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Production of conidia by Peronospora farinosa f. sp. spinaciae

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Abstract

Conidium production by *Peronospora farinosa* f. sp. *spinaciae* (pathotype 3) was measured daily on colonies induced on leaves of spinach cvs Breedblad Scherpzaad (BS) and Huro. Distinction was made between colonies grown at temperatures of 10 and 15 °C. Spore production was expressed as number of spores produced per stoma. There was a significant difference between total spore production on lesions on 'BS' at 10 °C and that on lesions on the three other cultivar-temperature combinations tested. Also a significant difference in the average sporulation period was observed between lesions produced at 10 and those at 15 °C. The same significant difference in response to temperature was found in the sporulation per stoma in the course of time.

Additional keywords: sporulation, infectious period, downy mildew, spinach.

Introduction

Downy mildews reproduce by means of oospores and conidia. During the crop-growing season several generations of conidia can develop, causing a rapid spread of the fungus if environmental conditions are suitable. The epidemic character of the disease is, among others, determined by the relative rate of spore production and the length of the sporulation period. It has never been observed that downy mildews caused by species of the genus *Peronospora* grow systemically, like downy mildews caused by species of f.i. the genus *Peronosclerospora* (Duck et al., 1987). So, the spore production on a lesion of non-systemically growing downy mildews is a main factor in calculations on the reproductivity of these fungi.

Cohen and Rotem (1971) studied the sporulation of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow in relation to lesion development on cucumbers. They estimated the production of sporangia per cotyledon in time at different temperatures. Maximum sporulation was observed three days after the first sporulation at high temperatures (20-30 °C), and 5 to 6 days after the first sporulation at low temperatures (10-15 °C). Inaba and Kajiwara (1971) measured the asexual sporulation of the same fungus on days 4-5, 7, and 11-12 after inoculation. Sporulation started on day 4 or 5 after inoculation, and maximum sporulation was reached on day 7 after inoculation. No indication was given about the sporulation period in the individual lesions.

Thomas (1970) demonstrated definite differences in sporulation potential of *P. cubensis* on two watermelon cultivars equally infected with the pathogen. Kato and Kozaka (1974) investigated the influence of temperature on sporulation by *Pyricularia*

oryzae Cav. and found that sporulation was maximal at 20 °C. The sporulation curves for the two lowest temperatures showed a relatively slow start with maximum sporulation between 9 and 13 days after first sporulation. The sporulation curves for the two higher temperatures showed a quick start with maximum sporulation only 3 days after first sporulation. In all cases the sporulation period lasted about 30 days.

The present paper aims at a more detailed investigation of the conidium production of *Peronospora farinosa* (Fr.) Fr. f. sp. *spinaciae* [Byford] to estimate the potential threat of the fungus at optimal temperatures. The greater and the longer the conidium production, the greater the epidemic increase supposing that all other factors are equal.

Materials and methods

Plant material. Two cultivars of spinach were used, viz. Huro (from Nunhem's Zaden B.V., Haelen) and Breedblad Scherpzaad (BS, from Rijk Zwaan B.V., De Lier). Both are susceptible to pathotype 3 of *P. farinosa* f. sp. *spinaciae*. In the Netherlands, 'BS' is recommended for the winter season in unheated glasshouses, and 'Huro' for early spring and late summer in the open.

Environmental conditions. The plants were grown in plastic pots placed in growth chambers at a constant temperature of 15 °C. After inoculation of the first true leaf pair, the plants of the two cultivars were evenly distributed in two growth chambers with temperatures of 15 °C (Huro-15 and BS-15) and 10 °C (Huro-10 and BS-10), respectively. The r.h. in the growth chambers was 70 to 80% and the daylength was 16 h. For initiating spore germination the pots were placed in closed plastic nursery trays during the first night after inoculation, and this was repeated at the seventh day. The r.h. in these trays was kept near saturation keeping water on the bottom of the trays.

Inoculation. The first true leaf pair was inoculated with a conidial suspension of pathotype 3 of *P. farinosa* f. sp. *spinaciae*. The spore density was 10^4 ml^{-1}. The inoculum was placed on the leaves in droplets (ca. 4 mm diam.) by means of a cotton swab (Rapilly, 1968). The average numbers of spores per droplet were 16.0 for 'Huro' and 16.3 for 'BS'.

Spore collection. Beginning with the first day of sporulation, spores of each individual colony were collected daily (Monday through Friday) by rolling a wet cotton swab along the colony surface. The collected spores were suspended in 2 ml deionized water. To assure the detachment of spores from the cotton swab, a Vortex vibrator was used.

Spore counting. Before counting, the spore suspensions were diluted 10 times with a NaCl solution of 8 g l^{-1} deionized water; 0.5 ml of the diluted suspension was used for spore counting with a Coulter counter.

As the sporophores of downy mildew grow through stomata, spore counts were related to the number of stomata present on the total lesion area on both sides of the leaf. The following formula was used:

$$SPS = [(TSL/14700) + (TSU/8100)]/LA$$

in which: SPS = spore production per stoma, TSL = total spore production of lower lesion surface, TSU = total spore production of upper lesion surface, LA = lesion area. 14700 and 8100 are the numbers of stomata per m^2 on lower and upper leaf surface, respectively (A.J. Termorshuizen, pers.comm.).

Statistical analysis. Curves, representing the cumulative spore production per stoma (SPS) for each individual lesion, were described by means of a function for growth curves (Corsten, 1985), which iteratively estimates the parameters of a function on its optimum value. This function is:

$$y = \left[y_1^b + (y_2^b - y_1^b) \cdot \frac{1 - e^{-a(i - t_1)}}{1 - e^{-a(t_2 - t_1)}} \right]^{1/b}$$

where y_1 and y_2 (spore production at beginning and end of the sporulation period respectively), and a and b (shape parameters) represent the parameters to be estimated, t_1 and t_2 the time in days of y_1 and y_2, and i the total production period in days. In our experiment, t_1 and y_1 equal 0; thus the function can be simplified into:

$$y = y_2 \cdot \left[\frac{1 - e^{-ai}}{1 - e^{-at_2}} \right]^{1/b}$$

In this function three parameters (y_2, a and 1/b) remain to be estimated. The estimates of y_2, a, 1/b, and the sporulation period (i) were subjected to an analysis of variance (ANOVA). Significance of differences between cultivars and temperatures was established by means of Student's t-tests.

Results

Spore germination. To determine the germination percentage of the spores, ten inoculations were done on each cultivar. The average percentage of spore germination was 68 on 'BS' as well as on 'Huro'.

Latency period. Sporulation was initiated from the seventh night onward. This led to a latency period of 7 days for five of the 29 colonies of the Huro-15 series. The latency period for all other colonies of the 15 °C series ('Huro' and 'BS') was 8 days, while that for those of the 10 °C series was 10 days.

Spore production. Spore counts were made of 102 colonies; 29 in the Huro-15, 21 in the Huro-10, 31 in the BS-15, and 21 in the BS-10 series. For all curves, representing the cumulative spore production for a colony per stoma in time, the three parameters y_2, a and 1/b were calculated. The average cumulative spore production per treatment was plotted against time (Fig. 1).

Spore production of Huro-15 started in the night preceding day 7, that of BS-15 in the night preceding day 8. The two series showed very similar exponential curves. Spore production began rapidly, reached its cumulative maximum in 12 to 13 days, and had an average of about 5.9 conidia per stoma. Neither total spore production per

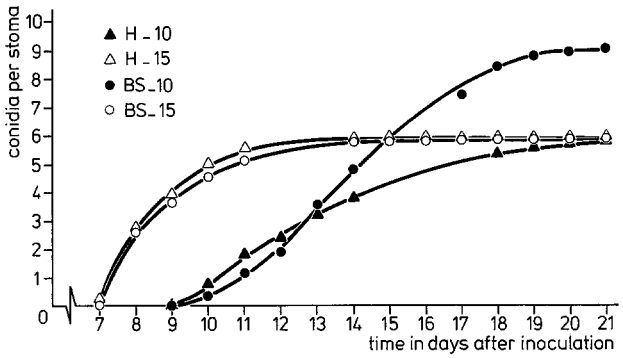

Fig. 1. Spore production by *P. farinosa* f. sp. *spinaciae* expressed as the average cumulative number of conidia produced per stoma in days after inoculation.

Entries: H-10 and H-15 = cv. Huro at 10 and 15 °C; BS-10 and BS-15 = cv. Breedblad Scherpzaad at 10 and 15 °C.

stoma (y_2), nor the shape parameters of the curve (a and 1/b) were significantly different (Table 1).

Spore production in the BS-10 as well as in the Huro-10 series started in the night preceding day 10. The shape of both curves was asymmetrically sigmoid. Total spore

Table 1. *P. farinosa* f. sp. *spinaciae* on spinach. Presence (+) or absence (−) of significant differences ($p \leqslant 0.05$) between treatments for the parameters y_2, a and 1/b of the spore production curves of Figure 1.

Treatment	y_2	a	1/b
Huro-15 and Huro-10	−	+	−
Huro-15 and BS-15	−	−	−
Huro-15 and BS-10	+	+	+
Huro-10 and BS-15	−	+	−
Huro-10 and BS-10	+	−	+
BS-15 and BS-10	+	+	+

Table 2. *P. farinosa* f. sp. *spinaciae* on spinach. Average sporulation period ($\bar{i}$) of pathotype 3 (two cultivars at two temperatures). n = number of colonies observed, w = range width, sd = standard deviation of i.

Treatment	n	$\bar{i}$ [1]	w	sd
Huro-10	21	10.0 a	14	3.8
Huro-15	29	5.8 b	8	2.1
BS-10	21	10.1 a	15	3.3
BS-15	31	6.6 b	8	2.2

[1] Values followed by a different letter are significantly different ($p \leqslant 0.01$).

production per stoma (y_2) for BS-10 and Huro-10, i.e. 9.2 and 5.8 spores, respectively, differed significantly. The shape of the curve (1/b), with the exception of the first part (a), also differed significantly. Spore production ceased around day 21 in both cases.

Sporulation period. The average sporulation period (i) for all series is shown in Table 2. Significant differences at the 1% level existed between temperatures, but not between cultivars.

Discussion

Total spore production was converted to spore production per stoma and based on the total area of the lesion at the end of the sporulation period. Lesion growth during the sporulation period was ignored. Lesion expansion might have been slowed down by the daily removal of spores and consequently of sporophores, which produce spores only once (R.Y. van der Weide, pers.comm.). When spores are removed as described, new sporophores can replace the old ones by using the same stomata. There is no need for the fungus to invade new stomata at the periphery of the lesion. So, lesion growth may be slowed. If spores and sporophores are not removed, the fungus is able to form new sporophores by utilizing new stomata at the periphery of the colony. In this way production of sporophores will enlarge the lesion area. In the field, liberation of spores from sporophores strongly depends on weather conditions. On some lesions, sporophores will lose their spores quickly after ripening, but on well-protected lesions, spores may remain present for a longer time. Small changes of r.h. can result in liberation of conidia (Pinckard, 1942), and this phenomenon is more the rule than the exception in a field situation.

In this study a comparison was made between the sporulation on lesions on cvs Huro and BS at two different temperatures. Huro-10, Huro-15 and BS-15 ended with the same average cumulative number of conidia per stoma; BS-10 showed a remarkably higher number. The reason might be the suitability of 'BS' to thrive at lower temperatures, and its higher susceptibility for *P. farinosa.* f. sp. *farinosa* at these temperatures. The differences in sporulation and latency periods between BS-15 or Huro-15 and Huro-10 have consequences for the development of an epidemic. Spore production was equal in these cases. This implies that the potential number of daughter lesions produced by one original mother lesion at the end of the sporulation period is equal in both cases, when the effectivity of the liberated spores is not considered. But at lower temperatures the total number of spores produced is liberated over a much longer period. This should result in an epidemic with a lower infection rate. The sporulation and latency periods of BS-10 and Huro-10 were equal. In this case, however, the sporulation potential of BS-10 is much higher and will result in a higher rate of disease progress.

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Samenvatting

Conidiënproduktie door Peronospora farinosa f. sp. spinaciae

De conidiënproduktie van *Peronospora farinosa* f. sp. spinaciae (fysio 3) werd dagelijks bepaald aan kolonies, welke op bladeren van de spinaziecultivars Breedblad Scherpzaad (BS) en Huro werden geïnduceerd. Daarbij werd onderscheid gemaakt tussen kolonies welke zich bij 10 en bij 15 °C hadden ontwikkeld. De sporenproduktie werd omgerekend naar het aantal sporen per huidmondje. Er was een significant verschil tussen de totale sporenproduktie op lesies op 'BS' bij 10 °C en die op lesies op de drie andere getoetste cultivar-temperatuur combinaties. Er kon ook een significant verschil in de gemiddelde sporulatieperiode worden waargenomen tussen de lesies ontstaan bij 10 en bij 15 °C. Een dergelijk significant verschil in temperatuurreactie kon ook worden gevonden in het verloop van de sporulatie per huidmondje in de tijd.

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