An improved spreader row method of inoculation of Philippine downy mildew of maize, *Pernosclerospora philippinensis*

Two inoculation methods have generally been used so far for the purpose of screening varieties or lines for resistance to downy mildew. One consists of spraying or dropping conidial suspension at prescribed concentration of conidia on each test material. The other is the spreader row method, in which test materials are planted in the field where infected plants (spreader) are growing in rows and at borders as a source of inoculation.

Exconde (1970), and Yamada and Aday (1977) showed that the whorl drop method is effective. Kajiwara (1974) indicated that spray or rubbing method can be recommended for inoculation of downy mildew of maize. On the other hand, Chang and Chou (1968) suggested that the natural epidemic inoculation seems practical. Furthermore, Chang and Wu (1976) developed a more practical method, in which test materials in pots are set between infected sugarcane plants for a week.

However, when several thousands of seedlings are to be inoculated at the same time, most of these methods are very tedious and laborious. The problem of conidial germination is also encountered. In such cases, the last method (Chang and Wu, 1976) may be practical. Indeed it would be very helpful if many seedlings could be exposed under the epiphytotic conditions at the same time and at any time during the needed period. For this purpose, a new inoculation method consisting of transferring seedlings in the nursery boxes into the inoculation field was devised. In this study, three experiments were conducted at the campus field of the University of the Philippines at Los Baños, Philippines, in 1977–1978 to obtain some basic information regarding the inoculation method.

The first experiment was to know the effect of seedling age and duration of exposure to spreader rows on the intensity of downy mildew infection. Mimies and La Granja Popcorn × UPCA Var. 3 were used as resistant and susceptible varieties, respectively. Seeds of these varieties were planted in nursery boxes under downy mildew free conditions on June 1, 1977. Then seedlings were brought into the row space of spreader in UPLB Downy Mildew Screening Nursery at different ages, 5, 7, 9 and 11 days after planting, and exposed to the spreader rows for 1, 2 and 3 days, respectively. The nursery boxes were placed in the middle of 5 m space between spreader rows. Each of the 24 treatments had two replications.

As shown in Table 1, Mimies showed a significantly lower infection rate than La Granja Popcorn × UPCA Var. 3. Percent in-

Table 1. Percent infection with downy mildew of Mimies and La Granja Popcorn × UPCA Var. 3 at varying seedling ages and durations of exposure to spreader rows

Variety	Duration of exposure (day)	Seealing age (days after planting)				
		5	7	9	11	Mean
Mimies	1	93.7	11.1	4.7	6.1	28.9
	2	70.2	65.4	32.3	18.0	46.5
	3	81.7	41.1	24.6	7.7	38.8
	Mean	81.9	39.2	20.5	10.6	38.1
La Granja Pop. × UPCA Var. 3	1	100.0	85.2	89.7	50.6	81.4
	2	100.0	98.5	93.5	56.1	87.0
	3	100.0	98.5	69.5	75.6	85.9
	Mean	100.0	94.1	84.2	60.8	84.8

fection decreased as seedling age increased in both varieties. In Mimies, a significant increase in downy mildew infection was observed when seedlings were exposed for 2 or 3 days as compared with only one day.

However, in La Granja Popcorn × UPCA Var. 3, there was no difference in infection among the different durations of exposure. This suggests that inoculation can be fairly effective with an exposure of only one night if the environmental conditions are suitable for the infection.

The second experiment was conducted to investigate the effects of seedling age, duration of exposure and distance from spreader rows on disease development. Cotabato White Flint, a moderately resistant variety, was used as test material. Three different distances between spreader rows were selected in the nursery, namely 2.25 m, 3.75 m and 5.25 m. Seeds were planted in nursery boxes as unit plots on December 23, 25 and 27, 1977. Nursery boxes with seedlings aged 6, 8 or 10 days after planting were placed between the spreader rows on January 2, 1978, and exposed for 2, 4 and 6 days. Each unit plot was replicated 3 times. A set of three graduated glass slides were placed on a stand at a height of 8 cm from the ground level at two points along the center line of each row space for counting conidia during the inoculation.

As shown in Table 2, percent infection of seedlings at different ages was highly significant. The 6-day old seedlings were shown to be most susceptible with a mean of 88.9% infected plants, followed by the 8- and 10-day old seedlings. Distance between spreader rows also gave significant F values showing the highest mean infection at 3.75 m. No significant differences were observed among the different durations of exposure. This may be due to the occurrence of strong rain and wind from the second to the fifth day of exposure. Dew is the most essential factor for sporulation, and also, for spore germination[3, 6, 7], but on windy nights, there may be no dew, hence no sporulation[8, 9]. Therefore, for windy nights during the exposure period, this factor must be considered. Also, as to the effect of conidial density on infection under field conditions, Exconde (1974) showed that 100% infection occurred 6 days after the highest spore counts (5,522 conidia/4.8 cm^2 = 115/10 mm^2), but conidial density of 400–4,000/4.8 cm^2 (=8–83/10 mm^2) produced infection rates ranging from 21.5 to 100%.

Table 3 shows that no conidia were observed on the glass slide from the third to the fifth day of exposure, suggesting that

Table 2. Percent infection with downy mildew of a variety Cotabato for three different parameters

Spreader row interval(m)	Duration of exposure(day)	Seedling age (days after planting) 6	8	10	Mean
2.25	2	87.1	44.4	25.7	52.7
	4	78.1	36.5	47.5	54.0
	6	87.4	39.2	35.5	54.0
	Mean	84.2	40.0	36.2	53.5
3.75	2	96.9	59.6	41.2	65.9
	4	91.0	50.5	26.5	56.0
	6	88.7	44.4	38.2	57.1
	Mean	92.2	51.5	35.3	59.7
5.25	2	95.9	41.6	19.9	52.5
	4	86.5	32.3	34.4	51.1
	6	88.6	43.1	34.4	55.4
	Mean	90.3	39.0	29.6	53.0

Table 3. Number of conidia counted in 10 × 1 mm² graduations on glass slides placed at two points in the middle of spreader row spaces from January 3 to 8, 1978

Night	Point*	Spreader row space (m)			Remarks
		2.25	3.75	5.25	
1st	1	11.3	7.3	10.0	Fine weather
	2	11.7	13.3	8.7	Dew on glass slides
	Mean	11.5	10.3	9.4	
2nd	1	—	—	—	No count, because
	2	—	—	—	of rain and wind
	Mean	—	—	—	
3rd~5th	1	0.0	0.0	0.0	Successively
	2	0.0	0.0	0.0	strong wind
	Mean	0.0	0.0	0.0	No dew on glass slides
6th	1	33.0	32.7	16.0	Fine weather
	2	48.3	41.7	7.7	Dew on glass slides
	Mean	40.7	37.2	11.9	

* A set of three graduated glass slides were placed on a stand at a height of 8 cm from the ground level at two points along the center line of each row space. Numbers of conidia counted on three glass slides were averaged.

the treatments of 2- or 4-day exposure received conidia only on the first night. On the contrary, the 6-day exposure treatment received conidia on both the first and sixth nights. However, there were no significant differences among the three exposure periods. This indicates that a density of about 10 conidia/10 mm² on the first night might be enough for the inoculation.

The third experiment was conducted to compare and correlate the effectiveness of spreader rows and whorl drop methods of inoculation. Varieties and inbred lines shown in Table 4 were used as test materials, and inoculation was done at two different seedling ages, 8 and 11 days after planting, with three replications. Seeds of each variety were planted in separate nursery boxes on December 23 and 26, 1977, to prepare seedlings of diffent ages. For the spreader row method, the nursery boxes were placed in the middle of row space (3.75 m) on January 2, 1978, with the exposure period of 4 days. In the whorl drop method, the seedlings were inoculated with a suspension of 25,000 conidia per ml on the early morning of January 3.

As shown in Table 4, the spreader row method caused infection rates averaging 67.1 and 36.5% for the 8- and 11-day old seedlings of all varieties, respectively. The whorl drop method gave comparatively lower infection rates, 54.8 and 24.0%, respectively. Significantly high correlations were obtained between the two methods in evaluating the resistance of the different varieties, especially when inoculated at the earlier stage. These results, therefore, indicate that this improved spreader row method, with all its practical advantages, is a reliable technique which can be used in screening genotypes for the resistance to downy mildew.

Thus, it was found that this improved method can be practically and effectively used for screening the resistance to downy mildew. The advantage of this method is that each and every plant is provided with a more or less equal dose of the inoculum, without labors and inconveniences required for pre-

Table 4. Percent infection with downy mildew of ten varieties and inbred lines inoculated by spreader row or whorl drop methods at different seedling ages

Variety and inbred	Seedling age (days after planting)			
	8 days		11 day	
	Spreader	Drop	Spreader	Drop
Phil. DMR Comp. 1	7.1	16.0	1.0	2.9
Phil. DMR Comp. 2	8.9	10.9	0.0	4.1
Improved Tiniguib	37.2	9.7	16.0	22.6
Cotabato	59.4	42.9	17.9	32.3
Mimies	85.8	74.2	14.3	26.0
UPCA Var. 1	100.0	95.7	78.9	48.9
UPCA Var. 3	90.6	76.3	86.3	25.6
Ph9 DMR	91.5	63.8	22.6	13.9
EG 15B	98.9	90.1	70.3	40.7
Ph9 DMR × EG 15B	91.5	68.4	58.0	22.6
Mean	67.1	54.8	36.5	24.0
Correlation* coefficient	r=0.95 sig, at 0.001 level		r=0.78 sig. at 0.01 level	

* For the calculation of the correlation coefficients, percent infection was transformed into arc sin.

paring inoculum suspension and actual inoculation itself. Also, weather permitting, the spreader rows provide continuous supply of inoculum for several days. This method has already been adopted with success in some experiments conducted by the authors.

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Koji KANEKO *Tropical Agriculture Research center, Japan*

Bliss A. ADAY *Department of Agronomy, College of Agriculture, University of the Philippines*

(Presently, San Miguel Corporation, Philippines)