METHOD FOR COLLECTING NON-GERMINATED CONIDIA OF *SCLEROSPORA SACCHARI* AND TARC'S DOWNY MILDEW PROGRAM

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Artificial inoculation is one of the most important techniques for plant disease research. In the most cases with fungal diseases the conidia, produced on artificial media, are used as inoculum. *Sclerospora sacchari* Miyake is obligate parasite and its artificial culture has not been successful yet. However, it is relatively easy to collect a large number of conidia from infected leaves. Therefore, artificial inoculations have been conducted with conidia collected from naturally occuring diseased plants.

However, the conidia collected are not idea-for inoculum because they are composed of mature, immature and germinated ones; i.e., they are not uniform in stage and sometimes germ tubes are entangled and it is difficult to prepare a well-suspended inoculum.

Conidia of this fungi are produced every night on conidiophores thrusting from stomata. The mature conidia are readily detached from conidiophores and germinate right away. The elongation of germ tubes is very rapid and reach about five times as long as the conidial length at 80 minutes after germination. The conidial formation and dissemination continues for about 5 hours. The germ tubes of conidia disseminated in the first half are elongated more than 10 times as the length of conidia at the end of the dissemination period. If the conidia are collected by washing the infected leaves with brush before dissemination is complete, they are the mixture of germinated, non-germinated and, also, immature conidia. A technique to collect only non-germinated conidia has not been developed yet. Therefore, it is quite difficult to obtain conidia of a uniform stage and preserve them for accurate and effective inoculation tests.

Generally, germination of conidia is effected on an artificial medium. In the present studies, germination and pathogenicity of the conidia fallen on agar media, containing different levels of neutral salts and different pH values, was examined to obtain the non-germinated conidia for inoculation.

Materials and Methods

The experiments were conducted at the Nantou Downy Mildew (DM) Trial Field, Taiwan, from August to October, 1972.

Sugarcane leaves which had sporulated abundantly were collected from the field at around 5:00 PM. They were washed by running water to remove dust and fungus conidophores formed during the previous night and superfluous water drops wiped dry with cotton. Then, the leaf blades were cut to fit the inside of petri dish cover and set in the petri dish containing agar media at 8:00 PM. The dishes were wrapped in a wet plastic film bag to maintain sufficient humidity for sporulation. They were incubated under natural night conditions (20-25°C) for 12 hours (8:00 PM - 8:00 AM). Neutral salts media (1, 1/2 and 1/4 M) were solidified with 2%, sometimes up to 6%, agar. Different pH solution were prepared by McIlveine's buffer solution, and 4, 3, 2 and 1.5% agar were used for pH 4, 5, 6 and 7 to 8, respectively; 2% water agar medium was used as a control. All media were poured into petri dishes before inserting the leaves.

Twelve hours after the leaves were set, i.e. 8:00 AM in the following morning, the dishes were opened for counting germination. Those dishes

were left in laboratory for another 24 hours for further counting.

The conidia fallen on the test agar media were collected on the end of a glass rod and then streaked on water agar plate at 12 hours after setting the leaves. Conidial germination was counted 24 hours after streaking to examine their viability.

Inoculation tests were conducted with the conidia discharged on media to examine the effect of those media on conidial pathogenicity. For inoculations, corn seedling *(Zea mays* L., Tainan No. 5, DM susceptible*)* of which the third leaf is still unfolding, were used. The inoculation tests were carried out by water suspensions of conidia collected at 12 hours after setting, from agar media containing KNO_3, $CaCl_2$ and different pH values. Those conidia were washed on the filter paper to remove the effect of the media and then collected in flasks containing distilled water. The inoculum density was adjusted 10 to 20 conidia per low power (100×) microscopic field. For each test, a 0.5 ml conidial suspension was dropped into the spindle part of 20 corn seedlings. Local lesion appeared at the inoculated part was examined and recorded 10 days after inoculation.

Results and Discussion

As shown in Fig. 1-2, remarkable differences in conidial germination was found among various media with 1, $\frac{1}{2}$ and $\frac{1}{4}$ M of neutral salts and different pH values.

Germination of conidia on 1 M neutral salts media was completely inhibited. However, even if those conidia were transferred to the water agar media at 12 hours after setting, they germinated only 0 to 20%. It indicates that almost all of the conidia which fell on 1 M neutral salts media lost viability.

On the media of $\frac{1}{2}$ and $\frac{1}{4}$ M neutral salts, germination of conidia was inhibited to some extent. The conidia transferred to water agar media at 12 hours after setting from NaCl, KCl, $NaNO_3$ and KNO_3 media of $\frac{1}{4}$ M showed 100% germination, but they had already germinated more than 50% on respective media before transferring. The germination percent of conidia on $\frac{1}{4}$ M $Mg(NO_3)_2$, $\frac{1}{2}$ M NH_4Cl and $\frac{1}{2}$ M NH_4NO_3 media were lower than 0.6% at 12 hours after setting, and 1.0 to 5.8% even at 36 hours after setting. The conidia transferred to water agar media from each salt media at 12 hours after setting had a germination of more than 60%.

As shown in Fig. 2, on the media of pH 4, 7 and 8 conidial germination was strongly inhibited. According to germination tests on water agar media, it was found that conidia on pH 7 and 8 media almost completely lost their viability. Meanwhile, those on pH 4 media had 94.5% germination.

As shown in Table 1, almost all conidia taken from the media of 1 M KNO_3 and $CaCl_2$ had lost their pathogenicity. In $\frac{1}{2}$ M and $\frac{1}{4}$ M media of KNO_3 and $CaCl_2$, the severity of infection was a little less than that of the control, but those conidia were still able to infect the host plant.

As shown in Table 2, no significant difference in pathogenicity was found between conidia at pH 4 and the control. Pathogenicity of conidia on the media of other pH values was inferior to that of the control.

Thus, it can be concluded that agar media containing some neutral salts or adjusted at certain pH values can inhibit the germination of conidia without causing loss of pathogenicity. The test of pathogenicity was conducted only for conidia taken from media of KNO_3, $CaCl_2$ and pH 4 to 8. Judging from the results of germination tests, the favorable media to collect non-germinated conidia for inoculum were $\frac{1}{4}$ M $Mg(NO_3)_2$, $\frac{1}{2}$ M NH_4Cl, $\frac{1}{2}$ M NH_4NO_3 and pH 4 media.

As the next step of research, preservation of those non-germinated conidia became the important problem. If we can preserve the conidia for some period of time, it will be a great help in studies on the DM diseases of corn.

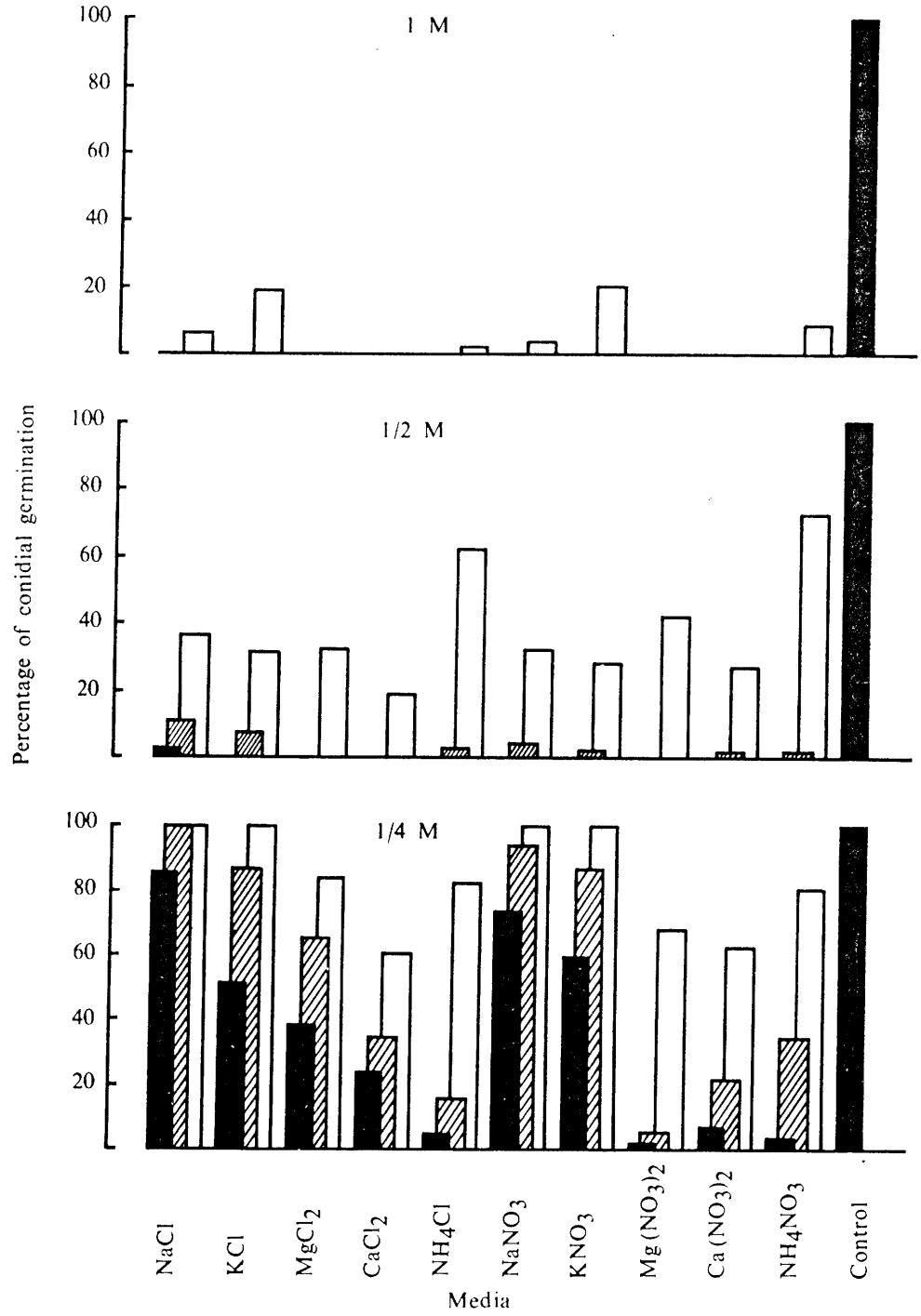
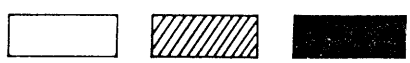


Fig. 1. Germination (%) of conidia of *S. sacchari* fallen on agar media containing different concentrations of salts and transferred onto water agar media.

 : See Fig. 2.

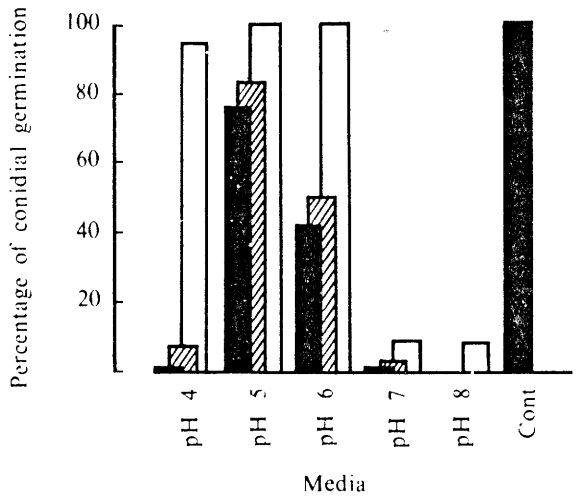


Fig. 2. Germination (%) of conidia of *S. sacchari* fallen on the agar media with different pH values and transferred on water agar media.

: Germination of conidia on the salt media at 12 hours after setting.

: Germination of conidia on the salt media at 36 hours after setting.

: Germination of conidia transferred on water agar media at 24 hours after transfer.

Table 1. Pathogenicity to corn seedling of conidia fallen on the media containing different concentrations of KNO_3 and $CaCl_2$.

Media	Replications							
	I				II			
	Concentration of Media (Mol)							
	1/4	1/2	1	Cont.	1/4	1/2	1	Cont.
KNO_3	++~+++*	++	–	+++	+++~++++	+++	+	+++
$CaCl_2$	++	+	–	+++	+++	++~+++	+	+++

*Degree of infection. – : No infection, +~++++ : Number of plus signs shows the degree of infection.

Table 2. Pathogenicity to corn seedlings of conidia fallen on the media of different pH values.

Media	Replications	
	I	II
pH 4	++++ *	+++~++++
pH 5	+++	+++
pH 6	+++	+++
pH 7	+++	+++
pH 8	+	+~++
Cont.	+++~++++	+++~++++

* Degree of infection. –, +~++++ : See table 1.

Research Program of TARC, Japan, for Downy Mildew Studies

The objective of the Tropical Agricultural Research Center (TARC) is to contribute something towards the development of tropical agricultural technology by extending research activities both at the Center and abroad. The most important activity to achieve this objective is to undertake joint or cooperative research programs with scientists of institutions in tropical and subtropical countries.

Downy mildew(DM) of corn is also one of the biggest concerns for TARC, Japan. Until now, TARC has dispatched several research scientists to research institutions in Taiwan, Indonesia and the Philippines as visiting scientists to undertake cooperative research programs on DM of corn.

The classification of DM fungi has attracted special interest recently. Up to the present, nine species of *Sclerophthora* and *Sclerospora* have been reported to attack corn. However, the classification of these nine species is not necessarily fully revealed. As you know, many problems on it had been also pointed out at the DM symposium held in Japan in September 1974. However, I am not intending to discuss them here. I would like to point out that some part of the data used in the classification were derived from the results of observation under different natural conditions. It is generally recognized that the morphology, pathogenicity, etc. of fungi are changed by cultural conditions. In the case of fungi which grow on the artificial media it is easy to examine their morphology and physiology under the same condition. All DM fungi of corn are obligate parasites and artificial culture of them has not been successful yet. If we can collect these fungi in one place it will be possible to make a large part of experimental conditions uniform. However, from plant quarantine viewpoints, it needs a large scale isolated greenhouse to conduct such kinds of experiments. It is also quite difficult, for prevention of epidemics, to collect these fungi in a country where the disease may break out.

TARC has fortunately constructed the isolate greenhouse in the new institution at Tsukuba, Japan. We have no problems for importation of pathogens from foreign countries. If you permit us to collect the fungi of DM of corn from each country, we will be able to import them, and the cooperative research will be possible on morphology and physiology of these fungi.