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Postponed germination of Puccinia recondita urediospores deposited on wheat seedlings. II. Infectivity of urediospores after postponed germination

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Abstract

Urediospores of *Puccinia recondita* f.sp. *tritici* were applied to wheat seedlings. Inoculated plants were placed in a growth chamber to expose the spores to dry periods from zero to nine days at near-optimal temperatures (ca 18 °C). The dry period was followed by a wet period varying from 2 to 24 hours for germination of spores and infection of plants. Infection results were subjected to analysis of variance. The main effects dry period, wet period, and temperature were highly significant. The dry period × wet period interaction was significant. The interaction implied that the effects of post-detachment ripening of germinable spores appeared in their resulting infectivity. There were two forms of post-detachment ripening, a slow ripening during the dry period and a faster ripening during the wet period. The two forms of ripening showed a non-additive compensatory interaction. The effect of post-detachment ripening on infectivity of germinated spores was more pronounced at 15 than at 18 or 20 °C; the effect was strongest during the first day of the dry period. At dry periods of over 6 days, infectivity of germinated spores decreased, especially at the higher temperatures. Prolonged exposure of spores to a dry period apparently damages the spores even though they are still able to germinate.

Additional keywords: brown rust, infectivity, latency period, pustule formation, ripening, rust.

Introduction

In an earlier paper (Zadoks and Van Hees-Boukema, 1986) on postponed germination and longevity of urediospores of brown rust *Puccinia recondita* f.sp. *tritici*) the effect of postponed germination on germination capacity was studied. Primary leaves of wheat seedlings were dry-inoculated in a settling tower. The inoculated plants were placed in growth chambers for up to nine days (dry period) before exposure to leaf wetness, in order to postpone germination. During the subsequent wet period spores were allowed to germinate. After exposure of the spores to wet periods of varying duration, the fraction of germinated spores was determined. Two conclusions were drawn. (1) When urediospores were placed on the primary leaves of wheat seedlings, their germinability was maintained for many days. (2) Post-detachment ripening of urediospores placed on wheat leaves and subjected to postponed germination was manifested as a temporary increase of the germinated spores.

Infectivity of urediospores rather than germinability is a criterium for the epidemiological importance of postponed germination. This paper reports on infectivity of urediospores subjected to postponed germination, as measured by the frac-

tion of resulting pustules and their latency period, using the same experiments as discussed by Zadoks and Van Hees-Boukema (1986).

Materials and methods

Experiments. For the description of the different experiments, indicated by a two-digit code, and the conditions in the walk-in growth chambers, the reader is referred to Zadoks and Van Hees-Boukema (1986). Some points are briefly repeated here.

Plants. Seeds of the winter wheat cv. Rubis were used. Chlormequat was added to the pots to keep plants short.

Urediospores had been stored in liquid nitrogen. For inoculation, fresh spores were used.

Inoculation. Primary leaves of wheat seedlings were inoculated at both sides in a settling tower by means of a CO_2-gun.

Dry period. Postponement of germination was obtained by exposing the inoculated plants to the current growth chamber environment(s) (see above and Table 1) during a varying number of days. The period of delay, called dry period (DP), is measured in days.

Wet period. Following the dry period, leaf wetness was applied for germination of the spores. The duration of the leaf wetness period, here called wet period (WP), was measured in hours. It varied from 2 to 24 hours. Leaf wetness was obtained by placing the pots with inoculated plants in improvised dew chambers, one for each pot, after spraying them lightly with tap water. Leaf wetness was applied at current growth chamber temperatures.

Spore germination. Following the wet period, spore germination was determined. Spores were called germinated when the germ tube length surpassed the smallest spore diameter. Per strip at least 10 microscope fields of 1.25 mm^2 were examined. The results are expressed as the fraction of germinated spores relative to the total number of spores (GTR according to Zadoks and Schein, 1979).

Latency period. Uredinia do not appear and open all at the same time. From the flecking stage onwards, leaves were periodically inspected for open pustules with a hand lense. The operational definition of latency period used in this study was the period in days from the beginning on the leaf wetness period until the finding of the first open pustule on the primary leaves of the plants in one pot. Thus, latency period was determined for each pot separately; it is the shortest observed latency period (p_{min}) per pot. Pot values were used as the basic statistical units.

Pustulation rate. The process of the appearance of pustules, here called 'pustulation', proceeds with a measurable speed, here called 'pustulation rate'. To determine that rate, pustulation at 20 °C in Experiment 3.2 was monitored twice a day by counting

open pustules on the adaxial side of the leaf over 1 cm length of leaf at 2 cm distance from the leaf tip. Counts were made on two leaves per pot, using a dissecting microscope, and expressed as mean number of pustules per cm^2. Pustulation roughly follows a sigmoid curve (Mehta and Zadoks, 1970), which can be transformed into a probit line. The slope of that line is a measure for the pustulation rate (Zadoks and Schein, 1979). The period from the beginning of leaf wetness until 50% pustulation is indicated by $t_{0.5}$; it is a measure of the median latency period per pot ($p_{0.5}$).

Density comparisons. The number of spores deposited per unit area, the number of germinated spores per unit area, and the number of pustules per unit area are here referred to as 'densities'. Over the time span between the end of the wetness period and the counting of the pustules the length and width of the leaves may not remain constant. During handling of detached leaves they may shrivel. Comparisons between total spore density, germinated spore density, and pustule density cannot be made by using the 'microscope field' as the reference area. Therefore, the number of stomata per unit area or 'stomatal density' was determined. All densities were then expressed as numbers of total spores, germinated spores and pustules per area with thousand stomata. Reference areas measured but variable in time were replaced by a theoretical reference area of one thousand stomata, assuming that no stomata appeared or disappeared after leaf unfolding. All densities were determined at the upper (adaxial) leaf surface.

Colonization ratio. The colonization ratio (CR) is the proportion of germinated spores leading to pustules (Zadoks and Schein, 1979). Accurate assessment of CR can only be made by using stomatal density as a reference, see above, as done in experiment 2.1. As the method was excessively time-consuming, an apparent colonization ratio (ACR) was calculated in Experiments 3.1 and 3.2. ACR is the number of pustules per cm^2 divided by the number of germinated spores per cm^2. ACR is nearly proportional to CR.

Statistical considerations. In view of the wet period treatment, in which each pot was treated individually and – maybe – differently, the pot was used as the basic statistical unit. Data collected at sub-pot level (plant, microscope field) were compacted into one statistic per pot. Standard techniques for the analysis of variance (ANOVA) were applied. Experiment 3.2 was handled as a split plot experiment, with temperatures as plots and dry period × wet period combinations as sub-plots; this treatment is not completely correct because temperatures were not replicated.

Results

Latency period. In Experiment 3.1 (18 °C) the shortest latency period per pot (p_{min} was 7 days for all pots. In Experiment 3.2 p_{min} varied with temperature (Table 1). The difference at 18 °C between the two experiments is due to the fact that observations were made once a day in Experiment 3.1 and twice a day in Experiment 3.2.

Pustulation. Pustulation followed a sigmoid pattern (Fig. 1). Linearization of the pustulation curves by probit transformation was possible (Fig. 2). Intercept and slope

Table 1. Experiment 3.2. The effect of different temperatures on some variables.

T[1]	p^2_{min}	$p^3_{0.5}$	a^4_0	a^5_1
20	5.5	6.0	−12.6	2.9
18	6.6 (+20%)	7.1 (+18%)	−11.8	2.4 (−17%)
15	7.7 (+40%)	8.5 (+42%)	− 9.7	1.7 (−41%)

[1] T = temperature in °C.
[2] p_{min} = shortest laten period observed (per pot).
[3] $p_{0.5}$ = median latency period (mean of pots).
[4] a_0 = intercept of probit line with y-axis.
[5] a_1 = slope of probit line (in probit units per day).

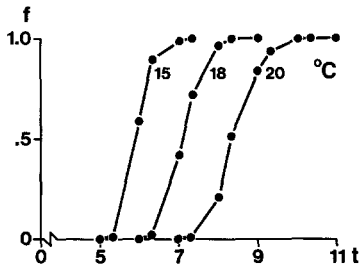

Fig. 1. Experiment 3.2, WP = 24. Pustulation curves for three temperatures. Abscissa: time in days from the beginning of the wet period. Ordinate: number of open pustules expressed as a fraction of the maximum number. Entries: temperature in °C.

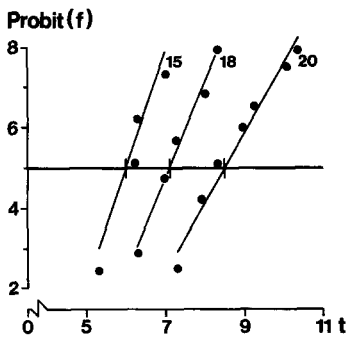

Fig. 2. Data from Fig. 1 after probit transformation. For intercept and slope of the probit lines see Table 1. Abscissa: Time in days from the beginning of the wet period. Ordinate: Probit value of the fraction of open pustules. Entries: Temperature in °C.

of the resulting probit lines are given in Table 1. At 20 °C pustulation began early and proceeded fast, at lower temperatures it started later and proceeded slower. Table 1 indicates that there was some degree of proportionality with temperature in the delays and retardations of pustulation, at least within the temperature limits of the experiment.

Colonization. The curve of the colonization ratio (CR) versus the dry period (DP) in Experiment 2.1, at 20 °C and with a wet period (WP) of 24 hours, shows an initial increase and subsequent steady decrease. The data, which are not suitable for statistical

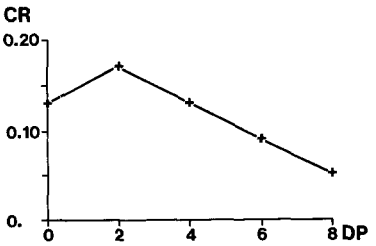

Fig. 3. Experiment 2.1, 20 °C, wet period WP = 24. Colonization ratio (CR) versus dry period (DP) in days.

Table 2. Experiment 3.1. Estimated colonization ratio (ACR) for various combinations of dry period (DP, in days) and wet period (WP, in hours). Entries represent the ^{10}log of ACR.

DP	WP				Mean
	2	4	6	24	
0	–	0.35	2.04	2.20	1.15 ab[1]
1	–	0.65	2.01	2.34	1.25 bc
2	–	0.44	1.96	2.33	1.18 b
3	–	0.40	1.58	2.25	1.06 a
4	–	0.54	1.80	2.25	1.15 ab
6	–	1.02	1.95	2.29	1.31 c
9	–	0.78	1.88	2.10	1.19 b
Mean	– A	0.60 B	1.89 C	2.28 D	1.19

[1]Means of rows and columns characterized by the samen letter do not differ significantly according to Bonferroni's test at $p = 0.05$. Critical difference for row means = 0.17, for column means 0.12 at $p = 0.05$.

Table 3. Data from Table 2, analysis of variance.

Effect	SS[1]	df[2]	MS[3]	F[4]	p[5]
Level	119	1			
Main effects					
DP = dry period	0.47	6	0.08	2.6	0.025
WP = wet period	71	3	24	790	<0.005
Interaction					
DP × WP	1.1	18	0.06	2.1	0.025
Residual	1.7	56	0.03		
Total	192	84			

[1] SS = sum of squares.
[2] df = degrees of freedom.
[3] MS = mean squares.
[4] F = variance ratio.
[5] p = significance level.

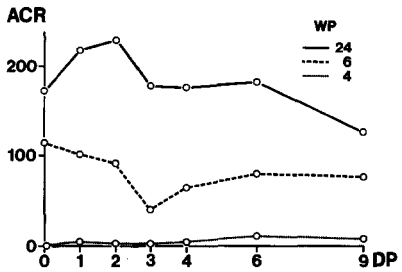

Fig. 4. Experiment 3.1, 18 °C. Apparent colonization ratio (ACR), expressed in number of pustules per 1000 germinated spores, versus dry period (DP) in days. Entries are wet periods (WP) in hours.

analysis, suggest that infectivity of germinated urediospores increases during the first two days of post-detachment pre-germination ripening, and subsequently declines (Fig. 3).

In Experiment 3.1 the main effects on ACR, dry period and wet period, were significant, with and without log transformation of ACR (Table 2 and 3). Interaction between dry and wet period was significant only after log transformation of ACR. It was

Table 4. Experiment 3.2. Apparent colonization ratio (ACR) for various combinations of temperature (T, in °C), dry period (DP, in days) and wet period (WP, in hours). Entries represent the ^{10}log of ACR.

DP	WP			T			Mean
	3	6	24	15	18	20	
0	–	1.56	1.84	0.89 A[1]	1.18 a	1.33 α	1.13
1	0.04	1.92	2.17	1.17 B	1.45 b	1.15 β	1.38
2	0.07	1.93	2.17	1.25 BC	1.39 b	1.52 β	1.39
3	0.06	1.87	2.20	1.29 CD	1.42 b	1.43 $\alpha\beta$	1.38
4	0.04	1.83	2.22	1.34 CD	1.37 b	1.38 $\alpha\beta$	1.36
6	0.06	1.84	2.24	1.36 CD	1.37 b	1.42 $\alpha\beta$	1.38
9	–	1.88	2.23	1.33 CD	1.43 b	1.35 α	1.37
Mean	0.04	1.83	2.15	1.23	1.37	1.42	1.34

T	WP			Mean
	3	6	24	
15	– A	1,67 B	2.03 C	1.23
18	– a	1.86 b	2,26 c	1.37
20	0.11α	1.97 β	2.17 γ	1.42
Mean	0.04	1.83	2.15	1.34

[1] Means of rows and colums characterized by the same letter do not differ significantly according to Bonferroni's test at $p = 0.05$.

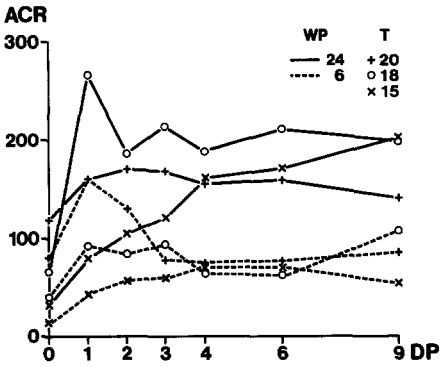

Fig. 5. Experiment 3.2. Apparent colonization ratio (ACR), expressed in number of pustules per 1000 germinated spores, versus dry period (DP) in days. Entries are wet periods (WP) in hours and temperature (T) in °C.

due to the initial increase of ACR at WP = 24 and the unexplainable dip in ACR at WP = 6 (Fig. 4). A wet period of 4 hours gave only minimal infection, whereas a WP = 24 produced about twice as much infection as a WP = 6.

In Experiment 3.2 (Fig. 5) all three main effects on ACR, viz. temperature, dry period and wet period, were significant (Tables 4 and 5). At 20 °C, peak values of ACR appeared after a dry period of about two days, at WP = 6 somewhat earlier, at WP = 24 somewhat later. The decline after the peak value was limited at 20 °C, amounting to about 37% over a period of about one week. At 18 °C ACR increased from DP = 0 to DP = 1, remaining approximately constant at longer dry period values. The increase was about 4.6 times. At 15 °C ACR increased with DP up to four days at least.

Table 5. Data from Table 4. Analysis of variance (split plot design).

Effect	SS[1]	df	MS	F	p
Level	340		1		
Main effects					
T = Temperature	1.2	2	0.59	59	<0.005
DP = dry period	1.4	6	0.23	23	<0.005
WP = wet period	164	2	82	8195	<0.005
One-way interactions					
T × DP	0.82	12	0.07	6.8	<0.005
T × WP	0.53	4	0.13	13	<0.005
DP × WP	0.60	12	0.05	5.0	<0.005
Two-way interaction					
T × DP × WP	0.75	24	0.03	3.1	<0.005
Residual	1.3	126	0.01		
Total	511	189			

[1] Symbols as in Table 3.

The total increase was over a fivefold. The one-way and two-way interactions were highly significant, with and without log transformation of ACR. The DP × WP interaction was marked at 20 °C at DP = 1 to 3 and also at 18 and 15 °C at DP = 6 to 9. The T × DP interaction appeared from the mainly descending curves at 20 °C and ascending curves at 15 °C.

Discussion

Latency period and pustulation responded to temperature as usual (Chester, 1946; Mehta and Zadoks, 1970). As they did not form the objective of the present study, they will not be discussed here.

Colonization. The CR and ACR values reveal some of the caracteristics of post-detachment ripening of urediospores. The Experiments 2.1, 3.1 and 3.2 share a common characteristic, a peak value after a dry period of one to two days plus a wet period of 24 hours, at 18 to 20 °C. At 15 °C, in Experiment 3.2, the peak was reached later, at about DP = 6. The results indicate that dry post-detachment ripening not only promotes germination (Zadoks and Van Hees-Boukema, 1986) but also infectivity. At 15 °C the ripening process seems to proceed more slowly than at 18 and 20 °C.

Wet periods. In Experiment 3.1, a wet period of four hours led to some infection at least. In Experiment 3.2 a wet period of three hours was insufficient for infection at 15 and 18 °C but sufficient for at least some infection at 20 °C (Fig. 6). Prolonged wet periods lead to high infection results, presumably because sufficient time is available for germ tube growth, appressorium formation (if any), and penetration. The results give no clue as to the existence of a post-detachment wet ripening favourable to infection.

Decline. In the present context, decline is the reduction in infectivity of the spores before postponed germination. It appears as a sloping down in the ACR versus DP curves, but this sloping down can mean either one or both of two things, viz. a decline in infectivity of the spores or a reduction of infectability of the leaves. Reduction of infectability might be expected specifically at the longer DP's, when the leaves are up to nine days older when the infection process begins. In Experiment 2.1, the plants were nearly wilting at the time of pustule counting and there was littele doubt that a

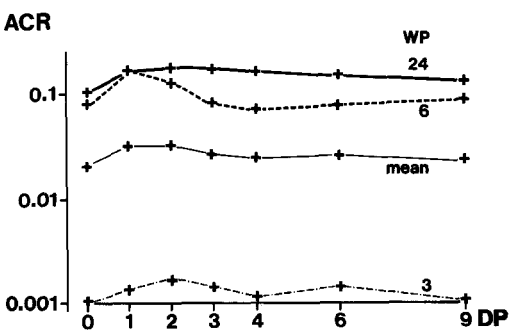

Fig. 6. Experiment 3.2, 20 °C. Apparent colonization ratio (ACR), expressed in number of pustules per germinated spore, versus dry period (DP) for three wet periods (WP). Abscissa: DP in days. Ordinate: ACR on a logarithmic scale. Entries: WP in hours.

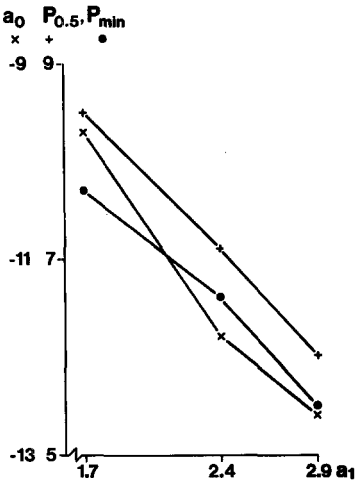

Fig. 7. Biological interrelation between slope and intercept of probit lines for pustulation (see Fig. 2, Table 1).
a_1 = slope of probit lines,
$p_{0.5}$ = intercept of probit lines with line y = 0.5 (probit y = 5),
p_{min} = shortest observed value of p,
a_0 = intercept with y-axis.

reduction of infectability, or at least of pustulation, had occurred due to the poor state of the plants. The effect was less obvious in Experiment 3.2 (Fig. 5), where plants were, seemingly, in good shape up to the end of the experiment. In Experiment 3.2 there was an initial decline of ACR shortly after its peak value, but at dry periods from four to nine days ACR remained nearly constant at any WP or T value. Results in Experiment 3.2 suggest that reduction of infectability is not a major point, at least not when the plants have been kept in a good state. In conclusion, at least part of the downward slope of the curves in Figures 4, 5 and 6 was due to decline of infectivity during prolonged postponement of germination.

Statistical and biological independence of derived responses. The data represented by the pustulation curves are primary responses. For linearization, they were subjected to a mathematical treatment, which yielded as secondary or derived responses a slope (of the probit line) and an intercept ($p_{0.5}$ with a horizontal axis or a_0 with the vertical y axis). Intercept and slope define a straight line. In a mathematical sense, intercept and slope are independent. Nevertheless, Fig. 7 suggests near-linear relations between intercept and slope. In a biological sense, they are not independent, as both reflect the response of pustulation rate to temperature. Biological interdependence of slope and intercept in linearly transformed S-shaped curves was also noted for logit lines (Rouse et al., 1981; Zadoks, unpublished).

Conclusion. Post-detachment pre-germination on-leaf storage of brown rust urediospores affects these spores in several ways. (1) The duration of the wet period strongly affects the apparent colonization ratio. (2) A reduction of infectivity during prolonged periods of postponed infection may affect the results. (3) A ripening process sets in which results in an increase of infectivity of germinated spores; the rate of this process depends on the dry period duration, wet period duration, and temperature. (4) A decline of infectivity occurs after the first or second day of dry period, except at the lowest temperature (15 °C); the decline may be proportionally stronger at the shorter wet period (6 hours).

Samenvatting

Uitgestelde kieming van uredosporen van Puccinia recondita afgezet op tarwekiemplanten. II. Infectiviteit van uredosporen bij uitgestelde kieming

Uredosporen van de bruine roest van tarwe *(Puccinia recondita* f.sp. *tritici)* werden over het eerste blad van tarwekiemplanten verstoven. De aldus geïnoculeerde planten werden in een klimaatkamer geplaatst bij ca. 18 °C om de sporen bloot te stellen aan droge perioden van 0 tot 9 dagen. De droge perioden werden gevolgd door natte perioden van 2 tot 24 uur om de sporen te laten kiemen en de planten te infecteren. Na de natte periode werden de planten bij verschillende temperaturen geplaatst om de latente periode en de vorming van sporenhoopjes te bepalen. De hoofdeffecten op de vorming van sporenhoopjes waren zeer significant: duur van de droge periode, duur van de natte periode en temperatuur. Twee vormen van sporenrijping werden gevonden bij rijping van sporen, die los op het blad lagen (rijping buiten de sporenhoopjes), een langzame rijping tijdens de droge periode en een snelle rijping tijdens de natte periode. Deze twee vormen van rijping vertoonden statistische interactie met enige wederzijdse compensatie. Het effect van deze sporenrijping (buiten de sporenhoopjes) op de infectiviteit van gekiemde sporen was bij 15 °C duidelijker dan bij 18 en 20 °C; het effect was het sterkst tijdens de eerste dag van de droge periode. Bij droge periodes langer dan 6 dagen daalde de infectiviteit, vooral bij de hogere temperaturen.

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