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Effect of environmental conditions and inocolum concentration on sporulation of *Peronospora destructor*

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Abstract. The effect of inoculum concentration, incubation time, relative humidity (RH) and temperature on sporulation of *Peronospora destructor* on onion leaves was analyzed under controlled environmental conditions in a greenhouse. The shortest period for spore germination and sporulation was on onion leaves inoculated with 10^6 spores/ml distilled water suspension. The intensity of infection and the sporulation of infected plants were affected greatly by the time and temperature of incubation. Exposure of inoculated plants at 15°C for 8 days and then at 22°C for 5 days resulted in the percentage of infection and the most abundant sporulation. Results of this experiment indicated that the period of *P. destructor* sporulation on one plant lasted 4–8 days.

Key words: incubation, onion, *Peronospora destructor*, relative humidity, sporulation, temperature

INTRODUCTION

Downy mildew on onion caused by *Peronospora destructor* (Berk.) Caspary, has a wide geographical distribution, which can be attributed to the adaptability of both host and pathogen to many types of climate, including temperate, tropical and subtropical zones (Palti, 1989). Onion cultivars differ greatly in their susceptibility to mildew, but no cultivar totally immune to the disease has yet been produced (Hubert & Kiessling, 1983). When weather conditions are favourable *P. destructor* sporulates prolifically on host leaves; the diseased plants become totally covered with spores, which may give rise to new rounds of infections on leaves and seed stalks (Mazelaitis & Stanevičienė, 1995). Cool temperatures, moderate relative humidity (RH), and low irradiance are more favourable for spore survival of *P. destructor* than are warm temperatures, high RH, and high irradiance (Hildebrand & Sutton, 1984b). Knowledge of the relationship between weather factors and sporulation is important for developing predictive models of downy mildew epidemics (Hildebrand & Sutton, 1984a). The most important factors affecting sporulation of the fungus on infected plants under controlled conditions are light, humidity, and temperature (Abd-Elrazik & Lorbeer, 1980). Relationships of weather variables and the infection cycle of *P. destructor* have been quantified in several studies. Even though the disease was first reported in Lithuania in 1928 (Mazelaitis & Stanevičienė, 1995), there is so far no information on the pathogen's sporulation under controlled conditions. The aim of the present study was to determine the effect of inoculum concentration, incubation time, relative

humidity (RH) and temperature on sporulation of *P. destructor* on onion leaves under controlled environmental conditions in a greenhouse.

MATERIALS AND METHODS

First experiment. Onion plants (cv. *Stuttgarter Rieser*) were grown in 0.5 kg plastic pots (two plants per pot) filled with garden soil in the greenhouse at the Lithuanian Institute of Horticulture in 2004. Spores of *P. destructor* were collected from surfaces of the onion leaves with a hairbrush and suspended in distilled water. Plants were inoculated with a fresh spore suspension (10^2, 10^3, 10^4, 10^5 and 10^6 spores/ml distilled water), applied with a hand atomizer (SOLO 454). Inoculated plants (20 plants for each suspension) were enclosed in polythene bags covered with black foil for the duration of 24 hr at 12°C, in order to maintain a high relative humidity, then returned to the light. Onions were grown at 15°C and the RH was 90% during seven days until the first disease assessment was carried out. At this time some leaves with spots were found and, on the following evening, all pots containing plants were sprayed with tap water and enclosed with black foil for 24 hours. Every morning the fresh spores which had formed during the night were rubbed with the brush to count the duration of *P. destructor* sporulation. Survival and infectivity of spores was assessed according to the number of leaves with sporulation.

Second experiment. To study the effect of incubation time and temperature on infection by *P. destructor*, onion plants were grown in the greenhouse and were inoculated with a spore suspension (10^6 spores/ml distilled water). Inoculated plants were incubated in a moist chamber at 15°C for 48 hr in darkness and then grown under a series of different temperatures in the greenhouse for 13 days. After the 13-day growth periods, sporulation was induced by placing the infected plants in a moist chamber at 12°C in darkness (overnight) for 12 hours. Twenty-five plants were used for each temperature treatment. Sporulation intensity was based on a scale of 0–4 (Žemės ūkio augalų kenkėjai, 2002). Each experiment was repeated twice with three replications. The data were analysed by 'ANOVA' statistical program. Duncan's test (at $P = 0.05$) was used for mean separation (Tarakanovas & Raudonius, 2003).

RESULTS AND DISCUSSION

First experiment. Hildebrand and Sutton (1984c) found that at high inoculum density the minimum latent period of infection was shortened and the time corresponding to the peak incidence of sporulating leaves was advanced; this resulted in a higher overall incidence of sporulating leaves. Our studies have shown that spores of *P. destructor* may survive for 4–8 days when environmental conditions are favorable. Sporulation on 10% of plants was seen after 7 days on onions inoculated with 10^6 spores/ml distilled water suspension; it was the shortest period of sporulation on leaves (Fig. 1). The longest period of sporulation, which lasted from 6- 8 days, was on leaves inoculated with suspension of 10^2 and 10^3 spores/ml of distilled water. On the basis of these results it can be concluded that the inoculum concentration seems to be an important factor influencing sporulation of *P. destructor* on inoculated onion leaves.

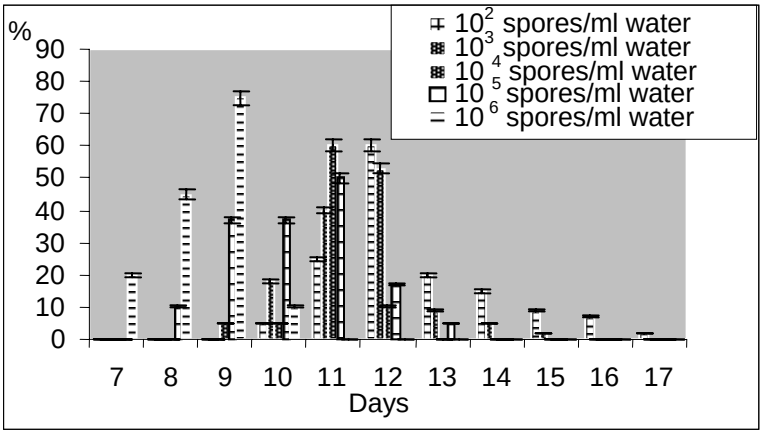

Fig. 1. The period of *P. destructor* sporulation on onion leaves.

Second experiment. Spores are able to survive under a wide range of weather conditions during the day but they are frequently killed at night due to alternating cycles of wetness and dryness, especially when these cycles are associated with low dew deposition (Maude, 1998). We found that temperature greatly affects the rates of sporulation as well as the survival of spores. The percentage of infection and the sporulation levels of *P. destructor* on infected plants were greatly affected by the time and temperature of incubation (Table 1). The highest percentage of infection (43%) and abundant sporulation (disease rate = 1.31) were observed on inoculated plants at 15°C for 8 days and then at 22°C for 5 days. The lowest percentage of plants infected and the lowest level of sporulation occurred on plants incubated at a continuous temperature (at 20°C) for 13 days in a growth chamber. The pathogen did not sporulate on plants incubated at a continuous temperature (at 22°C) for 13 days. Palti (1989) reported that the temperatures of 10–12°C are apparently optimal for most stages, 14–18°C is still quite favourable, but temperatures above 22°C rapidly become unfavorable, especially at lower humidity levels.

Table 1. Effect of incubation time and temperature on infection of *P. destructor* on onions 'Stuttgarter Rieser'.

Incubation time and temperature	Infection[a] (%)	Sporulation[b] (%)
10 days at 10°C +3 days at 22°C	31 b	0.19
8 days at 12°C +5 days at 22°C	36 b	0.27
8 days at 15°C + 5 days at 22°C	43 a	1.31
11 days at 18°C + 2 days at 22°C	19 c	0.2
13 days at 20°C	7 d	0.12
13 days at 22°C	0	0

[a]Means followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.
[b]Sporulation is rated on a scale of 0–4.

It has been shown by Viranyi (1988) that production, sporulation and germination of released sporangia and penetration of leaf tissues are favored by temperatures of 10–13°C, high relative humidity and prolonged periods of leaf wetness. Each of the stages of pathogen development (sporulation in all its phases, spore discharge, dissemination and survival, host invasion and colonization) has its own particular water relationship (Sutton & Hildebrand, 1985). The dynamics of most epidemics of foliar diseases are complex, and, in general, some facets are not well understood. Controlled conditions are required to induce sporulation in the greenhouse, especially in quantitative studies or when large amounts of inoculum are desired.

CONCLUSIONS

1. Inoculum concentration is an important factor influencing sporulation of *P. destructor* on inoculated onion leaves. The shortest period of spore germination and sporulation was 4 days on onion leaves inoculated with 10^6 spores/ml distilled water suspension.
2. The highest percentage of infection (43%) and the most abundant sporulation (grade = 1.31) were observed on inoculated plants at 15°C for 8 days and then at 22°C for 5 days.

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