Protocol to Evaluate the Metalaxyl Sensitivity Level in Isolates of *Plasmopara halstedii*

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Observations in France in 1995 (Lafon *et al.*, 1996) of *Plasmopara halstedii* isolates exhibited atypical response to metalaxyl led us to proposed a protocol for determined the metalaxyl sensitivity level of these isolates. This protocol which took account both aerial contamination and infection through roots was described in this paper.

Sunflower

Sunflower genotype used was a population cultivar Peredovik susceptible to all known French downy mildew races.

Fungus

Plasmopara halstedii isolates used as inoculum were :
- the A isolate, sensitive to metalaxyl, collected in 1988 (Tourvieille *et al.*, 1988) and maintained on EL64 seedlings,
- the ApR2 isolate, collected in 1995 (Penaud *et al.*, 1997) and showing a reduced sensitivity to metalaxyl. This isolate was maintained on Peredovick seedlings treated with the registered rate of metalaxyl : 2100 mg active ingredient (a.i.) per kg of seed.

Chemicals

Chimicals used in this study were Apron 35 (35 % of metalaxyl a.i.), Apron without metalaxyl (=charge) supplied by Ciba-Geigy.

Seed treatment

The Peredovik seeds used were free of chemicals. In order to determine the EC50, it was necessary to test a range of different metalaxyl rates. So seven metalaxyl rates were prepared : - 21 000 mg a.i. kg^{-1} = 10 g Apron 35 + 50 ml distilled steril water

- 2 100 mg a.i. kg^{-1} = 5 ml of 21 000 dose + 45 ml of charged water (13 g of charge per litre)
- 210 mg a.i. kg^{-1} = 5 ml of 2 100 dose + 45 ml of charged water (13 g of charge per litre)
- 21 mg a.i. kg^{-1} = 5 ml of 210 dose + 45 ml of charged water (13 g of charge per litre)
- 2.1 mg a.i. kg^{-1} = 5 ml of 21 dose + 45 ml of charged water (13 g of charge per litre)
- 0.21 mg a.i. kg^{-1} = 5 ml of 2.1 dose + 45 ml of charged water (13 g of charge per litre)
- 0 mg a.i. kg^{-1} = 30 ml of charged water (13 g of charge per liter)

The quantity of water required to treat 100 g of Peredovik seeds was 30 ml. This volume allowed a good dressing of seed. The seeds were treated in a glass jar, shaken for a time ensuring complete fungicide cover and dried at room temperature. Treated seeds were then kept in a glass jar in the dark at 12°C.

Artificial infecion through roots (Fig. 1)

Seeds were allowed to germinate in sterile moist filter paper in a saturated atmosphere in the dark at 25°C for 48 h. They were then inoculated with *P. halstedii* using the method described by Cohen and Sackston (1973). For each metalaxyl rates, series of 20 germinated seeds with 3- to 15- mm-long- rootlets were placed in pill boxes and just covered with a suspension of 10^5 zoosporangia per milliliter of distilled water for 5 h in the dark at 18°C ± 1. Untreated seeds were infected as the same in order to check the success of contamination. Each series was then planted in 1 liter pots filled with soilless compost (16 seedlings per pot with undamaged root initials). The remaining suspension (zoosporangia + diluted fungicide) was used to water the pot.

The seedlings were then placed in a growth chamber at 18°C ± 1, with a relative humidity of 70%, a photoperiod of 16 h and a light intensity of about 200 µE m^{-2} s^{-1}. The light was provided by 250 watt Osram mercury vapour lamps.

Pots were individually sprayed with 50 ml of water after 48 h, and 100 ml every 4 days. These volumes didn't lead fungicide loss by percolating.
After an abundant watering, 11-day-old infected seedlings were covered with transparent polythene bags to provide a saturated atmosphere. Three days later, the percentage of seedlings bearing zoosporangia on their cotyledons and/or their young leaves was noted (Table 1).

Artificial aerial contaminations (Fig. 2)
Peredovik seeds were metalaxyl-treated and allowed to germinate as previously. For each metalaxyl rate, 8 seedlings were planted in 1 liter pots filled with soilless compost. After the first pair of leaves had expanded (15 days after planting), a suspension of 5.10^4 zoosporangia per milliliter of distilled water was applied on leaves with a very small spray so as to distribute inoculum homogeneously. The amount of spray was such as to completely cover the whole shoot without dripping. The pots were then covered with transparent polythene bags to maintain a saturated atmosphere for 48 h. Thirteen days after infection, after 24 h in a saturated atmosphere, the percentage of seedlings bearing chlorotic spots and zoosporangia on the leaves was noted (Table 2).

Calcul of EC50
As primary and secondary infections data are proportions of plants showing sporulation, data were analysed by a generalised linear model (glm), using the canonical links and variance functions for binomial distribution. This method is also called the log-logistic regression, since the metalaxyl concentrations are transformed in a log-scale. A dose-response model is thus fitted to observed data. Approximate confidence intervals of EC50 were computed according to Collett (1991). For primary infections, the EC50 of ApR2 isolate was 12800 (confidence interval : 10 000 < 12 800 < 16 400) mg.a.i. metalaxyl kg^{-1} whereas that of sensitive isolate was 22 (19 < 22 < 27) mg a.i. metalaxyl kg^{-1}.
For aerial infections, the EC50 of A isolate was 67 (45 < 67 < 99). This concentration could not be calculated with reliability for the ApR2 isolate because 91% of seedlings showed disease symptoms with the 21 000 mg a.i. kg^{-1}.

Accuracy of this proposed protocol (Fig. 3)
It was estimated from 10 replicates of 16 sunflower seedlings (cv Peredovik) treated with the seven metalaxyl rates and infected by the A and ApR2 isolates. This test was reliable because we observed, on average, 98

Figure 1. Test using primary infection to determine the level of metalaxyl sensitivity of *Plasmopara halstedii* isolates.

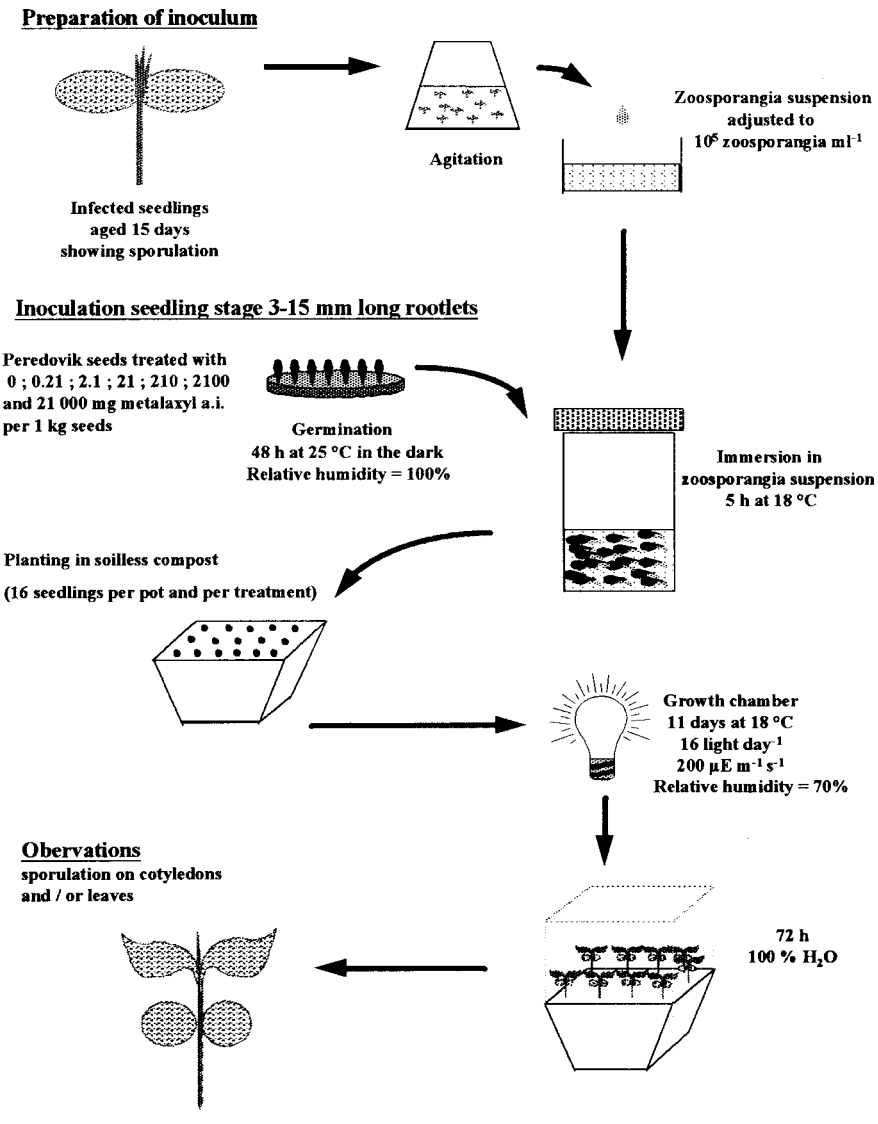


Figure 2. Test using aerial infection to determine the level of metalaxyl sensitivity of *Plasmopara halstedii* isolates.

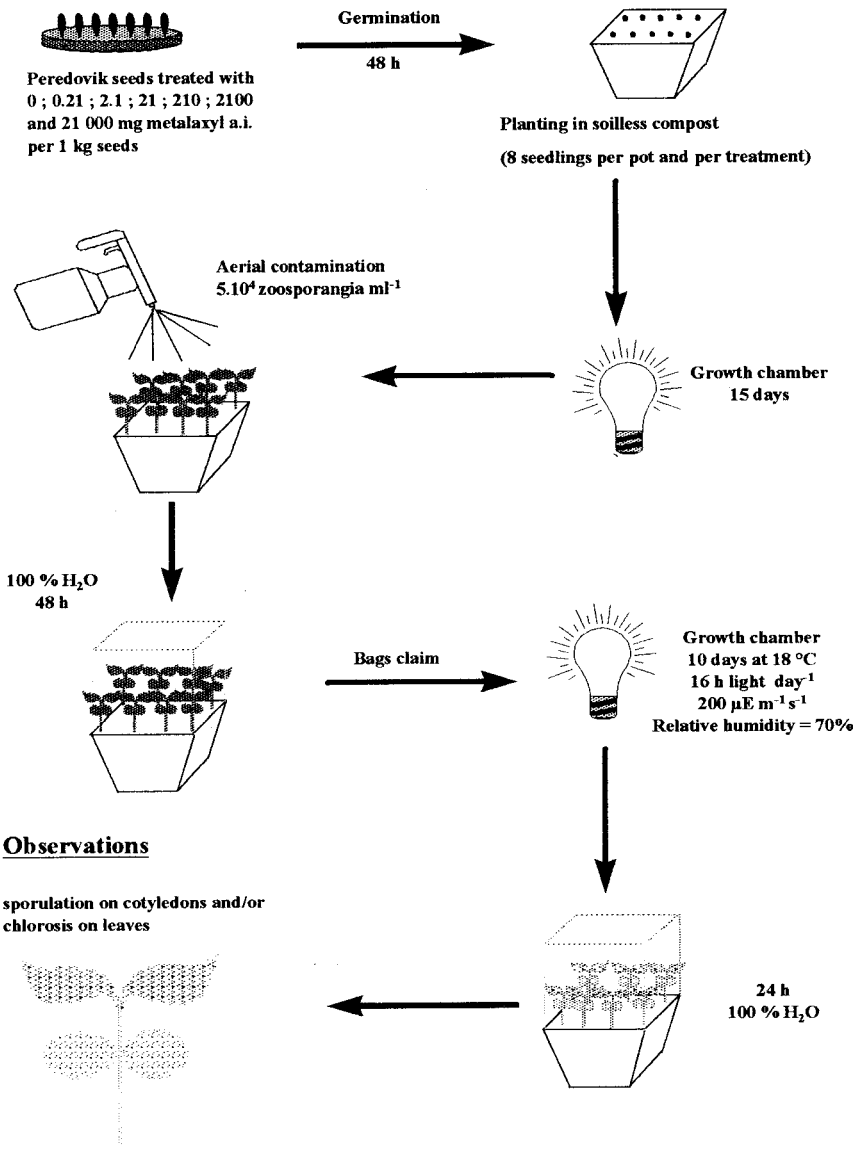


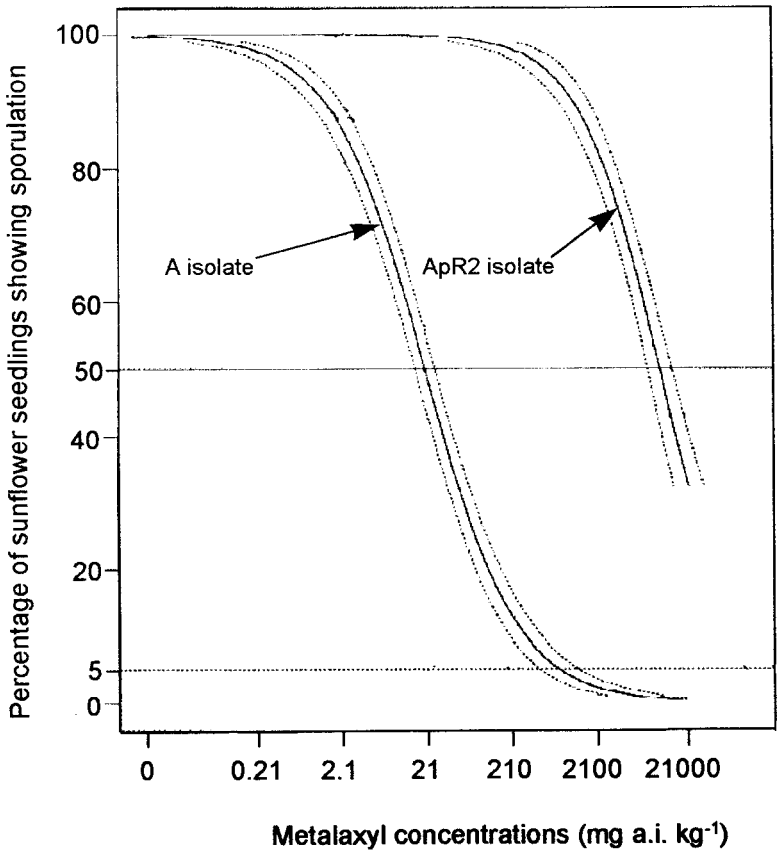
Table 1. Mean rates (± standard deviation) of sunflower seedlings (cv. Peredovik) treated with seven metalaxyl rates, showing sporulation after primary infections (mean calculated on 10 replicates of 16 seedlings) of A and ApR2 *Plasmopara halstedii* isolates.

	Untreated control	Metalaxyl rates (mg a.i. kg^{-1})						
		0	0.21	2.1	21	210	2 100	21 000
A isolate	98.0 ± 4.5	95.6 ± 5.9	98.0 ± 3.2	86.9 ± 14.3	51.6 ± 28.3	7.0 ± 10.9	0.0	0.0
ApR2 isolate	100.0	100.0	100.0	98.8 ± 3.9	98.1 ± 5.9	98.8 ± 2.6	91.3 ± 12.9	20.8 ± 21.1

Table 2. Mean rates (± standard deviation) of sunflower seedlings (cv. Peredovik) treated with seven metalaxyl rates, showing sporulation after aerial contaminations (mean calculated on 7 replicates of 8 seedlings) of A and ApR2 *Plasmopara halstedii* isolates.

	Untreated control	Metalaxyl rates (mg a.i. kg^{-1})						
		0	0.21	2.1	21	210	2 100	21 000
A isolate	100.0	98.2 ± 4.4	98.2 ± 4.4	98.2 ± 4.4	88.7 ± 12.5	17.8 ± 45.8	0.0	0.0
ApR2 isolate	100.0	100.0	100.0	100.0	98.2 ± 4.4	100.0	98.2 ± 4.4	91.1 ± 17.3

Figure 3. Percentage of sunflower seedlings treated with seven metalaxyl concentrations, showing sporulation after primary infections with A and ApR2 *Plasmopara halstedii* isolates. (.... correspond to confidence interval at P=0.05).



% of untreated seedlings showing sporulation with A isolate and 100 % with ApR2 isolate. Confidence intervals showed that the distance between upper limit and lower limit was the same in all spots both of the A and ApR2 curves. So, this protocol gived weak variability in results. Thus, it was relatively precise.

Acknowledgements
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