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RELATION BETWEEN DATE OF INFECTION OF SUNFLOWER DOWNY MILDEW (*Plasmopara halstedii*) AND SYMPTOMS DEVELOPMENT

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SUMMARY

Secondary infection of *Plasmopara halstedii* was studied at several growth stages under controlled conditions or under netting cages in the field. Infection was conducted by placing fresh zoosporangia of race 100 on flower bud or on top of shoot with a micropipette. Percent of successful infection, symptom development and agronomic characters of seeds were observed macroscopically. Progression of fungus in organs of infected plants after inoculation was observed microscopically.

Sunflower plants naturally infected by downy mildew were observed at flowering and classified into four categories according to the intensity of symptoms. Capitula of infected plants were harvested for agronomic character analysis. These results were compared with those obtained with artificial infections.

Secondary infection can occur on leaf tissues or on the flower bud; and symptoms after artificial infection on 28- and 42- day-old plants correspond with symptoms observed in naturally infested sunflower at flowering.

The economic risks of secondary infection of *P. halstedii* can be divided into three categories: 1) loss of plants infected at the age of 7 and 14 days, 2) plants infected at the age of 28 days may produce no seed, 3) plants infected at the age of 35 or 42 days may produce a reduced quantity of contamined seeds.

Key words: economy, downy mildew, seed, sunflower, symptoms

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INTRODUCTION

Plasmopara halstedii (Farl) Berl. & de Toni=*Plasmopara helianthi* Novot., the causal agent of downy mildew of sunflower, can infect plants by two means: primary and secondary infections. **Primary infection**, by definition, is an infection of plants after germination of resting spores (oospores) and it normally occurs on plants roots early in the season (Louvet and Kermoal, 1966; Delanoe, 1972; Wehtje and Zimmer, 1978). **Secondary infection**, on the other hand, is caused by an asexual propagules of the pathogen and can take place on both roots and shoots (Louvet and Kermoal, 1966).

Several authors have reported artificial methods of secondary infection (Gossen and Sackston, 1968; Cohen and Sackston, 1973; Sackston and Vimard, 1988).

The pathogen can cause different types of symptoms such as damping off (De Guenin, 1990; Gindrat *et al.*, 1998), systemic symptoms on leaves with sporulation of pathogen and stunted plants (Cohen and Sackston, 1974; Kurnik *et al.*, 1976; Delos *et al.*, 1997), leaf necrosis (Doken 1986, De Guenin, 1990), capitulum malformation (Kurnik *et al.*, 1976), and basal gall independent of systemic symptoms (Zimmer, 1973).

Downy mildew symptoms are usually used as an indicator to predict economic risks caused by the disease. Some estimations of losses due to downy mildew are presented in Table 1.

Table 1: Incidence of sunflower plants naturally attacked by downy mildew and the damage relative to the disease in several countries

Country	Year	Economic damage (%)	Author
Canada	1994	trace to 60%	Rashid, 1995
China	1986	4-15%	Shouen *et al.*, 1996
France	1997	0-70%	Perny, 1998
Hungary	1975	56%	Kurnik *et al.*, 1976
India	1984	up to 30%	Patil and Mayee, 1988
Maroc	1997	trace to 20%	Achbani *et al.*, 1998
U.S.	1970	to as high as 90%	Zimmer, 1971
	1981	50%	Carson, 1981

Surveys generally use observations of shoots only, with no information whether the damage was attributed to primary or secondary infections. To obtain an improved understanding of the economic risks caused by secondary infection of this pathogen, we report here symptom appearance and development produced by artificial secondary infections in comparison with natural secondary infections.

MATERIALS AND METHODS

In this study, artificial infections were conducted in growth chamber (Tourvieille *et al.*, 1988) and in 7 m x 9 m netting cages in the field (Tourvieille *et al.*, 1986) and a survey was carried in a naturally secondary-infested field.

Materials

The sunflower genotype used for artificial infection was the hybrid AIRELLE. Plants aged 7 days (cotyledons stage), 14 days (two pairs of leaves), 28 days (5 to 6 pairs of leaves), and 35 and 42 days (flower bud)** after germination were used. Nine naturally infested hybrids coded 8608, 8422, 8018, 7811, 7825, 8709, 7221, 7307, and 8513 were observed.

Inoculum

The fungal inoculum used was downy mildew race 100 (Tourvieille, 1999) freshly prepared following the method of Mouzeyar *et al.* (1993).

Inoculation was conducted by placing inoculum on flower bud or on top of shoots with a micropipette. On the 7, 14 and 28 day-old stage, the volume of inoculum was 100 µl containing 100 000 zoosporangia per ml, while on the flower bud the volume was 200 µl of the same concentration.

Growth chamber experiment

This experiment was conducted in randomized block design with 6 replications. For each replication in the block, 8 plants samples and two controls were used.

Netting cage experiments

Plants aged 14, 35 and 42 days after plant emergence were inoculated. Three netting cages (with 0.9 mm insect proof mesh, generally used as isolation plots) served as blocks and each block contained two plots each with an area of 4.5 m x 7 m. In each plot, 8 rows with a distance of 75 cm from each other were prepared for the experiment. In each row, plants were planted with 20 cm distance. Ten plants in each row were sampled for infection. The border plants were used as control. Capitula of infected plants were harvested.

Survey

A survey was made in a naturally secondary infested field located about 10 km from Clermont Ferrand. A survey was conducted at flowering. The plants were divided into four categories: plants without symptoms (0), local lesions with sporulation on leaves (1), a systemic-like symptom on leaves without any symptom

** CETIOM-tournesol 1997.

observable on the stem (2) and a systemic stunted symptom (3). In this survey, 5 plants of 9 genotypes of each category were harvested.

Observations

Percent of successful infection and the type of symptom appearance were observed macroscopically. Agronomic characters of the seeds produced in successful infection from the netting cage experiment and from survey were measured: capitulum diameter, seed number and 1000 seed weight. Progression of symptoms was observed microscopically on the plants infected in growth chamber experiments 15, 21 and 30 days after inoculation.

Data treatments

Data were treated with a Statgraph plus version 7 to obtain analysis of variance. For successful artificial infection, data were transformed using arcsin. Mean differences were compared with least significance difference (LSD) in the same program.

RESULTS

A. PERCENT OF SUCCESSFUL INFECTION

In the growth chamber experiment, the percentage of successfully infected plants was highest in young plants, 97.7% and 66.7% in 7 and 14 day-old plants, respectively, decreasing to 33.3% and 14.5% in 28 and 42 day-old plants, respectively. The results in the netting cages experiment indicated that the stage of the plant at the time of infection was not significant but there was more than 30% infection of flower buds of 42-day-old plants.

B. SYMPTOM DEVELOPMENT

1. Macroscopic observations

Infection on 7, 14 and 28 day-old plants produced stunting. These symptoms were observable three weeks after inoculation in both experiments. All the 7- and 14-day-old plants showed stunting symptoms; of the 28-day-old plants, some that were successfully infected did not produce the stunted appearance, the symptom on leaf was halted and gave a local lesion symptom only. This lesion, yellowish in color, tended to sporulate.

On 35- and 42-day-old plants, symptoms were observable macroscopically only 30 days after inoculation. The symptoms were atypical for downy mildew and the plant almost has a normal height. The capitulum was malformed with darker color than normal and the fertile surface did not turn downwards after seed development as in healthy plants. Sometimes, symptoms progressed along the stem up to 10 cm below the capitulum. The stem and the leaf on the affected zone were darker and the petiole was more fragile than in healthy plants. Stem diameter was slightly

reduced, but leaf size was the same as in healthy plants. When petioles were cut, there was a dark point at the site where the petiole was attached. Leaves with this symptom, when placed in a plastic bag under 100% humidity, did not sporulate. To differentiate from a systemic symptom with a stunted plant appearance produced from 7-, 14- and 28-day infections, the symptoms produced by 35- and 42-day infections were named late systemic symptoms.

2. Microscopic observation

Observed on cotyledons, zoosporangia germinated to give zoospores which produced a long germinating hyphae. The latter penetrated the tissue directly or passed through stomata openings. After penetration, the mycelia grew in extracellular cavities and developed haustoria which penetrated plant cells. From the point of infection on a leaf, the mycelium progressed to the stem through petioles. When the mycelium arrived in the stem, it developed in two directions both towards the apical meristem and towards the roots. The conducting tissues in the infected zone had dark color. Three weeks after inoculation of the 7- and 14-day-old plants, the mycelium was observed close to, but not in, the apical meristem. On 28 day-old plants, mycelium was found in the flower bud. After inoculation of the flower bud (35- and 42-day-old plants), the fungus was found to develop in all parts of the capitulum, and sometimes reached one or two upper leaves.

Observations of young seed showed that three possibilities can be encountered: (1) the mycelium penetrated all parts of the seed, (2) the mycelium penetrated the seed, but it did not reach the embryo and (3) seed escape infection. On mature seed, mycelium could be observed in the inner layer of pericarp and outside of the tegument, but it never passed into the interior part of the tegument. When the inside of the tegument was observed, no mycelium was seen.

C. RELATION BETWEEN SYMPTOMS OBSERVED AND YIELD

Seeds were only produced on 35- or 42-day-old infected plants. The comparison of mixed seeds obtained from infected plants and control is presented in Table 3.

The natural infection survey indicated that the plants with pronounced symptoms, systemic stunted plants (category 3) and a systemic like symptom on leaves without any symptom observable on the stem (category 2) reduced yield significantly whereas yield parameters were not affected by downy mildew if the infection only produced a local lesion on leaves (category 1) (Table 4).

DISCUSSION

A. PERCENT OF SUCCESSFUL INFECTION AND SYMPTOM DEVELOPMENT

The symptoms produced by artificial infection at all stages were comparable with symptoms observed on naturally infected plants. This agrees with Cohen and Sackston (1973) except that they found that systemic symptoms rarely occurred after the eight-leaf stage, whereas in our result (Table 2) there was 15% and 33%

successful infection at the flower bud stage in the growth chamber and netting cages, respectively.

The degree of successful infection decreased significantly with age in the growth chamber, but the difference was not significant under netting cages. This suggests that successful infection not only affected by plant age, but also by weather conditions at the time of inoculation.

Table 2: Percentage of diseased sunflower after artificial infection with downy mildew in two experiments (means of six replications)

Plant age (days)	Mean of percent of succesful infection (%)	
	Growth chamber	Netting cage
7	97.66 a	-
14	66.67 b	46.17 a
28	33.33 c	-
35	-	29.17 a
42	14.50 c	33.33 a

- = not observed, values with the same letter in the same column were not significantly different ($P<0.05$)

The present results show that the fungus can penetrate the leaf tissues or the flower bud. According to Veena Rao *et al.* (1998) zoospores that germinate on leaf tissue enter plant tissues through upper epidermis and initiate the process of infection.

Microscopic observation of systemic symptoms on leaves of 28-day-old plants showed that mycelium was only found in the petiole. Macroscopically, infection at this stage could produce stunted plants. This indicated that the stunted appearance was produced after the fungus arrived in the stem. Symptom development from flower bud infection confirmed this result. This observation may explain the different types of downy mildew symptoms encountered in naturally infected plants in the fields as cited in the introduction. This observation also gives a reason for utilization of category two in our survey observations.

B. RELATION BETWEEN SYMPTOM OBSERVED AND ECONOMIC RISKS DUE TO DOWNY MILDEW

The symptoms observed in naturally infected fields at flowering appear to correspond with those produced in mid-late and late artificial infections. Plants showing such symptoms at flowering may have been infected when aged between 28 and 42 days. If the plants were infected earlier, they generally died before observation.

Table 3: Comparison of sunflower capitula between controls and artificially downy mildew infected in netting cages (30 capitula)

Capitula	Capitula diameter (cm)	Number of full seed	Full seed (%)	1000 seed weight (g)
Diseased	15.67	118	24.5	40.263 a
Healthy	20.2	276.6	32.45	66.831 b

Values with the same letter in the same column were not significantly different ($P<0.05$)

The results presented in Table 3 indicated that yield decrease caused by downy mildew could follow infection of 35- and 42-day-old plants. The same result was obtained on naturally infected plants category 3 or category 2 (Table 4). These two data sets indicated that yield was affected by the stage of the plant at the moment of artificial infection or the degree of symptoms observed at the moment of survey.

Table 4: Comparison of sunflower capitula naturally infected based on symptom category (means of 5 plants per category of 9 genotypes)

Category	Capitula diameter (cm)	Number of filled seed	1000 seed weight (g)
Without symptom (0)	18.01 c	1586.06 c	55.03 c
Local lesion (1)	17.93 c	1623.92 c	57.63 c
Systemic symptom on leaf only (2)	15.30 b	1307.18 b	47.12 b
Stunted symptom (3)	9.90 a	695.06 a	17. 67 a

Values with the same letter in the same column were not significantly different ($P<0.05$)

Yield decrease caused by downy mildew may correspond to a reduced quantity of seeds as indicated by smaller capitulum diameter, number of filled seed and 1000 seed weight. The reduction in the number of full seed was probably correlated with the seed abortion observed in immature seed.

Based on plant stage at the moment of infection, three types of economic risks caused by downy mildew can be distinguished:

1. Reduction in the number of plant at maturity. This could happen after early secondary infection (14-days-old plants or less). The symptoms produced at this stage were similar to primary infection reported by Delanoe (1972);
2. Plants do not die, but can not produce any seed. This happens after infection of plants aged 28-days or less (mid-late secondary infection);
3. Plants can produce seeds but in a reduced quantity. This happens after infection of 35- and 42-day-old plants (late secondary infection).

Another economic risk of secondary infection is the dissemination of the disease by seeds. Observation of mature seeds showed that mycelium was present in the inner layer of the pericarp and the outside of the tegument in agreement with Doken (1989). Work on possibility of seed-borne propagules as a mode of dissemination is in progress.

CONCLUSION

Secondary infection of downy mildew can occur up to the flower bud stage and produce various symptoms.

The economic risks of downy mildew secondary infection depends on plant stage at the moment of infection.

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RELACIÓN ENTRE LA FECHA DE INFECCIÓN DE *Plasmopara halstedii* Y EL DESARROLLO DE SÍNTOMAS DEL MOHO DEL GIRASOL

RESUMEN

La infección secundaria de *Plasmopara halstedii* fue estudiada mediante la infección de plantas de varios estados de crecimiento en cámara de cría y bajo túneles de red. La infección se realizó colocando esporangios frescos de la raza 100 con una micropipeta sobre la yema floral o en la punta del tallo de las plantas. El porcentaje de infección exitosa, el desarollo de síntomas y caracteres agronómicos de las semillas fueron observados macroscópicamente.

Girasoles infectados naturalmente por el moho fueron observados al estado de floración y clasificados en cuatro categorías de acuerdo a la intensidad de los síntomas. Capítulos de plantas infectadas fueron cosechados para analizar caracteres agronómicos. Estos resultados fueron comparados con aquellos obtenidos con infecciones artificiales.

La infección secundaria puede ocurrir sobre tejidos de hojas o sobre la yema floral. Los síntomas en plantas de 28 y 42 días de edad, inoculadas artificialmente, son similares a síntomas observados en girasol infectado naturalmente al estado de floración.

El riesgo económico de una infección secundaria de *P.halstedii* puede dividirse en tres categorías: (1) pérdida de las plantas inoculadas cuando tenían 7 o 14 días de edad, (2) plantas que no producen semillas al ser inoculadas cuando tenían 28 días de edad, y (3) menor producción de semillas en plantas inoculadas cuando tenían 35 o 42 días de edad.

RELATION ENTRE LA DATE D'INFECTION ET LE DEVELOPPEMENT DES SYMPTOMES DU MILDIOU *(Plasmopara halstedii)* SUR TOURNESOL

RÉSUMÉ

L'infection secondaire de *Plasmopara halstedii* a été étudiée à différents stades sur des plantes élevées sous conditions controlée et sous tunnel en filet au champ. L'infection a été réalisée en déposant des zoosporanges frais de la race 100 sur le bouton floral ou sur l'apex de la plante avec une micropipette. Les observations ont porté sur les taux d'infection, le développement des symptômes et sur les caractères agronomiques des graines. Des observations microscopiques ont permis de suivre la progression du champignon dans les organes de la plante après l'inoculation.

Des tournesols infectés naturellement par le mildiou ont été classés en quatre catégories selon l'intensité des symptômes. Les capitules des plantes infectées ont été récoltés et les caractères agronomiques analysés. Les résultats obtenus ont été comparés à ceux des infections artificielles.

L'infection secondaire peut se produire sur feuille ou sur bouton floral. Les symptômes après infection artificielle sur des plantes âgées de 28 et 42 jours correspondent à ceux observés sur les plantes infectées naturellement.

Les risques économiques liés aux infections secondaires de *P.halstedii* peuvent être divisés en trois catégories: (1) perte des plantes infectées à 7 et 14 jours, (2) plantes infectées à l'age de 28 jours ne produisant pas de graines, (3) plantes infectées à 35 et 42 jours produisant des graines en moindre quantité et contaminée.