# Sunflower downy mildew: symptomatology, epidemiology and economic risks of secondary infection

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Summary:

**Secondary infection** of downy mildew caused by *Plasmopara halstedii* may take place both in roots and shoots of sunflower plants. The aim of this study is to evaluate symptom development according to site of infection, plant stages, sunflower genotype, and seed dissemination. The site of infection was studied on the susceptible hybrid, Airelle, grown under controlled conditions or under netting cages in the field and in a leaf disk test. Symptom progression was studied at several growth stages of several sunflower hybrids. Inoculation was made with an inoculum of fresh zoosporangia of race 710 with a micropipette. Percentage successful infection decreased with vegetative stage, but infection is possible up to flower bud stage. With the leaf disk test, infection can take place on upper or lower sides of mature leaves, but in *situ*, infection was only observable when inoculum was placed on summit of the shoot or flower bud. The variety effect analysed on six hybrids showed important qualitative rather than quantitative differences in symptom progression. This study also showed that contaminated seed harvested from diseased plants after secondary infection can transmit the disease. A method of seed evaluation is proposed.

**Key words: artificial and natural infection, *Plasmopara halstedii*, seed, dissemination**.

Résumé **:**

Les infections secondaires du *Plasmopara halstedii*, agent mildiou du tournesol, peuvent se produire sur les racines et sur les parties aériennes de la plante. Cette étude a pour objectif d'évaluer l'évolution des symptômes en fonction du site d'infection, du stade de la plante, du génotype et d'apprécier les risques de dissémination du pathogène. Les études sur les sites d'infections et sur les stades végétatifs ont été réalisées sur la variété Airelle, en chambre de culture, en plein champ sous tunnel en filet et sur rondelles de feuille en laboratoire. Les infections sont réalisées en déposant une suspension fraîche de zoosporanges de la race 710 à l'aide d'une micro-pipette. Les taux d'infections réussies diminuent avec l'âge de la plante mais la contamination reste possible après le stade « bourgeon étoilé ». Sur rondelles de feuille en laboratoire, la pénétration peut se faire sur la face supérieure ou inférieure des feuilles mâtures. Par contre, *in situ*, l'infection ne s'observe que sur les feuilles juvéniles et sur bourgeon floral. L'effet variétal analysé sur six hybrides montre des différences importantes dans l'évolution des symptômes tant au niveau qualitatif que quantitatif. Enfin, nos expériences ont montré que les semences contaminées récoltées sur des plantes montrant des symptômes d'infection secondaire de mildiou pouvaient disséminer la maladie. Une méthode d'analyse du risque est proposée.

**Mots clé : infection artificielle et naturelle, *Plasmopara halstedii*, semences, dissémination.**

## Introduction

Sunflower downy mildew can be caused by both primary and secondary infections with *Plasmopara halstedii* (Farl) Berl. & de Toni. The first is an infection from overwintering oospores (sexual form of the propagule) and essentially takes place on roots in the spring (Allard, 1978). The second is caused by zoospores which are formed by asexual propagation, and can take place on both roots and shoots. These infections can occur simultaneously. According to Allard (1978) primary infection always gave a systemic stunted symptom. Secondary infection, not only produces the same symptoms as primary infections, but also produces contaminated seed (Meliala and Tourvieille, 1998).

In the field, farmers grow many different hybrids varieties and new races of downy mildew may appear in a country or area where they were never observed before. It was suggested that the occurrence of new races in Europe may have been introduced on imported seed rather than developing from a mutation (Tourvieille *et al.*, 1996).

To understand more about the economical consequences of secondary infection, we report here progression of downy mildew symptoms according to different sunflower genotypes, site of infection, vegetative stages, and disease dissemination.

## Material and methods

In this study, artificial infections were conducted in a growth chamber and in 7 m x 9 m netting cages in the field. In addition, a natural secondary infection was observed in the field.

Materials:

The old downy mildew susceptible sunflower variety, Airelle, and experimental hybrids resistant to race 100 coded A1 to A10 were used.

The fungal inoculum used was downy mildew race 710 of *P. halstedii* (Tourvieille, 1999) freshly prepared. Inoculum of 100 µl or 200 µl containing 100 000 zoosporangia per ml for artificial inoculation and 20 µl of 50 000 zoosporangia for leaf disk test, were used.

Method:

*1.* ***Artificial secondary infection***

To study age effects, the inoculum was placed with a micropipette on top of the shoot of 7, 14, 28 and 42 day-old plants (cotyledon stage, two pairs of leaves, 5 to 6 pairs of leaves and flower bud). Infection sites of *in situ* test were 1) top of the shoot, 2) upper side and 3) lower side of the upper leaves of 28 day-old of Airelle. Infection sites of the leaf disk test were upper and lower side of mature leaves of 28 day-old and 42 day-old plants.

*2.* ***Natural secondary infection***

Trials were sown in a previously infested field. Seeds were sown at double density and thinned to 60 000 plants per hectare.

*3.* ***Seed dissemination***

Contaminated seeds from three capitula or more were mixed and then treated as follows: 3 days in refrigerator (-5°C), 4 days at 4°C, and 7 days in refrigerator (-5°C). About 150 seeds were sown in a tray of 45 x 30 cm filled with compost and grown for 4 weeks. Mineral nutrition was added only if necessary in second plantation and so on. To induce fungal sporulation, plants were covered with tranparent polythene bag for 48 hours. If no sporulation was observable on the seedlings, the compost was replanted with other samples of the same seeds.

Growth chamber experiment:

Except for seed dissemination, all experiments were conducted with a factorial block design with 3 replications. In each replication of *in situ* test, 8 plants samples and two controls were used. In the leaf disk test, ten leaf disks from each leaf side or genotype were placed in a Petri dish containing a tissue paper imbibed with demineralized water to constitute each block.

Netting cage experiments:

The same experimental design as in the growth chamber was applied. Three netting cages served as blocks which were divided into two plots with an area of 4.5 m x 7 m. In each plot, there were 8 rows with a distance 75 cm. In each row plants were separated by 20 cm distance. Ten plants in each row were sampled for the infection. The border plants were used as controls.

Observation:

Percentage of successful infection was counted in all **artificial infections**. For **natural secondary infection**, three successive observations were made at two pairs of leaves, 4 to 6 pairs of leaves and flower bud stage. At two pairs of leaves, all diseased plants were counted, whereas in the following observations, plants without systemic symptom were noted.

For seed dissemination, the number of seed planted and germinated and the number of plant showing sporulation was counted.

Data treatments:

Data were analysed with Statgraph plus version 7. For percentage of successful artificial infection, data were transformed using arcsin. Mean differences were compared with Newman-Keuls test of the same programme.

## Result

Percentage of successful infection:

Table 1. Percentage of diseased sunflower after artificial infection of downy mildew in two experiments. Means of three replications

| Plant age (days) | Mean of percent of successful infection (%)* | |
|---|---|---|
| | Growth chamber | Netting cage |
| 7 | 90.27 **a** | - |
| 14 | 79.16 **ab** | 18.33 **b** |
| 28 | 56.94 **b** | 39.17 **a** |
| 42 | 13.89 **c** | 3.33 **c** |

Note: * = observed non-transformed data
Figures with the same letter were not significantly different according to Newmann-Keul test ($P < 0.05$)

Percentage of successful infection was affected only by vegetative stage both in the growth chamber and in netting cages. Younger plants gave a higher percentage than the older ones (Table 1). Genotype differences were not significant (data not presented).

Site of infection:

Inoculum placed on the top of the shoot of *in situ* test gave downy mildew symptoms, none developed when inoculum was placed directly on the leaf surface. In the leaf disk test, inoculum placed both on upper and lower sides of the leaf surface gave sporulation but on only 16 out of 660 disks and no statistical analysis could be made.

Symptom progression:

Table 2. Number of plants without systemic symptoms after second plantation (from 30 plants per rows) in netting cages plot. Means of three replications

| Hybrid | Plant stages | |
|---|---|---|
| | 4-6 pairs of leaves | flower bud |
| | Mean of normal growth plant | Mean of normal growth plant |
| A2 | 26.67 **a** | 22.00 **a** |
| A4 | 23.16 **b** | 19.08 **b** |
| Airelle | 11.17 **c** | 5.00 **c** |

Figures with the same letter were not significantly different according to Newmann-Keul test ($P < 0.05$)

The effect of sunflower genotypes on symptom progression was clearly observable three weeks after inoculation in growth chamber. All 7 day-old infected plants gave systemic stunted symptoms, but leaves of Airelle only begin to collapse (16%), there was leaf chlorosis (33%) on A3, and scab-like symptoms (75%) on A5. In 14 day-old plants, there was 53%, 16%, and 63% of systemic symptoms in Airelle, A3 and A5 respectively. In natural infection, more than 90 per cent of plants were contaminated at the 2 pairs of leaves stage. Nevertheless, at fourth to sixth leaf stage and flower bud stage, the number of A2 and A4 plants without systemic symptoms were significantly higher than in the case of Airelle (Table 2).

Seed dissemination:

Table 3. Number of germinated and sporulated plants after first and second plantations of downy mildew contaminated seed.

| Hybrids | Number of plants | | | | | |
|---|---|---|---|---|---|---|
| | First planting | | | Second planting | | |
| | planted | germinated | sporulated | planted | germinated | sporulated |
| Airelle (healthy) | 80 | 80 | - | 80 | 80 | - |
| Airelle (diseased) | 160 | 137 | - | 120 | 97 | 16 |
| A6 | 122 | 97 | - | 208 | 187 | 52 |
| A7 | 165 | 156 | - | 140 | 114 | 1 |
| A8 | 165 | 160 | - | 140 | 128 | - |
| A9 | 165 | 122 | - | 140 | 119 | 2 |
| A10 | 165 | 118 | - | 140 | 103 | 1 |
| A10 | 165 | 134 | - | 165 | 138 | 5 |

Infected seed germination varied from 71 to 95%. In the first plantation, no sporulation of the fungus was observed even after all plants were harvested and placed in plastic bags. In the second plantation with inoculum which had not given symptoms for the first infection, artificial or naturally infected seed showed sporulation 28 days after sowing with percentages ranging from zero to 27.8%.

## Discussion

Percentage of successful infection:

The rate of succsessful infection decreased significantly with age (Table 1) and agrees with data from previous studies (Meliala and Tourvieille, 1998). In the growth chamber, the percentages of succsessful infections in two year experimentations are consistent, while in netting cages, it were lower than in previous studies. A lower level of natural humidity in 1999 may have affected the infection processus.

Symptom development:

The pathogen can penetrate and sporulate in mature leaves, in agreement with Sackston and Vimard (1988). In natural conditions, the most probable site of infection is where a small drop of water may persist such as juvenile leaves at the summit of the shoot and at the tip of leaves. Although mature leaf infection is possible, natural infection may only happen under similar humidity conditions to that in the leaf disk test. Qualitatively, progression of symptoms seems affected by sunflower genotypes. Slower progression of symptom or scab-like symptom in genotypes resistant to race 100 is evidence that their reaction to aggressiveness of the fungus was different from what may exist in Airelle. This phenomenon may correspond to a horizontal resistance in addition to vertical resistance as reported by Vear (1974).

Economic risks due secondary infection of Downy Mildew:

To calculate economic risks of downy mildew secondary infection, two factors must be considered : 1) sunflower genotype, and 2) vegetative stages at the moment of infection. Economically, study of different genotypes suggests that a variety resistant to race 100 could give a greater yield than those without any resistent gene. Those genotypes with the characters of the horizontal resistance might be used as a source of resistance to the downy mildew in the breeding programme. Nevertheless, if those genotypes resistant only to race 100 are commercialized and continuously grown by farmers in an area where a more virulent race is present, in the long run it could cause an epidemic of this race.

Aerial secondary infection not only reduces the number of plant at maturity when infection takes place on young plants, but also produces a reduced quantity of contaminated seed when infection takes place at flower bud stage. The latter may not have a great influence on yield, but it can cause spread of a more virulent race of the pathogen. Contaminated seed was proved as agent of dissemination and in agreement with the other observations (Anselme and Planque, 1972; Cohen and Sackston, 1974; Patil and Mayee, 1988).

To halt the dissemination of new races from one country to another through contaminated seed, it is advisable to evaluate all imported seed before commercialization in one country and the method of this study could be used.

## Conclusion

The principal sites of secondary infection of *Plasmopara halstedii* were on top of the shoot at any stage of the vegetation.

The economical risks of the downy mildew secondary infection can be divided into two categories: (i) reduction of yield and (ii) dissemination of new races of *P. halstedii* through contaminated seed.

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## References

Allard, C. 1978. Mildiou du tournesol *(Plasmopara halstedii)* (Farl) Berl. et de Toni). Recherches concernant les modalités de l'infection naturelle. Informations Techniques. CETIOM. 62: 3-10.

Anselme, C., Planque, J.P. 1972. Problèmes posés par la transmission du mildiou (*P. helianthi* Novot) par les semences. Informations Techniques CETIOM, 29 (IV): 61-66.

Cohen, Y., Sackston, W.E., 1974. Seed infection and latent infection of sunflowers by *Plasmopara halstedii*. Can J. Bot. 52: 231-238.

Meliala, C., Tourvieille de Labrouhe, D. 1998. Infections secondaires de mildiou sur tournesol : symptomatologie et épidémiologie. Les Rencontres Annuelles du CETIOM-Tournesol, Paris, 1er Décembre 1998.

Patil, M. A., Mayee, C.D., 1988. Investigations on Downy mildew of sunflower in India. Proc. of the 12th. Int. Sunfl. Conf. Novisad. Yougoslavia. July 25-29, 1988. p. 42-47.

Sackston, W.E., Vimard, B. 1988. Leaf disc immersion (LDI) inoculation of sunflower with *Plasmopara halstedii* for *in vitro* determination of host-pathogen relationships. Plant disease 72: 227-229

Tourvieille, J., Roeckel-Drevet, P., Nicolas, P., Tourvieille de Labrouhe, D. 1996. Characterisation of sunflower Downy mildew *(Plasmopara halstedii)* races by RAPD. Proc. of 14 Int. Sunflower Conf. II. Beijing/Shenyang, China, 12-20 June 1996. p. 781-785.

Tourvieille de Labrouhe D., 1999. La nouvelle nomenclature des races de *Plasmopara halstedii*, agent du mildiou du tournesol, appliquée aux races françaises. OCL 6 (3): 219-221.

Vear, F. 1974. Studies on resistance to Downy mildew in sunflowers. Six$^{th}$ Conf Int. Sunfl. 22-24 July. Bucharest - Romania. p. 297 - 302.