Preservation of conidia of *Sclerospora philippinensis* Weston on artificial medium for the use in inoculation tests

For breeding varieties resistant to any disease, it is necessary to develop screening techniques which can be used effectively in breeding works. Techniques of handling inoculum must be established in order to preserve conidia on artificial medium and to induce their sporulation at any time when needed. These techniques are required for conducting artificial inoculation under a definite condition.

With downy mildew of maize, however, no *in vitro* culture technique has been developed yet. Therefore, the screening of the resistance has been practiced so far only by field tests by natural infection or by inoculation of conidia directly collected from naturally occurring diseased plants. 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. Although *in vitro* culture of some *Sclerospora* spp. and *Sclerophthora* spp. was reported to have been achieved, the *in vitro* culture of *Sclerospora philippinensis* has not been successful (Dogma, Jr. 1975). Therefore, the authors attempted to find out the method to preserve the conidia of *S. philippinensis* on an artificial medium without germination, but without causing the loss of pathogenicity.

Kimigafukuro and Leu (1973) found out that agar media containing some neutral salts were effective in keeping mature conidia from germination without causing the loss of pathogenicity with *S. sacchari*. In the

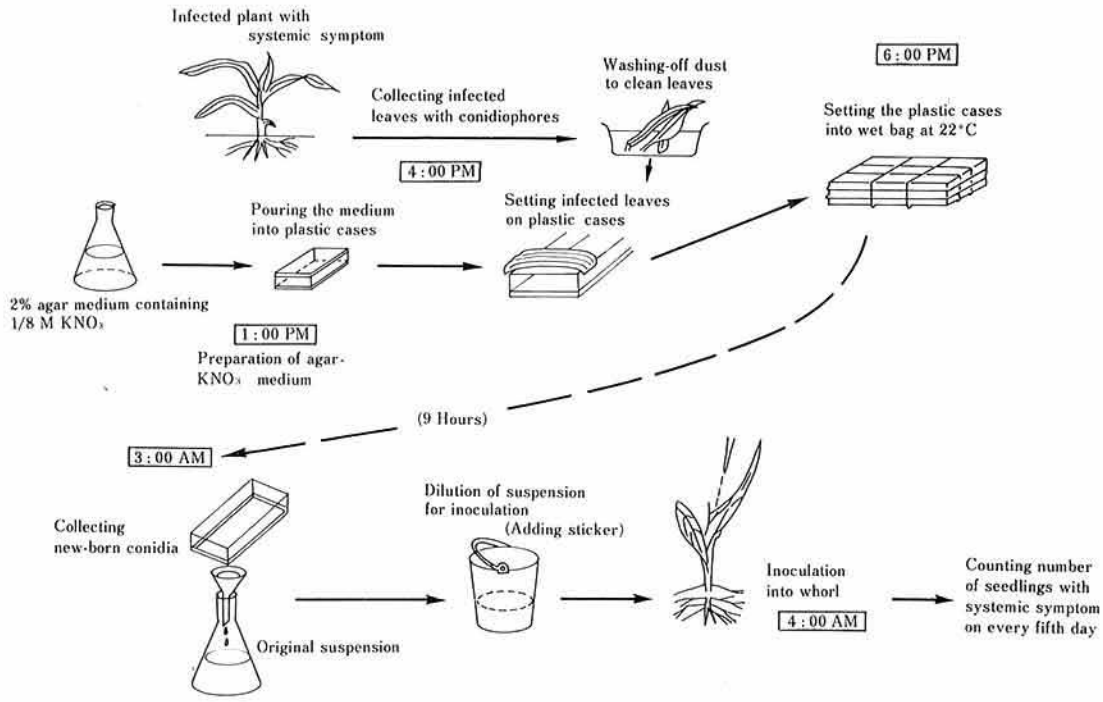

Fig. 1. Procedure of artificial inoculation of downy mildew, *Sclerospora philippinensis*, to maize for screening test at seedling stage.

present study, effect of KNO_3 contained in agar media was examined with *S. philippinensis*.

Procedure of the experiment: Infected leaves of UPCA VAR 3 (susceptible) were collected from the field in the evening, and washed in a water-bath by using a sponge to remove dusts and downy mildew conidiophores on the leaves. Leaf blades were cut into pieces, 10–11 cm long, and they were set on plastic cases containing agar media. Two levels of KNO_3 in the agar media (2% concentration), ie., 1/8 M and 1/4 M, were used with no-KNO_3 as a control. The plastic cases were then placed into vinyl-bags to keep the humidity for sporulation. They were kept in darkness at 22 or 23°C in a growth chamber for 10 hr (6:00 p.m. to 4:00 a.m.). After taking them out from the chamber at 4:00 a.m. germination and inoculation tests were conducted with conidia collected from each medium.

Germination and abortion of conidia were examined under the microscope at a magnification of ×100. For the pathogenicity test, 15 seedlings each of the two strains of maize were grown in a row 10 cm apart in nursery boxes (40 × 20 × 8 cm). Seedlings of (NE#1 × Ph 9 DMR) × MIT VAR 2 F_1 hybrid (resistant) and of La Granja Popcorn × UPCA VAR 3 F_1 hybrid (susceptible) were used. At the one-leaf-stage of the seedlings, conidial suspension (40×10^3 conidia/ml) was applied for inoculation. The inoculum density of conidial suspension from no-KNO_3 medium was not accurate, because only ungerminated conidia were counted. The experiment was carried out from February 25 to March 31, 1975 at UP Los Baños, College, Laguna.

1) Effect of KNO_3 concentration on conidia germination

Effect of KNO_3 concentration in the agar media on the germinability of conidia on the germination beds with different KNO_3 concentrations is shown in Table 1. Germination percentage was only 0.8 to 4.7% on the beds containing 1/4 M KNO_3, whereas it was 26.7 to 43.7% on the beds without KNO_3 or with 1/8 M KNO_3. Conidia preserved on the agar medium not containing KNO_3 showed 28.7% of germination on the bed with 1/8 M of KNO_3, although it gave 43.7% on the bed without KNO_3.

Germination and abortion of conidia that occurred in the course of the maintenance on the agar-KNO_3 media were examined at 12 hr and 24 hr (both including 10 hr for sporulation in a growth chamber at 22–23°C)

Table 1. Germination (%) of conidia of *S. philippinensis* collected and maintained on 2% agar media containing KNO_3 and their pathogenicity on maize seedlings

KNO_3 coucentration of germination bed	KNO_3 concentration of agar medium					
	OM (control)		1/8M		1/4M	
	Germination %	Infection %	Germination %	Infection %	Germination %	Infection %
OM (control)	43.7 (48)	R : 51 S : 100	37.5 (54)	R : 40 R : 90	27.1 (51)	R : 56 S : 100
1/8M	28.7 (43)	— —	35.2 (46)	R : 54 S : 100	26.7 (36)	— —
1/4M	4.7 (66)	— —	4.0 (40)	— —	0.8 (41)	— —

Note: Two diseased leaves trisected were set on different agar media with two replications. Conidia preserved on each of the agar media were transferred to a germination bed in three rows. Figures in perenthesis show average number of conidia examined in each row. Inoculation test was conducted with 4 repetitions and infection % was counted on 28th day after inoculation. R signifies resistant strain, and S susceptible one.

Table 2. Germination (%) and abortion (%) of conidia of *S. philippinensis* that occurred on agar-KNO_3 media

KNO_3 concentration of medium	Time after setting			
	12hr		24hr	
	Germinated	Aborted	Germinated	Aborted
OM (control)	19.3 (208)	1.8	60.5 (198)	10.6
1/8M	4.3 (198)	4.6	59.3 (162)	9.5
1/4M	0 (218)	6.8	23.7 (202)	16.0

Note: Trisected two diseased leaves were set on three plastic cases containing each of different agar media at 6 : 00 p.m. Germination was examined at three spots in each case. Figures in parenthesis shows average number of conidia examined at each spot.

after the setting of diseased leaves. As shown in Table 2, remarkable differences in germination and abortion of conidia were found among different media with different concentratoins of KNO_3 at 12 hr after setting. At 24 hr after setting, however, no difference was observed between the no-KNO_3 medium (60.5% of germination and 10.6% of abortion) and the 1/8 M KNO_3 media (59.3% and 9.5% respectively), but the medium with 1/4 M KNO_3 gave a germination percentage, 23.7%, less than half of other media, and the abortion percentage, 23.7%, higher than others.

2) Effect of KNO_3 on pathogenicity of conidia

Inoculation was carried out using water suspensions of conidia taken from agar and agar-KNO_3 media. In addition, conidial suspension in 1/8 M solution of KNO_3 was prepared using conidia collected from the medium containing 1/8 M KNO_3. On the 10th day after the inoculation, systemic symptoms of the disease appeared, and on the 28th day the percentage of infection reached its maximum in every case. As shown in Table 1, no significant difference in pathogenicity was found among different suspensions used.

Thus, it can be concluded that the agar medium containing 1/8 M KNO_3 is able to delay the germination of conidia without causing the loss of pathogenicity. Although the pathogenicity is maintained on the no-KNO_3 medium too, this medium is not useful because of difficulty in obtaining exact densities of conidial suspensions. As a result, the procedure shown in Fig. 1 is recommended. In fact, the authors could often collect 10^5 conidia per cm^2 on the medium by the procedure mentioned here.

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Behavior of nitrogen applied to upland field in Thailand

In 1974 a field experiment using ^{15}N tracer technique was carried out in the Phraputtabat Agricultural Experiment Station to clarify the behavior of fertilizer nitrogen applied to the upland field in relation to rainfall. The soil of the experimental field was Reddish Brown Lateritic soil, with a clayey texture throughout the profile, containing mainly kaolinite minerals with a moderate content of montmorillonite.

The experimental field was divided into following four plots:

Plot A: No crop was planted. When the plot had received a total of 300 mm of rainfall after fertilizer application, soil samples were taken.

Plot B: No crop was planted. Soil samples were taken when the plot had received a total of 600 mm of rainfall after fertilizer application.

Plot C: No crop was planted. The plot was not exposed to rainfall by being covered by a roof after fertilizer application, and soil samples were taken at the same time as in Plot B.

Plot D: Corn (DMR No. 6) was planted. Fertilizers were applied in the same way as other three plots. Soil and plant samples were taken at the time of corn harvest, that coincided with the date of soil sampling of Plot B and C.

In each plot a small sub-plot with an area of 0.225 m^2 (0.3 × 0.75 m), enclosed with galvanized iron sheets was placed, and fertilizers (^{15}N enriched nitrogen, phosphate and potassium) were applied in a band with a width of 10 cm to a depth of 3 cm below soil surface in each sub-plot. Nitrogen was applied in the form of ^{15}N enriched ammonium sulphate at a rate of 2,250 mg N/sub-plot as a basal dressing. Soil samples were taken quantitatively from successive soil layers as shown in Fig. 1.

Distribution pattern of applied nitrogen in the Plot A, which had received 300 mm of rainfall after fertilizer application showed that nitrogen concentration was very high at the site of nitrogen application, but it decreased with the distance from the site of application both in vertical and horizontal directions. At the depth of 50 to 90 cm, no nitrogen was recovered.

In the Plot B, which received 600 mm of rainfall, the nitrogen distribution was nearly the same as that of Plot A, although it showed a deeper downward movement of nitrogen due to more rainfall than Plot A. However, no accummulation of nitrogen was observed at the 50 to 90 cm of depth.

In the Plot C, which had been kept under a roof, the movement of nitrogen was extremely limited, showing high concentrations in the surface layer of 0 to 10 cm depth, particularly at the site of fertilizer application and adjacent portions as given in Fig. 1.

It is worthy to note that 50 to 60% of the total nitrogen applied was lost from the soil within a short period of two or three months after the application in the above three plots. Since it is evident that there was no nitrogen loss caused by leaching and absorption by crop, the result suggests the nitrogen loss in the form of gas.

Nitrogen distribution pattern in the Plot D after the harvest of corn is shown in Fig. 1. Balance sheet of applied nitrogen is given

Table 1. Balance sheet of applied fertilizer nitrogen in corn plot (Nmg/area*)

Amount of applied N	Fertilizer N taken up by plants (A)	Residual fertilizer N in soil after harvest (B)	A+B	Loss from soil	% of recovery of N
2250	235.5	201.5	437.0	1813	19.4

* Sub-plot surrounded by galvanized iron sheets

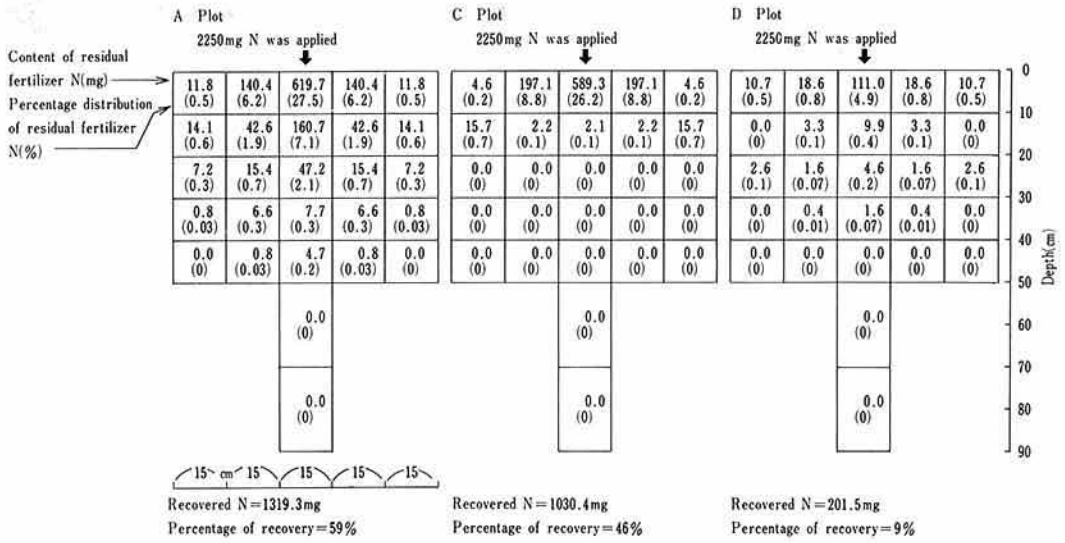

Fig. 1. Distribution of applied fertilizer nitrogen in soil layer

in Table 1, which indicates that about 10% of the total nitrogen applied was utilized by the crop, whereas residual nitrogen in the soil after the crop harvest was only 9%. This fact implies that as much as 81% of the total applied nitrogen was lost from the soil. This percentage of loss in the Plot D with crop culture was much higher than that of other plots without crop.

Further studies are needed to find out the mechanism of such nitrogen loss and methods to minimize it.

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