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Studies on the Methods for Testing the Effectiveness of Fungicides. I. The Use of Slide Glasses as a Mediun for Testing Spray Materials.*

By

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I. Introduction

An accurate evaluation of fungicides under controlled laboratory conditions has been the desire of many students of fungicide, especially by the ever changing and development of new products. If some forms of standard evaluating procedures are set forth and used in all routine evaluation of fungicides, much of the prevailing uncertainties as to the potentialities of many fungicides would be simplified. The present report is the first of a series attempting to present laboratory methods for such evaluation using minimum of facilities. The experiments discussed utilize slide glasses upon which the test fungicides are sprayed and inoculated with spores of test pathogens. The evaluating indices herein employed are the degrees of germination of spores and the extent of germ tube growth. Literatures dealing in some aspects of the present subject are listed at the end of this report.

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II. Materials and Methods

Fungi used in determining the degrees of preventive action of the fungicides against spore germination are listed below. They were of the pure cultures held in stock at the Institute.

Table 1. Fungi used for Testing the Effectiveness of Fungicides.

Disease and fungus	Stock number	Source	Location	Year isolated
Sesame-leaf-spot of rice, *Helminthosporium Oryzae*	1089	Blade	Kurashiki	1940
	1090	Seedling	Kurashiki	1941
Blast of rice, *Piricularia Oryzae*	1095	Seedling	Kurashiki	1941
	1091	Seedling	Kurashiki	1941
Scab of wheat, *Cibberella Saubinetii*	1256a	Panicle	Hokkaido	1936

Test fungicides include some of the commercial products sold on the market, their principal ingredients being copper, sulphur and zinc. For comparative purpose, in addition to the above, Bordeaux mixture of 1:1 combination and lime suphlur at Baume 33° were included. A list of commercial products is shown below.

Table 2. Commercial Products of Fungicides used for Testing the Effectiveness.

Product	Actve ingredients		Manufacturer
San Bordeaux		Cu 14.2%*	Nippon Nōyaku
Ōdo	$3Cu(OH)_2 \cdot CuCl$	Cu 39.8%	Nissan Kagaku Kōgyō
Kassei Bordeaux	$Al_2(SO_4)_3 \cdot CuSO_4$	Cu 9.2%	Yokohama Uyeki
Kupoid	$Cu(OH)_2, CuSiO_3$	Cu 17.2%	Sankyō Nōyaku
Colloidinoid			Sankyō Nōyaku
Soid			Sankyō Nōyaku

* The results of actual analyses.

1) Preparation of Slides.

Clean slides were sprayed with the test solutions of fungicides, using a pistol atomizer connected to a motor driven air compressor. With the type of nozzle used, a pressure of 5 pounds per square inch was enough to give a satisfactory mist. Slides were arranged on a base and sprayed for 5 seconds on the slides from a distance of 3 feet. All sprayings and subsequent drying of the slides were done in a glass enclosed compartment 6 feet by 6 feet in area.

2) Preparation of Spore Suspension.

In making up spore suspensions, each fungus was cultured in malt extract test tube slants for 14 days at 24°C. Conidia were produced in abundance at the close of this period, and were removed by suspending in sterile distilled water. Mycelial and agar fragments were separated from the spores by passing through a fine cheese cloth. The suspension was further diluted with sufficient water to give a desired concentrations of spores when observed under a microscope magnified to x 200. The approximate number of spores in a field for *Helminthosoporium Oryzae* was 30, and for *Piricularia Oryzae* and *Gibberella Saubinetii* of between 50 and 80.

The final suspension was immediately transferred to the prepared test slides, using a pipette which gives 0.05 ml to a drop. Two slides were used for each fungicide treatment with three drops to a slide. Slides were then set in large Petri dishes provided with U-shaped glass rod supports to suspend the slides away from the water saturated filter paper spread over the bottom for providing moisture in the chamber.

3) Recording of Results.

The degrees of germination was recorded in terms of number of spores germinated, as well as the extent of germ tube growth. From two different fields in each drop, the number of germinated spores were counted and was expressed in number 0 to 5, indicating germination of: 0, none; 1, 1-10 per cent; 2, 10-20 per cent; 3, 30-50 per cent; 4, 60-80 per cent; and 5, 80 per cent and over. Germ tube growth was expressed respectively by: 1, equal to or less than the length of the spore; 2, less than three times the length of the spore; and 3, anything longer than 2.

III. Experimental results

1) Inhibition of Spore Germination by Fungicides

Effect of spray residue on the spore germination was tested using ten fungicides at various concentrations. This gives indications of the fungicide and its concentration when determining the purpose and the effects of such application on the living plant under field conditions. Table 3 shows results with *Helminthosporium Oryzae*, *Piricularia Oryzae* and *Gibberella Saubinetii*. The figures are the averages of four replications.

Table 3. Inhibition of Spore Germination in *Helminthosporium Oryzae*, *Piricularia Oryzae* and *Gibberelle Saubinetii* from Ten Fungicides at Various Concentrations. Average of Four Replications.

Fungicide	Concentration in per cent	*Helminthosporium Oryzae*		*Piricularia Oryzae*		*Gibberella Saubinetii*	
		Germination	Germ tube	Germination	Germ tube	Germination	Germ tube
Bordeaux mixture	0.4	0*	0*	0	0	0	0
	0.1	1	1	3	1	2	1
	0.025	3	2	5	2	4	2
San Bordeaux	0.8	0	0	2	1	0	0
	0.4	0	0	3	1	0	0
	0.1	3	2	4	2	0	0
	0.025	5	3	5	3	5	3
Ōdo	0.8	2	1	5	2	0	0
	0.4	2	1	5	2	0	0
	0.1	4	2	5	3	1	1
	0.025	5	2	5	3	3	2
Kassei Bordeaux	0.8	0	0	2	1	0	0
	0.4	0	0	2	1	2	1
	0.1	2	2	4	2	4	2
	0.025	5	2	5	3	5	3

Fungicide	Concentration in per cent	*Helminthosporium Oryzae*		*Piricularia Oryzae*		*Gibberella Saubinetii*	
		Germination	Germ tube	Germinatube	Germ tube	Germination	Germ tube
Kupoid	0.8	1	1	4	1	0	0
	0.4	2	1	5	2	0	0
	0.1	3	2	5	3	2	1
	0.025	5	2	—	—	4	2
Kolloidinoid	1.6	—	—	3	2	—	—
	0.8	5	3	5	3	5	3
	0.2	5	3	5	3	5	3
Soid	1.6	—	—	4	1	—	—
	0.8	5	3	5	1	5	3
	0.2	5	3	5	1	5	3
	0.025	—	—	5	2	—	—
Larvacide	1.6	—	—	4	1	—	—
	0.8	5	3	5	2	5	3
	0.2	5	3	5	2	5	3
Lime sulphur	1.6	5	3	4	2	5	3
	0.8	5	3	5	2	5	3
	0.2	5	3	5	3	5	3
Blast	0.8	3	3	5	2	2	1
	0.3	4	3	5	3	5	3
	0.1	5	3	5	3	5	3
Control	Water	5	3	5	3	5	3

* As to the number consult (3) Recording of Results in p.2.

Table 3 shows spores of *Helminthosporium Oryzae* exhibited considerable resistance to the presence of fungicides. At the concentration used, there were no fungicide that inhibited the germination completely. There were also notable differences among fungicides in the concentration at which they were able to inhibit germination. At the concentration of 0.4 per cent, only Bordeaux mixtnre, San Bordeaux and Kassei Bordeaux were successful. Ōdo and Kupoid were only effective at the higher concentrations. The growth of the germ tube was greatly retarded at 0.1 per cent on all of the copper containing fungicides. Fungicides containing other than copper, such as the lime sulphur, Soid, Larvacide, and Blast showed negligible or no effect on the germination nor the subsequent growth of the germ tube.

Piricularia Oryzae appeared to be highly resistant to fungicides. Of the test fungicides used, only Bordeaux mixture at the concentration of 0.4 per cent was effective in inhibiting spore germination. In general, however, the elongation of the germ tube was somewhat suppressed even after germination, espeically in the copper containing compounds and to a slight extent in non-copper fungicides.

On *Gibberella Saubinetii*, copper containing fungicides inhibited spores from germinating at a comparatively lower concentrations than on the other two fungi. Bordeaux mixtur and San Bordeaux proved sufficient at 0.1 per cent, while other copper containing fungicides at the next higher concentration of 0.4 per cent. Germ tube was conspicuously retarded in growth at 0.1 per cent. Non-copper fungicides did not show any apparent indication of suppressing the spore germination.

Table 4. Analysis of Variance of the Spore Germination and the Germ Tube Growth of Five Copper Containing Fungicides in Table 3. *

Source of variation	Degrees of freedom	Sum of squares	Mean square
Spore germination			
Total	59	239	
Fungicides	4	19.91	4.98 **
Concentrations	3	109.66	36.55 **
Fungi	2	47.45	23.72 **
Error	50	51.98	1.039
Germ tube growth			
Total	59	66.60	
Fungicides	4	5.26	1,31 **
Concentrations	3	40.20	13.40 **
Fungi	2	8 40	4.20 **
Error	50	12.74	0.255

* In computing this table, 0 and 0 for 0.8% Bordeaux mixture for 3 fungi, and 5 and 3 in 0.025 Kupoid in *Piricularia Oryzae* were added.

** F valuces of mean square exceed tabular 1% value.

Significans of factors of fungicides, concentrations, and fungi used are brought out in Table 4. Minimum significant differences for the means of over all germination and germ tube growth in five copper containing fungicides at four different concentrations are shown in Table 5 and 6 respectively. A certain degree of differentiation among fungi and fungicides can be seen, but the omission of the factor of concentration apparantly inhibited a more precise distinction.

Table 5. Variation in Average SporeG ermination and Germ Tube Growth among *Helminthosporium Oryzae*, *Piricularia Oryzae*, and *Gibberella Saubinetii*. Combined results of Five Copper Containing Fungicides at Four Different Concentrations of Table 3.

	Helminthosporium Oryzae	*Piricullaria Oryzae*	*Gibberella Saubietii*	Significant difference for 5% level
Germination	2.15	3.70	1.60	0.64
Germ tube growth	1.20	1.80	0.90	0.32

Table 6. Variaton in Average Spore Germination and Germ Tube Growth among Five Copper Containing Fungicides. Combined Results of Four Concentartions and Three Fungi of Table 3.

	Bordeaux mixture	San Bordeaux	Ōdo	Kassei Bordeaux	Kupoid	Signifficant difference for 5% level
Germination	6.0	9.0	12.3	10 3	12.0	0.82
Germ tube growth	0.75	1.25	1.58	1.42	1.50	0.41

Interpretation of the results of Table 3 in terms of 100 per cent lethal dose, expressed as LD-100 is desirable at this point. A complete inhibition of germination is assumed in this report as the concentration being lethal to the spores. Since the exact values of LD-100 are not available with the concetrations tested, intermediate values showing the theoretical LD-100 were interpolated by the use of the table shown below.

Table 7. Observed Degrees of Spore Germiation at Limit Concentrations and Estimated Lethal Concentration. (LD 100)

Limits of concentration		Observed degrees of germination at concentration B				
A No germination	B Positive germination	1	2	3	4	5
0.8%	0.4%	0.45%	0.5%	0.6%	0.7%	0.75%
0.4 〃	0.1	0.125	0.15	0.2	0.3	0.35
0.1 〃	0.025	0.031	0.038	0.05	0.075	0.088

By utilizing the above table, lethal concentrations for different fungicides and for different fungi are tabulated in the third column of Table 8, under "Estimated lethal concentration, LD-100".

Table 8. LD-100 Expressed in Concentration of Fungicide, Content of Pure Copper, and Bordeaux Coefficient, as Modiffied by Different Fungi.

Fungus	Fungicide	Estimated lethal concentration (LD 100)	Percent pure copper (LD 100)	Bordeaux Coefficient (LD 100)
Helminthosporium Oryzae	Bordeaux mixture	0.113	0.026	1.00
	San Bordeaux	0 288	0.040	0.65
	Ōdo	0.663	0 260	0.10
	Kassei Bordeaux	0.116	0.016	1.62
	Kupoid	0.625	0.111	0.23
Piricularia Oryzae	Bordeaux mixture	0.175	0.044	1.00
	San Bordeaux	0.800	0.109	0.40
	Ōdo	0.800	0 320	0.14
	Kassei Bordeaux	0.800	0 075	0.59
	Kupoid	0 800	0.136	0.32
Gibberella Saubinetii	Bordeaux mixture	0.110	0.028	1.00
	San Bordeaux	0.088	0.013	2.14
	Ōdo	0.100	0.040	0.70
	Kassei Bordeaux	0.438	0.040	0.69
	Kupoid	0.194	0.033	0.85

Such lethal concentration when expressed as per cent pure copper is shown in next column under "per cent pure copper, LD-100". The column shows a rather

Table 9. Effectiveness of Fungicides as Influenced by Aging of Spray Residue, Tested with *Helminthosporium Oryzae*. Average of 2 Tests.

Fungicide	Concentration in Percent	Days after spraying, germination and germ tube growth													
		0		5		10		20		30		40		60	
		Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germinaiton	Germ tube	Germination	Germ tube	Germination	Germ tube
Bordeaux mixture	0.8	0	0	0	0	1 5	0.5	0	0	3	1	0	0	0	0
	0.4	0	0	1	1	2	1	1	1	4	2	0.5	0.5	0	0
	0.1	3.5	2	3.5	1	2.5	1.5	3	2	4	2	1	1	1	1
	0.025	4.5	3	5	3	4.5	3	4.5	3	5	3	5	2.5	5	3
San Borpeaux	1.6	0	0	0	0	0	0	0	0	—	—	0	0	0	0
	0.8	0	0	1	1	1.5	1	0.5	0.5	0	0	0.5	0 5	0	0
	0.4	0.5	0.5	1	1	1.5	1	1.5	1	2	1	1	0.5	1	1
	0.1	4	2.5	3.5	2	2.5	1	2.5	2	2	1	1.5	1	3	3
	0.025	4·5	3	5	3	4.5	3	5	3	5	3	4	3	5	3
Ōdo	1.6	2	2	2	1	2	1	2	1	—	—	2	1	2	2
	0.8	3.5	2	.5	1.5	2.5	1.5	2	1	2	1	1.5	1	1	2
	0.4	3 5	2	4	2	3	2	2.5	1.5	2	1	2.5	1	1	2
	0.1	3 5	2.5	4	3	3	2	3.5	2.5	3	1	3.5	2	2	3
	0.025	5	3	5	3	4	3	5	3	5	3	5	3	5	3
Kassei Bordeaux	1.6	0	0	0.5	0.5	1	1	1.5	1	1	1	0.5	0.5	1	1
	0.8	1	0.5	3	2	2	1	2.5	1	2	1	1	0.5	1	2
	0.4	2	2	2	1	2	1	2	1.5	5	3	2	1.5	2	3
	0.1	4.5	3	5	3	4.5	3	4.5	3	5	3	4.5	3	4	3
	0.025	5	3	5	3	4.5	3	5	3	5	3	5	3	5	3
Kupoid	1.6	3.5	2.5	2.5	1.5	4	1	3	1	1	1	1.5	1	5	1
	0.8	4	2.5	3.5	2	3	2	3	2	2	1	1.5	1	3	1
	0.4	4	2.5	4.5		3	2.5	3	2	3	1	3	1	2	3
	0.1	4.5	3	4.5	2.5	4	3	4	3	4	2	4	2.5	3	3
Soid	1.6	5	3	5	3	5	3	5	3	5	3	5	3	1	1
	0.8	5	3	5	3	5	3	5	3	5	3	5	3	5	3
Kolloidinoid	1.6	4.5	3	5	3	3.5	2.5	5	3	5	3	5	3	5	3
	0.8	5	3	5	3	4	2.5	5	3	5	3	5	3	5	3
Control	Water	5	3	5	3	5	3	5	3	5	3	5	3	5	3

Table. 10 Effectiveness of Fungicides as Influenced by Aging of Spray Residue, Tested with *Gibberella Saubinetii*. Average of 2 Tests.

Fungicide	Concentration in Percent	Days after spraying, germination and germ tube growth													
		0		5		10		20		30		40		60	
		Germination	Germ tube	Germination	Germ tube	Germination	Germ tbue	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube
Bordeaux mixture	0.8	0	0	0	0	0	0	0	0	—	—	0	0	0	0
	0.4	0	0	0	0	1	0.5	0	0	2	1	1	0.5	0	0
	0.1	1.5	1	1.5	1	3.5	1.5	5	2	5	3	3	1.5	2	1
	0.025	4	2.5	4	3	5	3	5	3	5	3	5	3	5	3
San Bordeaux	1.6	0	0	0	0	0	0	0	0	—	—	0	0	0	0
	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.4	0	0	0	0	0	0	0	0	3	1	0	0	0	0
	0.1	0	0	1	0.5	0.5	0.5	3	1.5	5	2	1	1	2	1
	0.025	5	3	5	3.5	5	2.5	5	3	5	3	5	3	5	3
Ōdo	1.6	0	0	0	0	0	0	2	1	—	—	2	1	0	0
	0.8	0	0	0	0	1	0.5	3.5	1	5	2	1	0.5	0	0
	0.4	1.5	0.5	2	1	1.5	1	4	1	5	3	3	1	0	0
	0.1	2	0.5	5	2	3	1	5	1.5	5	3	4	1	1	1
	0.025	3.5	2	2.5	1.5	5	2.5	5	3	5	3	5	1.5	5	3
Kassei Bordeaux	1.6	0	0	0	0	0	0	1	0.5	1	1	0	0	0	0
	0.8	0	0	0	0	0	0	4.5	2	5	3	3.5	1	0	0
	0.4	2.5	1	2.5	1	3	2	4.5	2.5	5	3	4.5	1	5	3
	0.1	3.5	2	5	2.5	5	3	5	2.5	5	3	5	3	5	3
	0.025	5	3	5	3	5	3	5	3	5	3	5	3	5	3
Kupoid	1.6	0	0	0	0	1	0.5	2	1	5	3	1	1	0	0
	0.8	0	0	0	0	1	0.5	2.5	1	5	3	2	1	0	0
	0.4	0.5	0.5	0.5	0.5	1.5	1	4.5	1.5	5	3	3	1	2	1
	0.1	3	1	3.5	2	3	2	5	2.5	5	3	4	2	5	3
Soid	1.6	5	3	5	3	5	3	5	3	5	3	5	3	5	3
	0.8	5	3	5	3	5	3	5	3	5	3	5	3	—	—
Kolloidinoid	1.6	5	3	5	3	5	3	5	3	5	3	5	3	5	3
	0.8	5	3	5	3	5	3	5	3	5	3	5	3	—	—
Control	Water	5	3	5	3	5	3	5	3	5	3	5	3	5	3

wide variation in the lethal copper concentration among materials and fungi.

An evaluation of fungicides in terms of Bordeaux mixture can be made, and such values are herewith expressed as Bordeaux Coefficient.

$$\text{Bordeaux Coefficient} = \frac{\text{LD-100 copper deposit of Bordeaux mixture (100)}}{\text{LD-100 active ingredient in spray deposit of an unknown (x)}}$$

Figures in column five show the "Bordeaux Coefficient, LD-100". Here, again it is apparent that the Bordeaux Coefficient of a fungicide is modified by the fungus in question. An example on San Bordeaux shows for *Helminthosporium Oryzae* a coefficient of 0.80, but on *Gibberella Saubinetii* it is 2.14; and likewise with Kassei Bordeaux, the values were 2.24 and 0.69, on respective fungi.

2) Effect of Aging of Spray Residue.

In order to determine whether there would be any decrease in the action of the spray residue upon aging, tests were made by observing spore germination on slides held for definite periods of time under room conditions. Results with *Helminthosporium Oryzae* and *Gibberella Saubinetii* are shown in Tables 9 and 10.

Tables 9 and 10 indicate that, although there appears to be some fluctuation in the spore germination, there is no definite tendency shown as influenced by the aging of the spray residue up to 60 days for any fungicide.

3. Effect of Washing of Spray Residue.

Sprays applied on plants are exposed to weathering under natural conditions, and lose the effectiveness in a course of time. One of the important factors to be considered under field conditions is the effect of rain. It is the purpose of this experiment to determine the effect of such washings, taking length of exposure to water as the item. Bordeaux mixture and San bordeaux were selected at the concentrations of 0.8 and 0.4 per cents. Sprayed slides were thoroughly dried in room and placed in glass containers filled with distilled water. The water in each container was renewed twice daily, once each in the morning and in the evening. This method, although differing from that expected from natural rain, was selected because of the fact that to replicate rain by means of spray would not only require elaborate equipment but also a large quantity of distilled water.

Washed slides were removed from the containers at the close of specified period of time and were dried in room prior to the germination test. Results of germinating with *Helminthosporium Oryzae, Piricularia Oryzae* and *Gibberella Saubinetii* are shown in Table 11.

The table shows that for Bordeaux mixture, a distinct difference in the germination between the two concentrations, 0.8 and 0.4 per cents is affected by the treatment, and the former shows a much lasting effect on the spores of all of the three test fungi. On San Bordeaux, the difference between concentrations was less and also appears to lose its effectiveness sooner than the Bordeaux mixture, particularly at the higher concentration of 0.8 per cent.

The tests showed that an extremely long exposure to water of between two and four days is required with Bordeaux mixture at 0.8 per cent to have any effect on germination. At 0.4 per cent, a continuous exposure up to 24 hours still retained

Table 11. Prolonged Washing of Spray Residue as Affecting Germination and Germ Tube Growth in *Helminthosprium Oryzae*, *Piricularia Oryzae* and *Gibberella Saubinetii*.

Fungus	Fungicide	Concentration in per cent	Period of washing, and germination and germ tube growth																		Clean slide	
			0		12 Hrs		24 Hrs		2 Days		3 Days		4 Days		5 Days		6 Days		7 Days			
			Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube	Germination	Germ tube		
Helmnthosporium oryzae	Bordeuax mixture	0.8	0	0	0	0	0	0	0	0	0	0	2	1	4	3	3	5	5	3	5	3
		0.4	0	0	0	0	0	0	4	2	4	2	4	3	4	3	4	3	5	3	5	3
	San Bordeaux	0.8	0	0	1	1	1	1	4	2	4	2	4	3	4	3	5	3	5	3	5	3
		0.4	0	0	1	1	1	1	4	2	4	3	4	3	4	3	5	3	5	3	5	3
Piricularia oryzae	Bordeaux mixture	0.8	0	0	0	0	0	0	1	1	3	1	4	1	5	3	5	3	5	3	5	3
		0.4	1	1	2	1	2	1	4	2	5	3	5	3	5	3	5	3	5	3	5	3
	San Bordeaux	0.8	1	1	2	1	2	1	4	1	5	3	5	3	5	3	5	3	5	3	5	3
		0.4	2	1	2	1	2	1	5	3	5	3	5	3	5	3	5	3	5	3	5	3
Gibberella saubinetii	Bordeaux mixture	0.8	0	0	0	0	0	0	0	0	0	0	0	0	2	1	4	2	4	2	5	3
		0.4	0	0	0	0	0	0	1	1	5	2	4	2	4	2	4	2	4	2	5	3
	San Bordeaux	0.8	0	0	0	0	0	0	4	1	4	2	4	2	4	2	4	2	4	2	5	3
		0.4	0	0	0	0	0	0	4	2	4	3	4	2	4	2	4	2	4	2	5	3

its effectiveness. The general tendency of reaction of the three fungi to the fungicides follow closely to that obtained in the previous germination tests.

IV. Discussion

Assay of fungicides by means of germinating fugus spores on slide glasses demonstrated peculiarities of each fungicides against different pathogens. The method, however, is only a part of the more extensive program in determining the effectiveness of fungicides, since other items must be considered when applications are made on living plants, such as the limit factor of concentrations tolerable by plants. The results attained indicated the effect of concentration upon spore germination which varied in different fungi. By correlating results with other more direct methods, one should be able to obtain a more general and practical application of this method of testing.

An expression of effective concentration of fungicides in terms of LD-100, either in concentration, copper content or Bordeaux Coefficient, is convenient for this type of testing.

There was no noticiable differences observed by aging of spray residues. This indicates that, by incorporating other factors such as sunlight, temperature, etc, enough differences may show, but this requires further consideration and study.

The effect of washing of spray residue by rain is available by the method outlined, which although differing from the natural rain, can become an indicator for such tests in laboratory. A combined test of aging and washing should serve a useful purpose in replicating weathering effects under natural conditions.

V. Summary

1. Testing spray fungicides by the using slide glasses is discussed in this report. The method consisted of spraying test fungicide solutions with an atomizer connected to a motor driven air compressor, adjusted to 5 pounds per square inch, upon a slide from a distance of 3 feet for 5 seconds. A pure culture fungus spores are then inoculated upon the dried spray residue on slides in drops of water suspensions using a small pipette. The concentration of the spore suspension was adjusted to give from 30 to 80 spores depending upon the size of the spore per field of an microscope magnified to x 200. Slides were placed in water saturated compartment for germination. Records of germination and germ tube growth are taken and the degrees of each are calculated and expressed in figures 0—5 for germination and 0—3 for germ tube length.

2. Tests on inhibition of germination by fungicides were made and the results distingished fungicides, concentrations, and fungi employed, both on the spore germination and the germ tube growth.

3. Each fungicide was evaluated on basis of lethal concentrations, expressed in per cent of spray material, per cent copper content, and Bordeaux coefficient.

4. As far as the method employed showed, there was no decided effect of

aging of spray residues upon the loss of effectiveness up to 60 days.

5. Washing off of spray residue by rain was tested by allowing slides to stand in distilled water renewed twice daily. The results showed a difference in the adhesiveness to slide by fungicides which affected the subsequent germination of spores. Also, the residual effect of such washing was shown to be modified by the concentration of the fungicide. Bordeaux mixture at 0.8% required from 2 to 4 days of constant exposure to water before losing its effectiveness, but at 0.4% this was reduced to 24 hours.

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