CONDITIONS OF THE SPORES OF THE SCABBED WHEAT EAR SUSPENDED IN RAIN DROPS

By

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Studies on the secondary dissemination of wheat scab disease, caused by the fungus, *Gibberella zeae* (Schw.) Petch, have been undertaken by the present writers for many years. The results reported previously suggested that the rain played an important role as a source of secondary dissemination of the disease. The present paper reports the results of experiments dealt with the rain drops, suspended with fungus spores, on the scabbed wheat ear.

I. Liberation of the conidiospores into the water

(1) The liberation-process of the conidiospores formed on the portions of wheat ear showing sign of scab into the rain drops

It has already been shown that the conidiospores formed on the scabbed wheat ear are suspended in water rapidly (1, 3). But, the effects of the quantity and the duration of rainfall upon the spore density in the suspension and its alteration by the lapse of time, have not yet been investigated. Therefore, experiments on the proccss of conidiospore-liberation from the spikelets into rain drops were done as described below.

Scabby ears of the wheat variety Norin No. 4 were collected from the experimental farm of the Institute. On each ear, only one or two spikelets showing a distinct salmon colored scab were kept, and the others were removed. Four or five of these prepared ears were bound so as to contain five spikelets. They were then atomized with water. Water drops on the spikelets, that have collected conidiospores, were allowed to drip and collect at intervals of designated time. Conidiospore bearing drops were collected, (contained in 30 seconds 9 to 11 drops, or 2 to 3 ml) and filled to 100 ml with water and stirred. Five droplets, each 0.01 ml, of the spore suspension were placed on a thin agar film settled on a slide glass with a pipette. The number of conidiospores in each droplet were counted under a low power microscope. The quantity of water atomized was in rainfall equivalent 20—25 mm/hr.

The results are givin in Table 1. Figure 1 shows the curve drawn by

Table 1. *Relations between the duration of water spraying and the spore liberation.*

Duration of water spraying second	0—30	30—60	90—120	180—210	300—330	600—630
A	14858	5030	1722	838	643	574
B	2971.6	1006.0	344.4	167.6	128.6	114.8
C	594.32	201.20	68.88	33.52	25.72	22.96

Water spraying to the ears were 20—25 mm/hr, 0.17—0.21 mm/0.5min., converted into rainfall.

Remarks A : Total number of liberated spores of 5 replications.

B : Average number of spores in 0.05 ml of suspension.

C : Number of liberated spores into 100 ml water per a spikelet, $\times 10^4$.

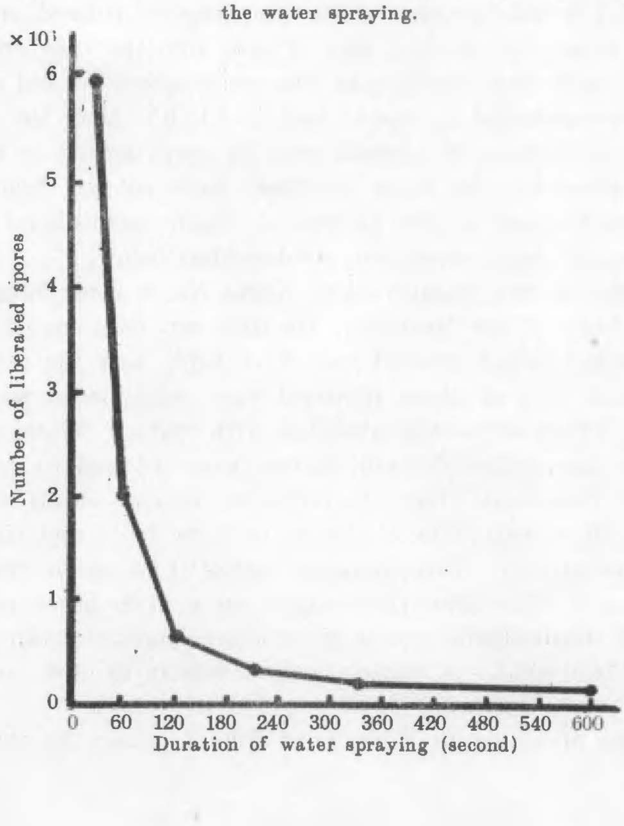
For the values in B,

LSD (0.05)=184.48, LSD (0.01)=229.71.

Analysis of variance of Table 1.

Item	Sum of squares	Degree of freedom	Variance	Variance ratio	Probability
Total	36175240.167	29			
Treatment	31426562.567	5	6285312.5134	32.2464	0.001>
Replication	850402.000	4	212596.25	1.0907	0.20 <
Error	3898275.600	20	194913.78		

Fig. 1. Alteration of the number of liberated spores during the water spraying.



the average number from 5 replications. The number of conidiospores suspended into 100 ml of water was converted into the value per scabbed spikelet. In all the experiments most of the conidiospores formed on the scabbed spikelets were liberated into the atomized water drops in the first 30 seconds. The number of conidiospores liberated during an unit period of time decreased rapidly within the first two minutes. And the degree of decrease in the spore number, liberated every 30 seconds, became smaller after 3—5 minutes. In the first minute of atomizing 20—25 mm/hr, 7.96×10^6 conidiospores were liberated from a spiklet into the water drops and dripped downward. Thereafter, however, the liberated spores decreased much in number.

(2) Liberation of conidiospores from the scabbed portion by the water

Following experiments were conducted to know in detail change in the condition of conidiospore liberation as affected time. A sheaf of 5 scabbed spikelets was soaked every 5 seconds one after another, and the number of conidiospores released into the water was counted under a microscope. In 15—30, 35—60, 65—120 and 125—300 seconds periods, however, the sheaf was moved after each given duration of water dipping. The results obtained are shown in Table 2 and Figure 2. Figure 2 shows the average number of five trials; the condition of the alteration of the spore liberation being

Table 2. *Relations between the duration of water dipping of scabbed spikelets and the number of spore liberation.*

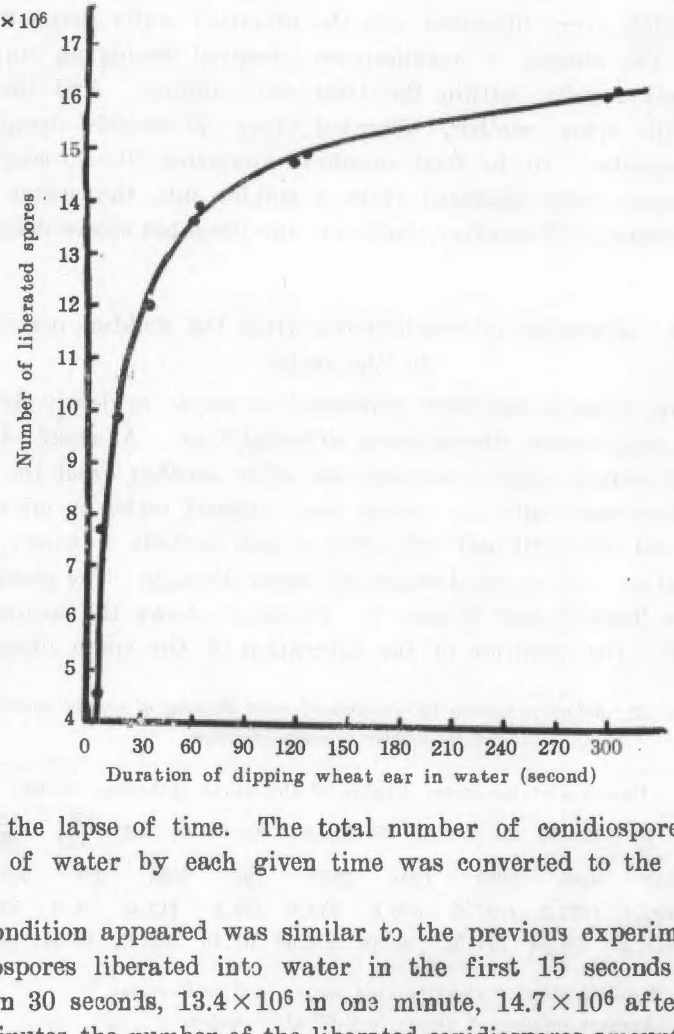
	Duration of the water dipping of the wheat spikelets. (second)										
	0—5	5—10	10—15	15—30	30—35	35—60	60—65	65—120	120—125	125—300	300—305
A	11234	8032	5166	5298	1504	2570	799	2068	271	2639	272
B	2246.8	1106.4	1033.2	1057.6	300.8	514.0	155.8	413.6	54.2	527.8	54.4
C	449.36	321.28	206.64	211.52	60.16	102.80	31.16	82.72	10.84	105.56	10.88

Remarks A: Total number of the liberated spores in 5 replications.
B: Average number of spores in 0.05 ml of water.
C: Number of spores liberated into 100 ml of water per a spikelet, $\times 10^4$.
For the values in B,
LSD (0.05)=242.93, LSD (0.01)=324.71.

Analysis of variance of Table 2.

Item	Sum of squares	Degree of freedom	Variance	Variance ratio	Probability
Total	26134365.96	54			
Treatment	24418346.33	10	2441834.633	67.5285	0.001>
Replication	269618.48	4	67404.62	1.8640	0.20 <
Error	1446401.15	40	36160.0288		

Fig. 2. Number of liberated spores and the durations of the dipping wheat ear in water.



figured in the lapse of time. The total number of conidiospores suspended into 50 ml of water by each given time was converted to the value of per spikelet.

The condition appeared was similar to the previous experiments; 9.8×10^6 conidiospores liberated into water in the first 15 seconds of dipping, 11.8×10^6 in 30 seconds, 13.4×10^6 in one minute, 14.7×10^6 after 2 minutes. After 5 minutes the number of the liberated conidiospores amounted to 15.7×10^6. The results show that the heavily scabbed spikelet of naturally infected wheat, variety Norin No. 4, carried at least 16×10^6 matured releasable conidiospores, and most of these spores seemed to have been liberated within a short time: about 84.4% of the conidiospores formed were liberated within the first minute of dipping.

II. The conidiospores move downward along the surface of wheat ear

The rain drops, as they suspend conidiospores, move downward along the surface of the scabbed ear. Following experiments were undertaken to know the amount of spores liberated into rain drops and that remains on the surface of the ear. Materials and experimental methods were similar in all the items.

(1) The amount of suspended conidiospore remaining on wheat ears

Healthy ears of the wheat, variety Norin No. 4, were collected from the experimental farm of the Institute. On both sides of the uppermost spikelet of the ear 2 ml of the water suspension of the conidiospores, produced on diseased spikelets, were dripped by an injector slowly. Spore suspension moved downward along the surface of the ear and fell down from the cut end of the base. It was collected and measured by absorbing into another injector. Table 3A is the results of experiments in which spore suspension, 5.5×10^4/ml in spore density, was applied to the ears with 19 and 5 spikelets respectively. Results of another experiments are shown in Table 3B, in which spore suspensions of 4.01×10^4/ml, 2.92×10^4/ml and

Table 3. *Alteration of the amount of spore suspension during its falling down from the top of the ear to the base.*

A*			B**		
Number of spikelets	ml[2)]	%[3)]	spore density ($\times10^4$/ml)	ml[2)]	%[3)]
19	1.59	79.3	4.01	1.62	81.0
5	1.77	88.7	2.92	1.68	84.0
			0.73	1.62	81.1

Remarks 1) 2 ml of spore suspension was applied.
2) Amount of spore suspension fell from the cut end of the ear.
3) Percentage of 2) to 2 ml.
* Spore density was about 5.5×10^4/ml.
** 16 spikelets wheat ear.
A and B are the average of each 5 replications. Between these averages no significant differences were presented.

0.73×10^4/ml were treated on the ear with 16 spikelets. There were no significant difference between the average in both the experiments A and B. No significant differences were noted in the number of spikelets of ears, in the spore density or in the volume of collected spore suspension. Therefore, about 20% of 2 ml conidiospore suspension remained on the surface of the ear by the treatment.

(2) Alteration in the spore density of conidiospore suspension by its movement downward along the surface of ears

Several experiments were undertaken to study the difference between the conidiospore suspensions applied on the uppermost part of an ear and those dripped down from the cut of the base. One ml of the spore suspension dripped from the cut end of the ear was diluted to 10 ml with tap water, and the spore number in 0.05 ml of the dilution was counted by the same way as the previous. Table 4A is the results of the experiments dealt with

Table 4A. *Relations between the number of spikelets and the alteration of spore density during its falling down from the top of the ear to the base.*

Experiment	I		II	
	a	b	a	b
	2764[1)]	552.8[1)]	2761[1)]	552.2[1)]
	1706	341.2	1538	317.6
	1725	337.8	1876	375.2
	1557	311.4	1814	362.8
	1621	324.2	1670	334.0

Remaks I. Result of experiment with the wheat ears of 19 spikelets.
II. Result of experiment with the wheat ears of 5 spikelets.
1). Value on the spore suspension dropped on the top of the ear.
a) Total number of the spores in 0.01 ml of the suspension fell down from the cut end of the ear.
b) Spore density of the suspension fell from the cut end of the ear.
For the values in b),
I: LSD (0.05)=99.24 II: LSD (0.05)=122.57
LSD (0.01)=152.63 LSD (0.01)=202.81
Significant differences were presented between the experiments I and II in the degree of decrease of spore density.

Table 4B. *Alteration of the spore densities of various suspentions during its falling down from the top of the ear of 16 spikelets to the base.*

Experiment	I		II		III	
	a	b	a	b	a	b
	2004[1)]	400.8[1)]	1460[1)]	292.0[1)]	364[1)]	72.8[1)]
	940	188.0	887	177.4	263	52.4
	1144	228.0	632	126.4	296	59.2
	1043	208.6	743	148.6	297	59.4
	1272	254.4	672	134.4	257	51.4
	791	158.2	797	159.4	234	46.8

Remarks Values surveyed 5 replications.
a) Total number of the spores in 0.01 ml of the suspension fell down from the cut end of the ear.
b) Spore density of the suspension fell from the cut end of the ear.
1) Value on the spore suspension fell from the cut end of the ear.
For the value of b),
I: LSD (0.05)=84.21 II: LSD (0.05)=60.02 III: LSD (0.05)=18.91
LSD (0.01)=139.33 LSD (0.01)=99.31 LSD (0.01)=29.59

the ears having 19 and 5 spikelets respectively. Significant difference were noted in the spore density before and after the treatment in both trials;

the spore density decreased by the downward movement of conidiospore suspension, and the decreased part of the spores in the suspension remained on the surface of the ears. In Table 5A, the proportion of the spore density after the treatment to the before is given in percentage, to show the degree of the decrease of the spore density as to the difference of the spikelet number of the ears. Between 59.77% and 62.46%, the average values in the cases of 19 and 5 spikelets respectively, there was no significant difference statistically. While moving down 2 ml of spore suspension of 5.5×10^4/ml in spore density, decreased to the 65% of the original density. The degree of decrease of it differed but little between the ears of 19 and 5 spikelets. According to the estimation from these valued, the number of spores remained on the ear are about 5.82×10^4 and 4.74×10^4 per ear, and is evaluated to 53% and 43% of all the spores in 2 ml of spore suspension respectively.

Another experiment was conducted to know the alteration of spore density when the suspensions of different spore density moved down along the surface of the ear. Number of spikelets of the ear was 16. Spore suspensions contained 4.01×10^4, 2.92×10^4 and 0.73×10^4 spores per ml were dripped with an injector to the top of the ear, and the number of spores in 0.05 ml of suspension that fell from the cut end of the ear was counted. The results shown in Table 4B were obtained. Spore densities decreased remakably after the suspensions streamed over the surface of the ear.

Table 5. *Alteration of the spore density during the suspension falling down from the top of the ear to the base.*

A*			B**			
Number of spikelets	%2)	θ3)		spore density ($\times10^4$/ml)	%2)	θ3)
19	59.77	50.7	I	4.01	51.80	46.0
5	62.46	52.3	II	2.92	51.11	45.6
			III	0.73	74.01	59.5

Remarks 1) 2 ml of spore suspension was applied.

2) Percentage of dripped suspension from the cut end of the ear to the applied suspension in their spore densities.

3) Transformation of 2) to $P=\text{Sin}^2\theta$ of Bliss.

* Spore density was about 5.5×10^4/ml.

** 16 spikelets wheat ear.

A and B are the average of each 5 replications. Significant difference is not presented between the averages in A.

For the value in B,

LSD (0.05)=5.96, LSD (0.01)=9.26.

Table 5B shows the decrease in the density after treatment, expressed as percentage of the original density. In the trials I and II, there was no significant difference between 51.80% and 51.11% respectively, but the percentage in the trial III is 74.01%, and the degree of decrease of spore density was less than the former two trials. According to these results, the less the density of the conidiospore suspension, the less the proportion of the number of spores remained on the surface on the ear. Estimations of the number of spores remained on the ear, from these values, were 4.65×10^4, 3.30×10^4 and 0.58×10^4; and their percentages evaluated are about 58.0%, 56.4% and 40.0% in the trials I, II and III respectively.

III. Discussion

Ishii and Koyama (1952) and the writers (1954) recognized the fact that the conidiospores produced on the scabbed wheat ear are not liberated so easily in dried condition. But, they become so when the diseased ear is soaked into the water. According to the writers' previous suggestion, the rain drops following down along the surface of the scabbed spikelets will play an important role in the secondary dissemination and infection of wheat scab. The result of the present paper, seems to clear the condition of the liberation of the conidiospores, produced on the wheat spikelet showing signs of the scab, into the rain drops that fall on the surface of the ear. A great many of the conidiospores abundantly formed on the scabbed spikelet are suspended into the water soon after in contact with the rain drops; and this condition continues as long as spores are produced. Figure 1 seems to show that the relations between the liberated spore numbers in every unit period and the duration of water spraying (duration of rainfall—rain drops streaming on the surface of the signed portion) are the function related with hyperbola. Similar relations are recognized in Figure 2. When diseased spikelets are soaked into the water, the matured spores that liberate into the water and play a part of secondary infection are formed as many as 16×10^6 per spikelet at least.

Numbers of these spores remained in high percentage in the water film on the surface of the ear, as shown in Tables 3, 4 and 5. The possibility of the above mentioned secondary dissemination and infection may be much greater than the dissemination by the wind in moist and rainy condition as previously investigated by the writers. As it has not yet been studied on the rain drops that will fall on the ear, it will be hard to draw a conclusion on the natural rainy condition. Moreover, experiment of the present paper, may be considered to differ from the natural conditions by the following reason. The spore suspension was dropped only on both sides of the uppermost spikelet and streamed downward, and it remained mostly in the inside surface and the spaces formed by the spikelets, rachides and rachillas. The spores thus remained were much more than those on the outer sides of the spikelets. But the density of the spore suspension decreased while it moved downward on the surface of the ears. In the experiment of Table 4A that used 5.5×10^4/ml of spores in suspension, 53% and 43% of the suspended spores remained on the ears of 19 and 5 spikelets respectively. And in those of Table 4B, when 2 ml of spore suspensions having densities of 4.0×10^4/ml, 2.9×10^4/ml and 0.7×10^4/ml were used, 58%, 56% and 40% of the spores remained on the ear surface respectively.

From the facts presented in this paper, it may be considered that these numerous conidiospores thus remain on the ear surface will play a large part in the secondary dissemination and infection unless they are washed away by the following rain. The rain drops that remain in the spaces in the spikelets and the rachides, moreover, do not evaporate so easily, and there are some possibilities of infection by the scab fungus.

It will be considered from the results of the experiments that infection of wheat scab will generally appear on the spikelets situate below the spikelets that show a signs of scab. In field observations the occurrence of the secondary infection distinct from the primary infection which accompanies ascospores, is not always observed, except in the case of epidemic prevalence. In the field near the Institute in 1954, the occurrence of the scabbed spikelets was distributed rather at random on the diseased ear and in the field; and it was hard to say in generally that the infection occurs on the spikelets situate below the scabbed one. The apparent occurrence of the sign of scab on the diseased wheat spikelets is observed chiefly at the time of nearing the milky ripening stage. There is an incubation period after for the infection to develop as the writers have shown previously, and its development is affected by climatic conditions (3). The conditions of wheat plant in the heading and flowering periods, as a host, are most affected by the fungus, and become more difficult for infection after these periods.

From these facts, it will be considered that the susceptibility of wheat to the scab may decrease but the probabilities of secondary infection may increase in the beginning of the rainy "tsuyu" season in Japan. And the visible symptoms may not develop so markedly. When the secondary dissemination sources are established in the early season, however, the possibilities of the occurrence of the secondary infection will be extremely increased.

The above mentioned discussions about the experimental results are on the condition of the season which the occurrence of the wheat scab is comparatively less. As to the epidemic prevalence, studies will be done from another aspect examining climatic and other factors.

IV. Summary

The present paper is a part of the studies on the secondary dissemination and infection of the wheat scab disease, caused by *Gibberella zeae* (Schw.) Petch, and deals with the experiments as to the drops of water

suspension of the fungus spores on wheat ears.

1) The relations between the duration of water spraying by an atomizer on the diseased spikelets and the alteration of spore liberation from the scab sign were studied. To know details on the alteration of the spore liberation in the process of time number of liberated spores into the water was investigated; scab-signed spikelets were dipped into the water in various length of time. A great deal of conidiospores were suspended into the water, in all the trials, just after the contact with the water. 11.3×10^6 conidiospores were suspended in the first 30 seconds of dipping, 13.0×10^6 in 1 minute, 14.5×10^6 in 2 minutes. After 5 minutes the number of the conidiospores liberated and suspended into the 100 ml of water, amounted to 15.7×10^6 per spikelet. It was found that at least 16×10^6 matured and liberatable conidiospores were formed on the scab diseased wheat spikelet used in the experiments.

2) Examining the alteration of the spore density and of the volume of the spore suspension during it's falling down along the surface of the ear, the amount of the remained spores on the spikelet was investigated. In all the experiments that used various spore densities and number of spikelets, 0.3—0.5 ml from 2 ml of spore suspensions used remained on the surface of the ear. Spore densities changed while the suspension moved downward along the surface of the ear. 5.82×10^4 and 4.74×10^4 spores remained on the ears when 2 ml of spore suspension of having a density of 5.5×10^4/ml was used on 19 and 5 spikelets respectively. The more the proportion of the amount of spores remained on the ear, the larger the spore density applied.

3) Discussions on the results of experiments mentioned above were made with respect to the secondary dissemination and infection of the wheat scab disease.

Literature

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