13. SOME EXPERIMENTS ON DOWNY MILDEW OF MAIZE

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Sporulation

Based on personal observations the downy mildew fungus of maize frequently forms a few spores, when a diseased leaf is detached from the plant in the morning, or on a cloudy and/or rainy day. Therefore, sporulation of the fungus was first studied to determine how to obtain enough spores for inoculation.

Although several trials failed, pretreatment of diseased leaves with glucose solution enhanced sporulation. Thus, naturally infected leaves (variety Harapan) collected from the field of CRIA (Bogor, Indonesia) at 1.00–1.30 pm were used for further studies. The diseased leaves were washed with tap water to remove old conidia and conidiophores, and the base parts dipped into glucose solution at concentrations of 4, 2, 1, and 0.5% for about 5 hours. Then they were removed from the glucose solution and kept in a moist chamber for about 14 hours at room temperature.

The results are shown in Table 1.

Table 1. Sporulation on diseased leaves pretreated with glucose solution

Repl.	Concentration of glucose solution (%)					Cont[2]
	4	2	1	0.5	0	
I	7,035[1]	5,990	8,741	3,146	23	897
II	3,560	2,774	2,271	1,502	192	1,547

1) Spore in numeric per cm^2

2) In control the diseased leaves were detached at 5.30 pm, and others at 1.00 pm

In this experiment, sporulation of diseased leaves increased remarkably by the pretreatment with 1–4% glucose solution.

Inoculation

Inoculation methods and suitable spore concentrations for inoculation were investigated using the spores formed on the lesions by the pretreatment with glucose solution.

(1) Inoculation Method

The following three methods of inoculation were investigated.

1) Spray method: The leaf surface of maize plants were rubbed with cotton wool dipped in distilled water to remove the wax substance. Spore suspension was then applied by spraying with a glass atomizer.

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2) Rubbing method: Leaf surface was rubbed with cotton wool dipped in the spore suspension.

3) Injection method: Spore suspension was injected into the funnel-shaped part of undeveloped young leaf with an injector.

The inoculated plants were kept in a moist chamber for 24 hours at 23–27°C.

The process of symptom development of the inoculated plants is given in Table 2.

Table 2. Process of symptom development

Days after inoulation	Symptom
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. Some of 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 leaf elongate and are clear.
14–25	Typical systemic symptoms appears on newly developed leaf.

1) Pale green spots were the most conspicious by the injection method
2) Symptoms, streaks, yellowing and wilting, appeared earliest in the spray method, then in the rubbing method, and latest in the injection method. The symptoms by the injection method started to appear about 2 days later than those by the spray method

All of the inoculated plants produced initial symptoms, small spots and yellowish streaks, appeared immediately on inoculated parts.

The development of systemic symptoms in each method are shown in Table 3.

Table 3. Relation between inoculation method and development of systemic symptoms

Inoculation method	Number of inoculated plants	Number of plants developing systemic symptoms (Days after inoculation)						Final % of systemic symptoms	Leaf stage systemic symptoms developed
		10	15	17	20	22	28		
Spay	32	0	11	28	31	31	31	97	6(5–8)
Rubbing	32	0	3	11	15	20	23	72	7(6–9)
Injection	32	0	0	1	5	9	10	31	7(6–9)

1) Variety: Harapan
2) Inoculation was carried out at 3–4 (av. 3.5) leaf stage, 10 days after seeding

Of the three method, systemic symptoms first appeared on plants infected by spraying. The percentage of plants developing systemic symptoms was also the highest in the spray treatment.

(2) Spore Concentration

Spore concentration of inocula was regulated at 80, 40, 20, 10, and 5 respectively in one field of microscope (Nikon 10×10). Maize plants (variety Harapan) at 2.2 leaf stage (6 days after seeding) were inoculated by the spray, rubbing and injection methods at these inocula concentrations.

Systemic symptoms appeared on all spray inoculated plants at the concentration of 80 spores, and at 80 and 40 concentrations by rubbing. The developmental rate of systemic symptoms decreased as spore concentration decreased (at 5 spores: 50–60%) in each inoculation method. In this experiment, the developmental rate of systemic

symptoms in rubbing was slightly higher than spraying. Injection was the lowest.

The results indicate, spray or rubbing method can be recommended for downy mildew of maize inoculation. Spore concentration of more than 80 spores are desirable per field of microscope at magnification 10×10 (about 4×10^4 spores per 1 ml).

Age of Plant Infection

The experiments were carried out to confirm susceptiblity of maize at different ages. Plants were inoculated at 0–8.5 leaf stages (4–29 days after seeding) in Exp. I and at 2.9–5.8 leaf stages (7–17 days after seeding) in Exp. II.

Initial symptoms, yellowish streaks, appeared at 7 days after inoculation on all inoculated leaves, except plants just emerging that had not developed leaves. Table 4 shows the development of systemic symptoms in Exp. I. The results of Exps. I and II were almost the same.

Table 4. Age of maize plants and development of systemic symptoms by inoculation

Age of inoculated plants[1]		Development of systemic symptoms		
Leaf stage	Days after seeding	Leaf Stage	Days after inoculation[2]	Final percentage
0[3]	4	—	—	0
1.1	5	4–5	10	100
2.5	7	6–7, 9	13	100
3.3	9	7–9	14	100
4.1	11	7–10	16	100
4.8	13	10–11	24	100
6.2	20	—	—	—
8.5	29	—	—	—

1) Six plants were inoculated in each plot using spray method.
2) First day systemic symptoms observed on inoculated plants.
3) Just after emergence. No leaf developed.

As shown in Table 4, the plants inoculated just after emergence and before leaf development did not produce symptoms. Plants inoculated at 1.1 leaf stage showed systemic symptoms at 4–5 leaf stage. Plants inoculated at 4.8 leaf stage showed systemic symptoms 24 days after inoculation, 10–11 leaf stage. Thus, as the leaf stage of inoculation advanced, incubation period for appearance of systemic symptoms became longer. When plants were inoculated after the 6.2 leaf stage (20 days after seeding), systemic symptoms were not observed, even if the initial symptoms, such as yellowish streaks, occurred on the inoculated leaves.

Also, in Exp. II, plants inoculated at 4.8, 5.4 and 5.8 leaf stages, systemic symptoms did not show 100% and decreased as leaf stage increased (75%–33%).

From the results of these experiments, the maize plant seems to be able to escape downy mildew damage when plants are at past the 6 leaf stage.