves per plant was lower than at 4 WAI because the first and second leaves died and were shed.

Average numbers of leaves with systemic chlorosis increased from 2 at 2 WAI to 4 at 3 WAI and 5 leaves at 4 WAI. At 5 WAI the number of leaves with chlorosis symptom had decreased to 1 leaf (Table V.2).

The average number of leaves with only conidia increased slowly during 2 and 3 WAI but increased rapidly by 4 WAI and decreased rapidly 5 WAI. The average number of leaves with both conidia and oospores did not vary during this time period but decreased after 5 WAI. Average number of leaves with oospores only began to increase at 3 WAI and increased rapidly at 4 WAI. The data on the percentage of various categories of infected leaves is shown in Table V.3.

At 2 WAI most infected leaves showed systemic chlorosis symptoms but the percent of leaves with only conidial production was about 50 percent of this as some of the infected leaves were not sporulating.

At 3 WAI, the percentage of leaves with systemic chlorosis was as high as the percentage at 2 WAI. Percent infected leaves with only conidia, conidia and oospores, and only oospores were not different from the percentage at 2 WAI.

At 4 WAI, the percentage of leaves with systemic chlorosis was not different from the second and third week but the percent infected leaves with conidia increased from 16 percent to 31 percent. The percent infected leaves with only oospores was increased from 21 to 34.

At 5 WAI, both infected leaves with systemic chlorosis and infected leaves with only conidia had decreased from 44 percent to 17 percent and 31 percent to 17 percent respectively. Only the proportion of the leaves with only oospores had increased (up to 55 percent).

The relationships between the proportion of leaves in various categories during this time period can be clearly seen from Figures V.1 and V.2.

DISCUSSION

From the results of this experiment, we can see (Figure V.2) that percent infected leaves with systemic chlorosis and conidial production was high at 2, 3 and 4 WAI and decreased by 5 WAI. The percent of leaves with only conidia 2 and 3 WAI was not very high because the plant was young and the systemic symptom leaves had just developed. When the plant was older the leaves with systemic chlorosis gave good

asexual sporulation so that the percent of leaves with only conidia increased from 3 WAI to 4 WAI. After 4 WAI the percentage decreased because subsequent leaves produced oospores and did not produce conidia.

In the case of infected leaves with both conidia and oospores, the proportion of these did not differ during this period because the systemically infected leaves which appeared during 2-3 WAI had not turned completely to oospores production. Only the base to the middle of the leaves showed oospore production but their tips showed systemic chlorosis symptoms with conidial production.

For the infected leaves with only oospores, the percentage increased steadily because most of newly developed leaves gave oospore production from 3 WAI.

CONCLUSION

The first systemically infected leaves to appear producedonly conidia and maximum conidial production occurred 3-4 WAI. Subsequent leaves produce both conidia and oospores and finally developing leaves produce only oospores. The change-over from mainly asexual reproduction to mainly sexual reproduction occurs between 4 and 5 WAI. By 5 WAI the leaves were producing mainly oospores with little conidial production.

What are the implications of these observations for utilising naturally produced conidia for field screening? We need to have young (5 day) seedlings exposed to conidial inoculum, and thus in order that the seedlings to be tested exposed at the most susceptible stage during the time of maximum conidial production, infector rows should be planted about 3 weeks before the test rows.

Table V.1. Total number of observed plants, total number of leaves, total number of leaves with chlorosis symptom, number of leaves with conidia only, number of leaves with conidia and oospores and number of leaves with oospores only at 2, 3, 4 and 5 weeks after inoculation.

Characters	weeks after inoculation			
	2	3	4	5
Total number of:				
observed plants	70	63	57	31
leaves	299	504	594	220
leaves with chlorosis symptom	130	225	259	38
leaves with conidia only	65	80	186	37
leaves with conidia and oospores	31	84	69	33
leaves with oospores only	65	105	203	121

Table V.2. Average value per plant of total number of leaves, total infected leaves, total number of leaves with chlorosis symptom, total number of leaves with conidia only, total number of leaves with conidia and oospores and total number of leaves with oospores only 2, 3, 4 and 5 weeks after inoculation.

Characters	weeks after inoculation			
	2	3	4	5
Total number of leaves:				
per plant	4.3	7.9	10.4	7.1
infected per plant	3.5	5.5	9.1	6.2
chlorotic per plant	1.9	3.5	4.5	1.2
with conidia only per plant	0.9	1.3	3.3	1.2
with conidia and oospores per plant	0.7	1.3	1.2	1.1
with oospores only per plant	0.9	1.6	3.4	3.9

Table V.3. Percent total infected leaves, infected leaves with chlorosis symptom, infected leaves with conidia only, infected leaves with conidia and oospore, infected leaves with oospores only at 2, 3, 4 and 5 weeks after inoculation

Characters	Days after inoculation (weeks)			
	2	3	4	5
Infected leaves:				
percent total	76	82	89	87
with chlorosis symptom	43	45	44	17
with conidia only	22	16	31	17
with conidia and oospores	10	17	12	15
with oospores only	22	21	34	55

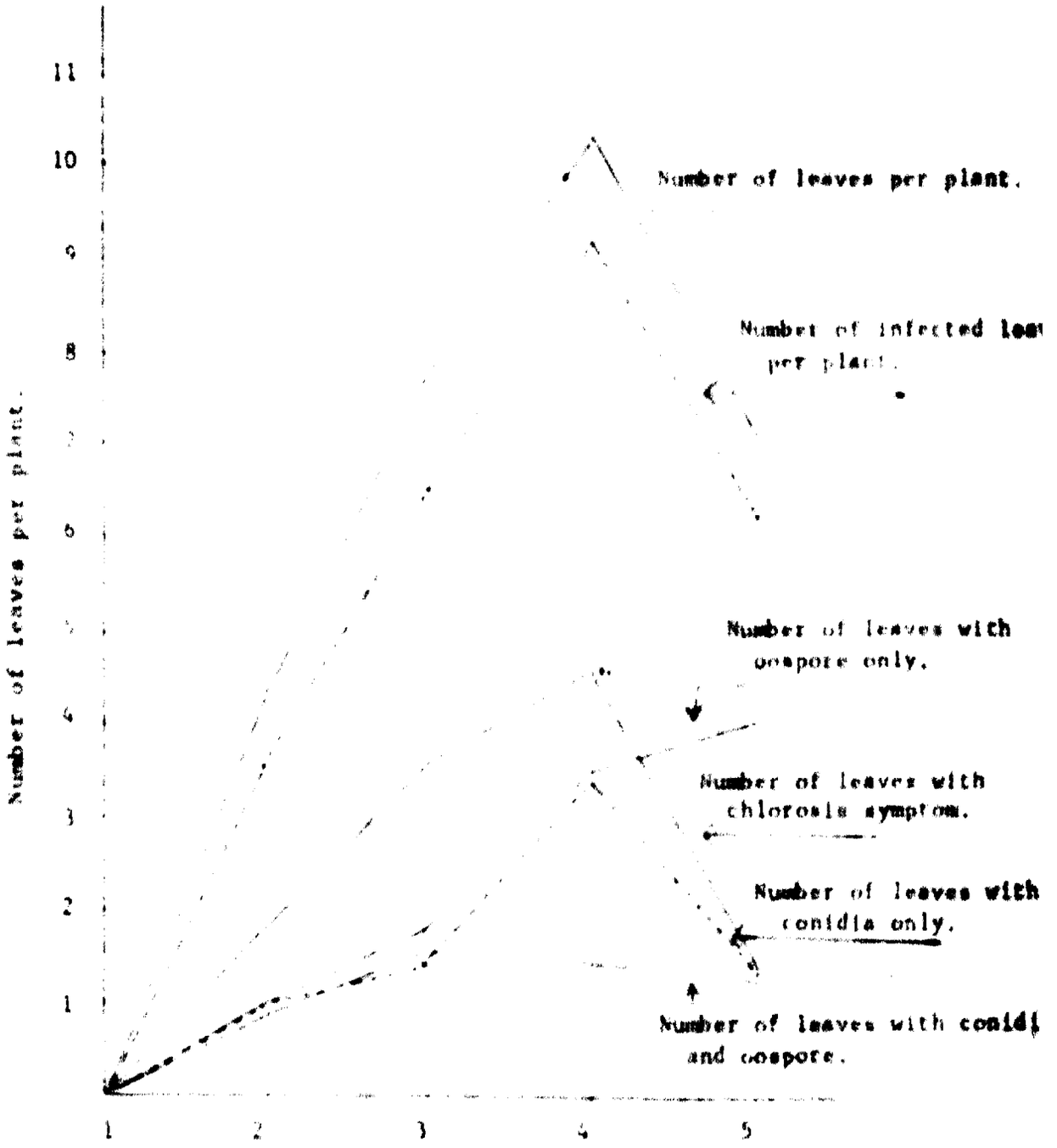

Figure V.1: Showed the average number of leaves with chlorosis symptom, conidial only, conidia and oospores, oospores only number of infected leaves and number of leaves per plant.

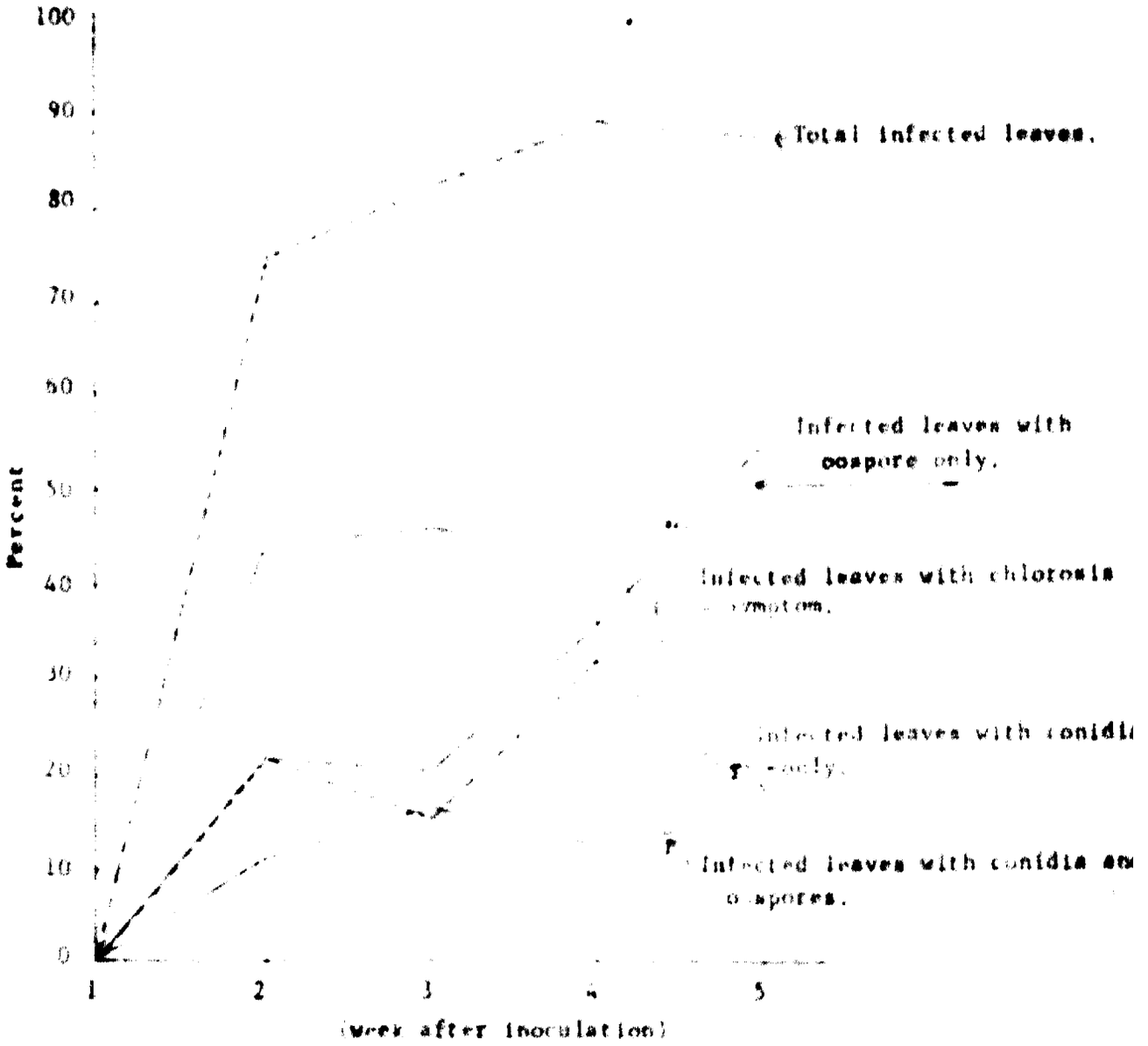

Figure V.2 The percent of leaves with chlorosis symptom, conidia only, conidia and oospore, oospore only and total infected leaves.

VI. COMPARISON OF REACTIONS OF 25 ISDN ENTRIES FOLLOWING INJECTION INOCULATION AT HYDERABAD AND NATURAL EXPOSURE TO FIELD INOCULUM AT DHARWAR AND THE EXAMINATION OF RELATIONSHIPS BETWEEN LOCAL LESION, LOCAL COLONIZATION AND SYSTEMIC SYMPTOMS

Sorghum plants produce three types of symptoms in response to conidial inoculations with the SDR pathogen, *Sclerospora sorghi*. There are local lesions at the point of inoculum placement, local colonization of tissue around the point of inoculum placement and systemic infection via infection of the growing point. One objective of this experiment was to determine if there were any relationships between the development of these three symptom types on a range of sorghum lines. The second objective was to compare the reaction of a large number sorghum lines after direct inoculation at Hyderabad with their reaction to field exposure to inoculum at Dharwar.

MATERIALS AND METHODS

Twenty-five sorghum lines from the 1977 ISDN (including the susceptible checks) were planted in the field in a randomized complete block design, with two replications and 20 plants per plot after thinning. Furadan was applied to the soil before planting and perfospray irrigation was applied daily at 1700 hr for 30 min throughout the experiment. In the field at Dharwar the same varieties were planted in a randomized complete block design, with two replications, and 50 plants per plot after thinning during the rainy season, 1977.

For the inoculation at Hyderabad inoculum was prepared by incubating infected leaves at 20°C and harvesting the mature conidia after 8½ hr. A conidial suspension was made in tap water at 28-32 x 10^4

conidia per ml. Syringe inoculation was done on seedlings 5 days after emergence during night from 2000 hr until 0200 hr. Fresh inoculum was made up after each 30 minutes and the needle of the syringe was injected very deep into the whorl of the seedlings.

At Dharwar, where there was natural infection, the epidemic developed both from overwintering oospores in the soil and from conidia produced on spreader rows which were inoculated by spraying with conidial suspension at the seedling stage.

At Hyderabad, the local lesion incidence was observed 5-7 days after inoculation and the local colonization incidence was observed 10-14 days after inoculation. The systemic infection incidence was observed 14-21 days after inoculation. At Dharwar, only the percent systemic infection was recorded which was two months after emergence.

Statistical analysis

The correlation among the % local lesion, % local colonization and % systemic infection were calculated in all combinations by the formula as follows:

$$r = \frac{Exy}{\sqrt{(E\,x^2)\,(Ey^2)}}$$

r = correlation coefficient

x = dependent variable

y = independent variable

The varieties were divided into three groups based on the percent

systemic infection expressed under artificial inoculation. Those three groups are as follows:

Group I: Resistant 0-17% systemic infection

Group II: Moderate susceptible 18-49% systemic infection

Group III: High susceptible, 50-80% systemic infection.

The correlation coefficient was calculated separately in each group also. The relationships between the rank value of four susceptibility characters was calculated by Spearman's coefficient of rank correlation method as the following formula.

$$rs = 1 - \frac{6 \Sigma d_i^2}{(n-1)\, n\, (n+1)} \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where rs is Spearman's rank correlation coefficient

n is the number of pairs

d_i is the difference for the i th pair

and the significance of the correlations were thus.

$$t = rs \sqrt{\frac{n-2}{1 - rs^2}} \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

RESULTS AND DISCUSSION

The complete data are given in Appendix Tables 1 to 14 and are summarised in Table VI.1.

Comparison of systemic infection at Hyderabad and Dharwar. The degree of systemic infection was higher at Hyderabad than at Dharwar for 21 of the 25 entries. The overall mean at Hyderabad was 43 percent and at Dharwar, 19 percent. At Hyderabad only two entries were in the resistant category (<10 percent) whereas at Dharwar 11 entries were in this

category. We do not consider this an indication of the existence of physiologic races; rather it is a reflection of different inoculation procedures, efficiency and, perhaps, weather. At Dharwar the ISDM entries were exposed to natural inoculum provided by soil-borne oospores and conidia from infector rows. At Hyderabad all plants of all entries were inoculated with a conidial suspension injected deeply into the whorls. Under these latter conditions of inoculation the opportunities for escape are minimized and it is thus to be preferred. However, injection of inoculum into whorls in the early hours of the morning is a tedious and labor intensive activity. The use of a spray inoculation of conidia warrants examination. Also, the efficacy of morning or evening inoculations need further examination.

It is most encouraging that even under the severe Hyderabad inoculation QL-3 remained completely free from infection, and CSV-4 developed only 5 percent (similar to the incidence at Dharwar).

The relationship between local lesion, local colonization and systemic infections. With the exception of QL-3, which had no local lesion development, all entries had high levels of local lesion incidence (47%-100%). However, no consistent relationship was found to exist between the local lesion incidence and incidence of other symptoms.

For 21 entries the incidence of local colonization was greater than the incidence of systemic symptoms indicating that possibly the inoculum had not reached the growing points of some plants which would have developed systemic symptoms. When the rank values are analysed we see (Table VI.3) that there is a high correlation between rank values

for systemic infection and high levels of local colonisation incidence (a range from 47 to 100% and there was no consistent relationship between local lesion incidence and incidence of systemic symptoms.

CONCLUSION

1. In this particular experiment the percent systemic infection from injection was higher than that from natural infection. The variety QL-3 was free from downy mildew both at Hyderabad and Dharwar.

2. There were relationships between local lesion, local colonisation and systemic symptoms after artificial inoculation with conidia by the injection method. The correlation coefficient indicated a high relationship existed between them. This means we can use this relationship as an indication of the percent systemic infection after inoculation. And we can estimate that the actual percent systemic infection should be higher than percent systemic which expressed in the field condition.

3. From a study of the rank correlations, we conclude that percent local colonisation should be used to a greater extent than local lesion for indicating the potential systemic infection. This is because we got a high correlation between the ranks of local colonisation and systemic infection from the injection method of inoculation. In addition, it was easier to observe local colonisation than local lesions.

Table VI.1. Mean value of percent local lesion, local colonisation and systemic infection from artificial inoculation and systemic infection from natural inoculation at Dharwar.

Varieties	% local lesion	% local colonisation	% systemic infection Artificial *	% systemic infection Natural **
CL-3	0	0	0	0
CSV-4	85	34	5	5
UCHV-1	47	35	12	5
IS 5273	73	46	15	7
IS 173	77	52	26	2
IS 2042	92	73	26	2
SC-239-14	75	57	27	19
UCHV-2	77	56	28	9
SC-108-14	85	79	28	5
CSV-5	54	24	29	7
SC-120-14	77	72	37	5
IS 2918	92	69	43	21
IS 3164	81	68	44	10
TAH-428	100	97	46	26
IS 3799	75	64	47	9
TAH-2566	97	92	55	15
SC-175-14	95	84	55	25
SC-120-6-88	78	62	59	31
SC-110-14	80	68	61	10
SC-414-12	87	67	65	17
SC-173-12	97	97	66	21
CSV-2	97	97	67	71
NSA-440-12	100	94	68	45
SC-1706-17	90	62	76	14
DMS-652	92	75	80	100

* At ICRISAT Hyderabad

** At Dharwar

Table VI.2. Correlation coefficient, mean, standard deviation, and significant of correlation coefficient among 25 varieties.

Characters	Local colonization	Systemic inf. (Artifi. Ino.)	Systemic inf. (Natural Ino.)	Mean	S.D.
local lesion	0.87**	0.64**	0.40*	80	21
local colonization	-	0.72**	0.46*	65	24
systemic infection (Artificial inc.)	-	-	0.65**	43	22
systemic infection (Natural ino.)	-	-	-	19	23

* significant at 5% level of probability = 0.40
** significant at 1% level of probability - 0.57

Table VI.3. Analysis data for rank values using Spearmans coefficient of rank correlation.

Character	Local colonization rs	Local colonization t-value	Systemic infection rs	Systemic infection t-value
local lesion	0.8	7.6	0.6	5.9
local colonization	-	-	0.9	0.3
systemic infection	-	-	-	-

t (0.05) = 2.1

Table VI.4. Correlation coefficient, mean, standard deviation and significance of correlation coefficient among 24 varieties (without variety QL-3)

Characters	Local colonization	Systemic infection (Artificial Ino.)	Systemic infection (Natural Ino.)	Mean	S.D.
Local lesion	0.84*	0.57**	0.44*	84	13.3
Local colonization	-	0.65**	0.44*	68	20.4
Systemic infection (Artificial Ino.)	-	-	0.65**	44	21.0
Systemic infection (Natural Ino.)	-	-	-	20	22.9

* significant at 5% level of probability = 0.40

** significant at 1% level of probability = 0.52

Table VI.5. Mean, standard deviation, coefficient of variation and mean squares of percent local lesion, local colonization, systemic infection of 25 sorghum varieties.

Characters	Mean	S.D.	% C.V.	Mean Squares	
				Variety	Error
% Local lesion	80	10	12	900**	99
% Local colonization	65	15	23	1167**	226
% Systemic infection (Artificial Ino.)	43	15	35	1007**	227
% Systemic infection (Natural Ino.)	19	9	47	1041**	84

** significant at 1% level of probability.

GENERAL DISCUSSION

The experiments conducted during this Research Fellowship have had a dual function in that they have contributed to training and to our research knowledge. The major research findings which will contribute to the SDM screening work at ICRISAT are: that by manipulation of incubation environment and timings mature infective conidia can be obtained at any time, that inoculations can be made at 1700 hr with a good level of success, thus eliminating the need for nocturnal inoculations; that the ISDMN set has been successfully inoculated at ICRISAT farm and the resistance of QL3 is confirmed under this severe test. As a result of this study we can plan for successful SDM screening at ICRISAT.

There remain however several unanswered questions which need attention. The inoculation method used in this study was the syringe injection of conidial suspension deep into the whorls, probably right to the vicinity of the growing points. This inoculation method is laborious and slow and will not allow large numbers of plants to be inoculated. We need to examine the possibility of using sprayers to deliver the inoculum to the plants. In this respect the effect of age needs to be further examined and it would have been better in this study to have concentrated on the ages between 1 and 10 days after emergence, rather than on the wide range from 5 to 30, as there was already ample evidence in the literature on the greater susceptibility of younger plants. A necessary study now is the effect of spray and injection inoculation on seedlings 1-5 days after emergence at various times of the day. As spray

inoculation is unlikely to get the inoculum deep into the whorls it is more likely to be successful if done at a time when the inoculum will not dry rapidly. The use of the perfospray irrigation system should help get over the latter difficulty.

Another major area which needs study is the relationship between the reaction to oospores, to conidia under natural inoculum provision (infector rows) and to conidia in artificial inoculations. There is some evidence in the literature that injection inoculation gives a higher level of infection in some cultivars than is obtained in the field under natural inoculum provision. Our inoculation method should not be so harsh that we eliminate sources of resistance which would be adequate under natural inoculum pressure in the field.

These and other questions will need to be answered before we will be fully competent to screen in the most meaningful way for SDM resistance.

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Appendix Table 1. Percent local lesions at 7 days after inoculation.

Variety	Replication I	Replication II	Average
DMS-652	90	94	92
PI-3	0	0	0
C.S.V.-2	95	100	97
C.S.V.-4	75	94	85
C.S.V.-5	55	53	54
S.C. 175-14	89	100	95
S.C. 120-6-88	79	78	78
S.C. 170-6-77	87	93	90
S.C. 414-12	75	100	87
S.C. 108-14	80	89	85
S.C. 110-14	95	67	81
S.C. 239-14	72	79	76
S.C. 173-12	95	100	97
S.C. 120-14	70	85	77
I.S. 2918	100	84	92
I.S. 3164	71	90	81
I.S. 173	65	89	77
I.S. 2042	90	94	92
I.S. 5273	67	89	78
I.S. 3799	59	91	75
UChv-1	30	65	47
UChv-2	75	79	77
TAM-2566	95	100	97
TAM-428	100	100	100
NSA440-12	100	100	100

Appendix Table 2. Analysis of variance of percent local lesions at 7 days after inoculation.

Source of variation	df	SS	MS	F
Uncorrected total	50	348559		
Correction for mean	1	327731		
Corrected total	49	20828		
Varieties	24	21600	900	9.06**
Replication	1	833	833	8.30**
Experimental error	24	2396	99	

Mean = 80.5

S.D. = 10.0

C.V. = 12.4%

L.S.D. (0.05) = 20.6

Appendix Table 5. Percent local colonization at 14 days after inoculation

Variety	Replication I	Replication II	Average
DKS-652	50	100	75
SL-3	0	0	0
C.S.V.-2	95	100	97
C.S.V.-4	35	33	34
C.S.V.-5	25	24	24
S.C. 175-14	73	95	84
S.C. 120-6-83	58	67	62
S.C. 170-6-17	60	64	62
S.C. 414-12	50	85	67
S.C. 108-14	80	79	79
S.C. 110-14	90	47	68
S.C. 239-14	61	53	57
S.C. 173-12	95	100	97
S.C. 120-14	65	80	72
I.S. 2918	75	63	69
I.S. 3164	62	75	68
I.S. 173	40	63	52
I.S. 2042	57	89	73
I.S. 5273	43	50	46
I.S. 3799	47	82	64
UChv-1	20	50	35
UChv-2	75	36	56
TAM-2566	85	100	92
TAM-428	100	94	97
RSA-440-12	94	94	94

Appendix Table .. Analysis of variance of percent lesion colonization at 14 days after inoculation.

Source of variation	df	SS	MS	F
Uncorrected total	50	[illegible]		
Correction for mean	1	[illegible]		
Corrected total	49	[illegible]		
Varieties	24	[illegible]	1167	5.17**
Replication	1	707	707	3.13NS
Experimental error	24	5414	226	

Mean = 65.2

S.D. = 15.0

C.V. = 23.0%

L.S.D. (0.05) = 31.0

Appendix Table 5. Percent systemic infection at 21 days after inoculation.

Variety	Replication I	Replication II	Average
DES-652	65	95	80
QL-3	0	0	0
C.S.V.-2	68	67	68
C.S.V.-4	10	0	5
C.S.V.-5	30	28	29
S.C. 175-14	26	84	55
S.C. 120-6-66	79	39	59
S.C. 170-6-17	80	71	76
S.C. 414-12	70	60	65
S.C. 108-14	30	26	28
S.C. 110-14	75	47	61
S.C. 239-14	22	32	27
S.C. 173-12	80	53	66
S.C. 120-14	30	45	37
I.S. 2913	55	32	43
I.S. 3164	43	45	44
I.S. 173	20	32	26
I.S. 2042	24	28	26
I.S. 5273	14	17	15
I.S. 3799	35	59	47
UChv-1	0	25	12
UChv-2	30	26	28
TAM-2566	60	50	55
TAM-428	47	44	46
NSA 440-12	53	83	68

Appendix Table 6. Analysis of variance of percent systemic infection at 21 days after artificial inoculation at Hyderabad.

Source of variation	df	SS	MS	F
Uncorrected total	50	1 [illegible]0850		
Correction for mean	1	[illegible]11[illegible]8		
Corrected total	49	29662		
Varieties	24	[illegible]4173	1007	4.43**
Replication	1	32	32	0.14NS
Experimental error	24	5457[illegible]	227	

Mean = 42.7

S.D. = 15.1

C.V. = 35.3%

L.S.D. (0.05) = 31.1

Appendix Table 7. Percent systemic infection at 60 days in the field at Dharwar

Variety	Replication I	Replication II	Average
[illegible]-652	100	100	100
QL-3	0	0	0
C.S.V.-2	52	89	71
C.S.V.-4	5	5	5
C.S.V.-5	4	11	7
S.C. 175-14	23	27	25
S.C. 120-6-88	23	39	31
S.C. 170-6-17	14	14	14
S.C. 414-12	17	18	17
S.C. 108-14	5	8	5
S.C. 110-14	9	12	10
S.C. 239-14	17	21	19
S.C. 173-12	34	7	21
S.C. 120-14	0	10	5
I.S. 2918	34	9	21
I.S. 3164	10	10	10
I.S. 173	0	5	2
I.S. 2042	0	5	2
I.S. 5273	10	4	7
I.S. 3799	10	8	9
UChv-1	0	10	5
UChv-2	11	7	9
TAN-2566	20	9	15
TAN-428	23	29	26
NSA-440-12	33	56	45

Appendix Table 8. Analysis of variance of percent systemic infection at 50 days at Dharwar

Source of variation	df	SS	MS	F
Uncorrected total	50	45667		
Correction for mean	1	17602		
Corrected total	49	27065		
Varieties	24	24985	1041	12.4*
Replication	1	72	72	0.87 NS
Experimental error	24	2008	84	

Mean = 19.3

S.D. = 9.1

C.V. = 47.4

L.S.D. (0.05) = 18.9

Appendix Table 9. The relationship between rank value for four susceptibility characters.

Varieties	Rank on % local lesion	Rank on % local colonization	Rank on % systemic infection	
			Artificial	Natural
QL-3	1	1	1	1
C.S.V.-4	14	3	2	6
UChv-1	2	4	3	5
I.S. 5273	9	5	4	8
I.S. 173	7	6	5	2
I.S. 2042	18	17	6	3
S.C. 239-14	5	8	7	17
UChv-2	6	7	8	11
S.C. 108-14	13	19	9	7
C.S.V.-5	3	2	10	9
S.C. 120-14	8	16	11	4
I.S. 2918	17	15	12	19
I.S. 3164	12	14	13	12
TAN-428	24	23	14	21
I.S. 3799	4	11	15	10
TAN-2566	22	21	16	15
S.C. 175-14	20	20	17	20
S.C. 120-6-88	10	10	18	22
S.C. 110-14	11	13	19	13
S.C. 414-12	15	12	20	16
S.C. 173-12	22	25	21	18
C.S.V.-2	21	24	22	24
NSA-440-12	24	22	23	23
S.C. 170-6-17	16	9	24	14
DMS-652	19	18	25	25

Appendix Table 10. Correlation matrix and t value of the relationship between rank value for four susceptible characteristics.

Characters	Local colonization		Systemic infection (Artificial Ino.)		Systemic infection (Natural Ino.)	
	rs	t-value	rs	t-value	rs	t-value
Local lesion	0.85**	7.64**	0.68**	3.95	0.34NS	1.7
Local colonization	-	-	0.94**	0.32	0.39NS	2.0
Systemic infection (Artificial Ino.)	-	-	-	-	0.79**	6.3
Systemic infection (Natural Ino.)	-	-	-	-	-	-

t (0.05) = 2.06
(0.01) = 2.81

Appendix Table 11. Mean, value of percent local lesion, local colonization and systemic infection of resistance varieties group (0-17% systemic infection).

Variety	% Local lesion	% Local colonization	% Systemic infection Artifical Ino.	% Systemic infection Natural Ino.
QL-3	0	0	0	0
C.S.V.-4	85	34	5	5
I.S. 5273	78	46	15	7
UCRiv-1	47	35	12	5

Appendix Table 12. Mean value of percent local lesion, local colonization, and systemic infection of moderate resistance variety group (18-49% systemic infection).

Varieties	% local lesion	% local colonization	% systemic infection	
			Artificial Ino.	Natural Ino.
C.S.V.-5	54	2[illegible]	29	7
S.C. 108-14	85	79	28	5
S.C. 239-14	76	57	27	19
S.C. 120-14	77	72	37	5
I.S. 2918	92	69	[illegible]3	21
I.S. 3164	81	68	44	10
I.S. 173	77	52	26	2
I.S. 2042	92	73	26	2
UChv-2	77	56	28	9
I.S. 3799	75	6[illegible]	47	9
TAM-428	100	97	46	26

Appendix Table 13. Mean value of percent local lesion, local colonization, and systemic infection of susceptible variety group (50-80% systemic infection).

Varieties	% local lesion	% local colonization	% systemic infection	
			Artificial Ino.	Natural Ino.
C.S.V.-2	97	97	68	71
S.C.-175-14	95	84	55	25
S.C. 120-6-88	78	67	59	31
S.C. 414-12	87	67	65	17
S.C. 110-14	81	68	61	10
S.C. 173-12	97	97	66	21
TAM 2566	97	92	55	15
NSA-440-12	100	94	68	45
DMS-652	92	75	80	100
S.C. 170-6-17	90	62	76	14

Appendix Table 14. Correlation coefficient, mean, standard deviation and significant of correlation coefficient of three groups of variety.

Characters	Group	Local lesion	Local colonization	Systemic infection (Artificial Ino.)	Mean	S.D.
Local lesion	I	-	-	-	52	39
	II	-	-	-	81	12
	III	-	-	-	92	7
Local colonization	I	0.90	-	-	29	20
	II	0.90	-	-	65	18
	III	0.87	-	-	80	15
Systemic infection (Artificial Ino.)	I	0.60	0.8[illegible]	-	8	7
	II	0.32	0.45	-	35	9
	III	0.14	0.16	-	65	8
Systemic infection (Natural Ino.)	I	0.92	1.00	0.86	4	3
	II	0.42	0.37	0.52	11	8
	III	0.28	0.22	0.60	35	29