

## Methods for the inoculation of *Chrysanthemum morifolium* with *Puccinia horiana*

R. Zandvoort<sup>1</sup>, C. A. M. Groenewegen<sup>2</sup> and J. C. Zadoks<sup>2</sup>

<sup>1</sup>Plant Protection Service (PD), Wageningen

<sup>2</sup>Laboratory of Phytopathology, Wageningen

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No data were available on inoculation methods for *Chrysanthemum* white rust, *Puccinia horiana* P. Henn. Inoculations can be non-quantitative or semi-quantitative and they can be performed by dusting with dry spores or spraying with spore suspensions.

Non-quantitative inoculation was performed by placing two leaves with sporulating lesions on a support at ca. 10 cm above the plants. Plants and inoculum were surrounded by polythene plastic foil to ensure a relative humidity close to 100%. The test plants were exposed to the inoculum during 1, 2, 4 or 8 days. Good results were obtained independently of the exposure period.

In semi-quantitative experiments, the number of resulting lesions is related to the area of sporulating lesions used to prepare the inoculum. The efficiency of a method can be expressed as the number of lesions per plant obtained per cm<sup>2</sup> of sporulating leaf surface, taking into account the dilution factors.

Approximately 25 cm<sup>2</sup> of leaves with a sporulating area of ca. 40% was cut into small pieces. The pieces were put into glass tubes containing 25 ml tap water. The tubes were placed in an ultrasonic vibrator and treated during 30 or 60 sec at 20 kHz. Leaf fragments and the supernatant suspension were separated by decantation. The spore-containing suspension was diluted by tap water up to volumes of 0.4 or 2.0 l. Plants were inoculated by spraying the spore suspensions over the leaves until run-off, using a De-Villbiss no. 4 sprayer.

Sporulating lesions were prepared as described above and put in a Bühler mixer with tap water. The mixture was treated at ca. 50,000 r.p.m. during 10, 30 or 60 sec. The suspension was diluted with tap water up to volumes of 0.2 or 1.0 litre. The spore-containing suspensions were used for inoculation as above.

Dry spores were obtained using a cyclone collector. Spores were sucked away from ca. 25 cm<sup>2</sup> sporulating leaf area and mixed with two parts of talcum powder. Plants to be inoculated were placed at the bottom of a sedimentation tower of 3 m height and 40 cm diameter. The spore-containing dust was blown in at the top of the sedimentation tower using a De-Villbiss powder blower. Plants were exposed to the sedimenting spore cloud for 10, 30 or 60 min.

Each treatment included five potted plants of cv. 'Juweeltje' with about eight leaves.

Table 1. Mean number of sporulating and necrotic lesions per plant, 3 weeks after inoculation

<i>Method</i>	<i>Time of treatment</i>	<i>Dilution (l)</i>	<i>Number of lesions (sporulating + necrotic)</i>	<i>Efficiency</i>
Ultrasonic vibrator	30 sec	0.4	0	0
		2.0	0	0
	60 sec	0.4	0	0
		2.0	0	0
Mixer	10 sec	0.2	1.4	1
		1.0	0.4	2
	30 sec	0.2	71.8	57
		1.0	33.8	135
	60 sec	0.2	45.2	36
		1.0	17.2	69
Cyclone collector*	10 min	—	26.2	1
	30 min	—	16.6	0.7
	60 min	—	30.8	1.5

\* Time of treatment is duration of placing in sedimentation tower

Tabel 1. Gemiddeld aantal sporulerende en necrotische vlekken per plant, 3 weken na de inoculatie

After treatment the plants of all three treatments were placed in a greenhouse at ca. 23°C. During the first 24 h after inoculation the plants were placed under a black polythene plastic foil in order to keep the relative humidity at about 100% and to exclude light (Yamada, 1955).

Owing to a mechanical defect between the third and eighth day after inoculation the temperature was during short periods over 30°C. The inoculation result was determined by adding together the numbers of sporulating and necrotic lesions (cf. Zadoks et al., 1967), see Table 1.

Results with the semi-quantitative methods were the following. Ultrasonic vibration was ineffective in producing good spore suspensions. Inoculations both by mixer-made suspensions and by cyclone-collected dusts were effective. With mixer-made suspensions, the 30 sec treatment was most effective. Curiously, the numbers of lesions counted were not proportional to the dilution factors used. Results with spore powder dusting were variable with regard to exposure time.

Finally, the efficiency (E) of the inoculation was calculated according to the formula:

$$E = \frac{N}{I} \cdot \frac{V}{v}$$

when N is the number of lesions per plant after inoculation, I the surface area of leaf used for the preparation of inoculum, V the total volume of suspension, and v the quantity of suspension used.

In dusting, all the inoculum from 25 cm<sup>2</sup> leaves with sporulating lesions is used, but only about 10 ml of spore suspension was needed to reach the run-off point. Computed on this base the efficiency of the spraying method is markedly high. Spraying a mixer-made spore suspension is also the easiest of the methods tested.

Further, it appeared that the best results were obtained with leaves with young sporulating lesions.

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## Samenvatting

### *Methoden voor het inoculeren van Chrysanthemum morifolium met Puccinia horiana*

Enige niet-kwantitatieve en semi-kwantitatieve inoculatiemethoden werden onderzocht. Onder niet-kwantitatieve inoculatie wordt verstaan het aanbrengen van blaadjes met sporulerende soren boven de te inoculeren planten; semi-kwantitatief zijn inoculaties waarbij de sporen van een bekende hoeveelheid sporulerend bladoppervlak in suspensie verspoten of als poeder verstoven worden over de te inoculeren bladeren. De efficiëntie van de inoculatie werd berekend als het aantal bladvlekken per plant verkregen per cm<sup>2</sup> sporulerend uitgangsmateriaal, waarbij rekening werd gehouden met de verdunningsfactor (zie Tabel 1). Sporensuspensies werden gemaakt met een ultrasone vibrator en met een huishoudmixer. Sporenpoeder werd verzameld met een cycloon. De mixer-methode is de meest efficiënte; bovendien is deze het eenvoudigst in de uitvoering. Het gebruik van bladeren met jonge sporenhoopjes verdient hierbij de voorkeur.

## References

- Yamada, S., 1955. Experiments on the epidemiology and control of Chrysanthemum White Rust, caused by *Puccinia horiana* P. Henn. Ann. Phytopath. Soc. Japan 20 : 148-154.
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