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ON LIGHT-SENSITIVITY IN GERMINATING UREDOSPORES OF WHEAT BROWN RUST[1]

Over de lichtgevoeligheid van kiemende uredosporen bij de bruine roest van tarwe

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Experiments consisted of three phases: spore production, spore storage and spore germination. Throughout the experiments temperature was near-optimal. In each phase various light and humidity treatments were given. The time at which a spore germinated was considered to be a stochastic variable. 3,000 lux inhibits germ tube elongation measurably; 7,600 lux inhibits germination measurably. Spores produced in darkness are more sensitive to light than spores produced in light, independent of the age of the pustules. Hydration of spores during storage increases light-sensitivity during germination. Samples of spores formed in darkness at low humidity are considered to consist of three sub-populations: a light-insensitive one presumably having profited from the foregoing light period, a light-sensitive sub-population and a group of spores which is inert or dead. The light-inhibited spores germinate rapidly after being placed in darkness with a germination rate little affected by the duration of light exposure. After four hours of light-exposure a gradually increasing proportion of the light-inhibited spores is killed, which means that the reversibility of the light-inhibition is limited.

INTRODUCTION

The physiology of uredospore germination has received much attention recently. GIVAN & BROMFIELD (1964 a,b), SCHRÖDER & HASSEBRAUK (1964) and SHARP (1965) published excellent studies on this subject. These writers have also given extensive surveys of the literature, which do not need to be repeated here. The influence of light on uredospore germination was studied among other subjects in the publications mentioned above. CARLILE (1965) prepared a stimulating review on the general photobiology of fungi. Some consequences for routine rust work were indicated by ZADOKS (1967).

The present paper deals with the effect of light and humidity on the uredospore germination. Emphasis has been placed on the treatment of the spores during the period of their production with respect to the subsequent effect of inhibition by light during germination. The writers considered that improvement of methods was needed and thus close attention was paid to methods of experimentation and interpretation.

The investigation is divided into three phases. Phase 1 is the "spore production period", during which uredospores were formed under various conditions of light and humidity. Phase 2 is the "spore storage period", during which the spores were stored under various conditions of light and humidity. Phase 3 is the ensuing "germination experiment", in which the germinating spores were exposed to light of various intensities and exposure times. Throughout the experiments the temperature was near-optimal for sporulation and germination.

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MATERIALS AND METHODS

The uredospores

The test object was a single spore culture of *Puccinia recondita*, 'Flamingo' race (race group 68/96/97; UN-race 12), isolated from cv. 'Heines VII' in the Wieringermeerpolder, the Netherlands, 1961 (isolate number 1042). The rust was grown on the primary leaves of cv. 'Rubis' in a conditioned greenhouse at temperatures varying between 18 and 23 °C. Several successive generations of rust have been grown in the greenhouse. For each experiment spores from one generation only were used. The spore population on a leaf at the time of sampling can be considered to consist of several sub-populations, formed during different periods under different environmental conditions and therefore differing in maturity and, consequently, in germination time and germination rate. Preliminary experiments showed that spore samples harvested with a cyclone collector contained a variable percentage of immature spores. In order to obtain a *homogeneous* spore sample the following procedure was adopted. Only those spores were used which were produced during a definite, short period, the spore production period, under adequately defined environmental conditions. At the beginning of the spore production period, the wheat leaves were gently tapped to free them from mature spores. At the end of the spore production period, the mature spores were harvested by gentle tapping of the leaves over a Petri dish (conform SCHRÖDER & HASSEBRAUK, 1964: 623).

Phase 1, the spore production period

Five pots each containing ca. 20 plants were placed in a polyethylene cage 15 × 15 cm wide and 30 cm high. At the top of the cage was an adjustable slit whereby the humidity in the cage could be regulated. The cages were placed in a ventilated tunnel with light sluices, either in a light or in a dark compartment. Over the tunnel a rack was mounted with 8 Philips TL-MF fluorescent tubes (40 Watt, color 33), see Fig. 1. Light intensity between plants was measured with the spherical light meter (WASSINK & VAN DER SCHEER, 1951). Relative humidity between plants was regularly measured with an El-Tronics humidity

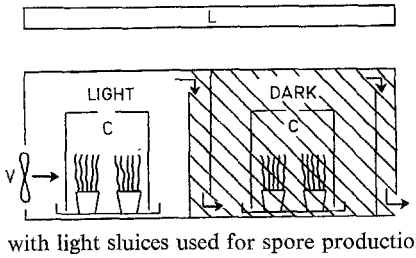

FIG. 1. Ventilated tunnel with light sluices used for spore production, schematical side view.
Geventileerde tunnel met lichtsluizen gebruikt voor de sporenproduktie, schematisch zijaanzicht.

L = Lamp rack/*Lampenrek*
C = Polyethylene cages in trays with adjustable slit in top
Polyethyleen kappen in bakken, bovenaan instelbare spleet
V = Ventilator blowing conditioned air into the cage
Ventilator die geconditioneerde lucht in de kap blaast
→ = Direction of air flow
Richting van de luchtstroom

senser. Temperatures between plants were measured with copper-constantan thermocouples connected with a Honeywell-Brown recorder.

The light intensity varied according to the sunshine, but the minimum light intensity between plants at 10 cm above soil level was ca. 10,000 lux (= ca. 51 mgcal. cm^{-2}. $sec.^{-1}$). A cycling program provided 16 hours light and 8 hours dark per day during the incubation and sporulation period prior to the onset of the "spore production period". During the spore production period either constant light or constant darkness was given. Temperatures varied between 17 and 23 °C in dependence of irradiation by sunlight.

The relative humidity in the open cages (dry) varied from 50 to 80%. In the closed cages (wet) the humidity was over 90%, too high to be measured by the available El-Tronics sensers. Heavy condensation on the polyethylene cover of the cage indicated a near-saturated atmosphere inside. During simultaneous dry and wet spore formation in light the temperature in the closed cage (wet) was ca. 1.5 °C higher than in the open one. During simultaneous dark and light spore formation in closed cages (wet), the temperature in the light cage was ca. 1 °C higher than in the dark one.

Phase 2, the spore storage period

Spores were collected on discs of high quality white cardboard fitted into 14 cm ⌀ Petri dishes. During the spore storage period the open Petri dishes were placed in a container covered with a glass plate. High humidity in the container was ensured by lining the walls and the bottom with wet filter paper. The container with its load of Petri dishes was placed in the dark during ten hours or more, unless stated otherwise. This treatment was called "hydration". The atmosphere in the Petri dishes was practically saturated, condensation droplets were formed on the glass parts and the cardboard was moistened, but no free water was seen in contact with spores. The storage was done at room temperature, which was 18 to 20 °C.

Unhydrated controls were submitted to the same treatment, except that dry filter paper was used to line the container. The relative humidity varied from 55 to 62%.

Phase 3, the spore germination experiment

Spores were germinated on filtered water agar in open 4 cm ⌀ Petri dishes. In each experiment the Petri dishes were filled from a single batch of agar. A regular deposit of spores was obtained by dusting the spores over the dishes in a 3 m high sedimentation tower, using a DeVilbiss Powder Blower.

Light was obtained from a rack with fluorescent tubes as described above. Germination in darkness was obtained by enveloping the Petri dish in aluminium foil and placing it amongst the other Petri dishes in a randomised order. The experiment was shielded from any other light source by a black plastic cover. Light intensity was 40,000 lux, as measured with the spherical light meter at the site of the germinating spores, unless stated otherwise. For comparison with American data, obtained with a flat lux meter, the figures given here should be divided by 2.

Temperature control during germination was obtained by means of a special arrangement (Fig. 2). The Petri dishes were placed on wet filter paper in zinc trays. The trays were mounted in containers filled with water and kept at con-

stant temperature by a thermostat. To intercept the heat eradiated by the lamps, a 2cm thick water filter was mounted 6cm above the tray bottom. The water flowing through the filter entered at a temperature of 15°C. Temperature was checked with mercury thermometers. The mercury reservoir was shielded by a small piece of aluminium foil in order to reduce heat radiation effects. In each experiment, the temperature difference between any two randomly chosen spots was 0.1°C or less.

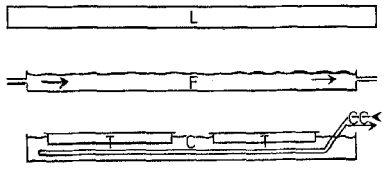

FIG. 2. Technical arrangement of spore germination experiments, schematical side view.
Opstelling van de sporekiemproef, schematisch zijaanzicht.

L = Lamp rack/*Lampenrek*
F = Heat filter with glass bottom, filled with flowing water / *Warmtefilter met glazen bodem, gevuld met stromend water*
C = Container filled with water / *Vat gevuld met water*
CC = Copper coils with water at constant temperature coming from a thermostat / *Koperen buizen met water van constante temperatuur, afkomstig van thermostaat*
T = Zinc trays with wet filter paper on bottom and glass cover / *Zinken bakken met nat filtreerpapier op de bodem, afgedekt met glasplaten*

The "germination period" is defined as the period in hours from the application of spores onto the agar by dusting until spore fixation. Fixation was done by placing the Petri dishes with spores in a dessicator with 40% formaldehyde for ten minutes. Between fixation and spore counting the Petri dishes could be stored for one or two days in a refrigerator.

The spore counting

Counting of germinated and ungerminated spores was done under a binocular microscope at a magnification of times 80 or 125. When spore clusters were present, they have been disregarded. Only those spores were counted which were separated from their neighbours by a distance of one spore diameter or more. For each Petri dish 500 spores were classified as either germinated or ungerminated. A spore was considered to be germinated when the germ tube was longer than the smallest diameter of the spores (conform SHARP, 1965, who, however, used the largest diameter as a criterium).

Statistical methods and definitions

The "germination percentage" is the number of germinated spores expressed as a percentage of the total number of counted spores. Since each counted spore is classified in either one of two classes, germinated and ungerminated, the estimates of the germination percentage obtained from one Petri dish will vary according to the binomial distribution. Tests of the significance of a difference between two estimates have been made graphically using binomial probability paper No. 31.298 of Codex Book Company, Inc. (FERGUSON, 1960).

The moment at which a spore "germinates" according to the working

definition is difficult to assess. The moment of germination of individual spores in a sample varies a great deal. It is assumed, that the normal distribution adequately approximates to the frequency distribution of the moment of germination in time. Under this assumption the probit analysis permits the estimation of the "germination time" of a spore sample, expressed in hours from the application of the spores onto the Petri dishes until the moment when half of the spores have germinated. The regression line of the probit of the germination percentage on time is called the "probit line". The "germination rate", defined as the tangent of the angle included by probit line and time axis, is expressed as probit units per hour.

In probit analysis as applied to mortality problems, a weighing method is usually applied because of decreasing accuracy of mortality estimates at the extremes of the distribution due to a limited sample size. The weighing method is not used here because the statistical effect of sample size at the extremes of the distribution is counteracted by another statistical effect in the central range of the distribution arising from researchers' fatigue. The decision, whether a spore has germinated or not, was more fatiguing and consequently less accurate when the number of germinated spores approximately equals that of ungerminated spores.

In some samples, not even after a long waiting period, do all spores germinate. In these cases it can be useful to compute the "relative germination percentage" in the following way:

$$\text{relative germination percentage} = \frac{(100-1).\ \text{germination percentage}}{\text{terminal germination percentage}}$$

The multiplier (100−1) is used instead of 100 to avoid difficulties in probit analysis.

RESULTS

General remarks

The specifications described under the section "Materials and methods" were developed during a set of preliminary experiments which did not meet all the said specifications. In the actual series of experiments the conditions were conform to the specifications. Variables have been tabulated in Table 1.

Preliminary experiments

When spores are placed on agar in darkness, germ pores start to bud after 30 minutes. With side light buds are usually formed at the shadow side of the spores. Germ tubes are negatively phototropic and hydrotropic. The phototropic reaction dominated over the hydrophobic reaction and in strong side light germ tubes grew near to or at the agar surface.

The "spore deposit" is the number of spores per square millimeter. In spore germination experiments a large spore deposit may cause self-inhibition of the spores. According to HOYER (1962) the threshold value for self-inhibition is about 25 spores per mm^2. Tests showed this value to be approximately correct. In the experiments reported in the next paragraphs the spore deposit varied from 3 to 18 spores per mm^2.

From the preliminary experiments it appeared among other things that the light regimen during spore production has a great effect on the germination

TABLE 1. Environmental conditions during the experiments.

Milieu-omstandigheden tijdens de proeven.

Experiment	Phase 1 Spore production			Phase 2 Spore storage			Phase 3 Spore germination	
	Duration in days	Light	Humidity	Duration in hours	Light	Hydration	Spore deposit[2]	Temperature in °C
09[1] a	2	L	d	12	D	+	10	18.0
b	2	L	d	12	D	−	10	18.0
c	2	D	d	12	D	+	10	18.0
d	2	D	d	12	D	−	10	18.0
10 a	2	L	w	01	L	−	15	20.0
b	2	D	w	01	L	−	15	20.0
11 a	2.4	L	d	14	D	+	11	20.0
b	2.4	L	d	14	D	−	11	20.0
c	2.4	D	d	14	D	+	08	20.0
d	2.4	D	d	14	D	−	11	20.0
12	1	D	d	01	L	−	18	20.0
13	3	D	d	01	L	−	04	23.0
14 a	2	L	w	11	D	+	15	19.0
b	2	L	w	01	L	−	15	19.0
c	2	L	d	11	D	+	15	19.0
d	2	L	d	01	L	−	15	19.0
15 a	2.1	D	d	10	D	+	04	19.0
b	2.1	D	d	01	L	−	03	19.0

D = Dark w = Wet + = Hydrated
L = Light d = Dry − = Non-hydrated

[1] In experiment 09 light during germination was 18,000 lux.
[2] Spores per mm²

TABLE 2. The influence of light conditions during the spore production period on the germination percentage and the germ tube length after 3.0 hours germination.

De invloed van lichtomstandigheden tijdens de sporeproduktieperiode op het kiempercentage en de kiembuislengte na 3,0 uur kieming.

Phase 1 Spore production	Phase 3 Spore germination			
	Light intensity in lux			
	19,600	12,000	3,000	0
	Germination percentage			
Light	94 a[1]	93 ab	94 a	92 b
Dark	0.0	0.2 e	72 d	88 c
	Germ tube length			
Light	16.0 f	16.9 fg	18.5 fg	20.0 g
Dark		4.0 h	13.1 i	19.1 j

[1] Letters indicate significance groups, each letter joining together those germination percentages which do not differ significantly at the 5% level.
[2] In scale units, average of 50 germ tubes.

percentage and the germ tube length. The results of one experiment are shown in Table 2. Germination is inhibited by light when the spores have been produced in the dark. Germ tube growth is retarded at light intensities as low as 3,000 lux, that do not affect germination percentage. The germination percentage is not reduced by light up to at least 7,600 lux. The light-sensitivity of germinating spores due to spore production in the dark is independent from pustule age, as is shown in Table 3.

TABLE 3. The influence of the age of the spore producing pustules and of light conditions during the spore production period on the germination percentage of the spores after 2.5 hours.

De invloed van de leeftijd der sporenhoopjes en van de lichtomstandigheden gedurende de sporeproduktieperiode op het kiempercentage na 2,5 uur.

Phase 1 Spore production	Phase 3 Spore germination		Age of pustules in days at spore harvest
	Light intensity in lux		
	18,000	0	
Light	64.4[1] c	69.8 b	3
	65.0 c	75.8 a	9
Dark	2.4 e	53.2 d	3
	0.4 f	72.6 ab	9

[1] Figures represent germination percentages, letters represent significance groups.
Note: Spores produced in light and germinated in light show a small but significant fall in germination percentage when compared to spores germinated in the dark. The slight increase in light-sensitivity may be due to an unexpected hydration during the spore storage period (phase 2) of 16 hours in darkness at ca. 6°C.

Hydration during the storage of spores greatly increases the light-sensitivity of germinating spores (Table 4). Exposure of spores to light during storage without hydration does not seem to influence their germination behaviour as the following experiment indicates. A spore sample was stored during 44 hours, one part in light of 18,000 lux, the other part in the dark. After a germination

TABLE 4. The influence of light conditions during the spore production period and of hydration during the spore storage period on the germination percentage of the spores after 2.0 hours.

De invloed van de lichtomstandigheden tijdens de sporeproduktieperiode en van de hydratatie tijdens de sporebewaarperiode op het kiempercentage van de sporen na 2,0 uur.

Phase 1 Spore production	Phase 2 Spore storage[1]	Phase 3 Spore germination	
		Light intensity in lux	
		18,000	0
Light	+	[2].1 f	98. 6a
	−	[2]93.4 c	97.8 ab
Dark	+	2.0 e	96.8 ab
	−	[2]40.5 d	95.8 b

[1] + = Hydrated
− = Non-hydrated
[2] 1,000 spores counted

period of three hours in light of 18,000 lux the spores stored in the light had a germination percentage of 39 whereas those stored in the dark showed a germination percentage of 41. The difference is not significant.

These preliminary experiments yielded a new fact, light-sensitivity of germinating spores formed in the dark. In the following experiments this effect was studied under more rigourously controlled conditions.

Results after two hours of germination

All combinations of light and humidity treatment during spore production, hydration treatment during storage and light treatment during germination were tested. Results after two hours of germination are presented in Table 5. Darkness during germination always resulted in a germination percentage of nearly 100, though spores formed in the dark under wet conditions seem to be weakened. Spores formed in light and not hydrated during storage do not show light-sensitivity. The germination of spores formed in darkness at high humidity, and not hydrated subsequently, is completely inhibited by light; the germination of spores formed in darkness at low humidity, without subsequent hydration, is partially inhibited by light. Hydrated spores show a nearly 100 per cent inhibition of germination by light, independent from the conditions under which they have been produced.

The course of spore germination in time

The study of the spore germination in the course of time revealed several interesting facts. In all experiments part of the spores was germinated in darkness in order to have "dark controls". These dark controls germinated with approximately equal germination time and germination rate. All relevant data have been combined in Fig. 3. The resulting probit line with a germination time

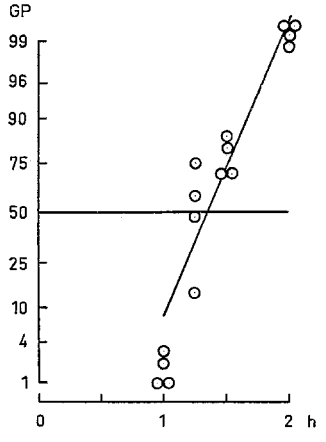

FIG. 3. Probit line of uninhibited spore germination; germination in darkness, various pretreatments (Experiment 11).
Probitlijn van de ongeremde sporekieming; kieming in duisternis, diverse voorbehandelingen (Proef 11).

GP = Germination percentage / *Kiempercentage*
h = Hours / *Uren*

of 1.35 and a germination rate of 4.05 can be considered as representative of all dark controls and of other uninhibited spore samples irrespective of their treatment prior to germination.

Spores, produced in the light and hydrated during the storage period, show a considerable light-inhibition (Fig. 4). The germination time is retarded with a factor 4.7 to 8.7; the germination rate is slowed down with a factor 5.1 to 14.5. The retardation varies greatly between experiments. Therefore the difference in the results of experiment 14 between the conditions wet and dry during spore production cannot be interpreted properly. The results of experiment 14 have been represented by straight probit lines for the sake of simplicity only.

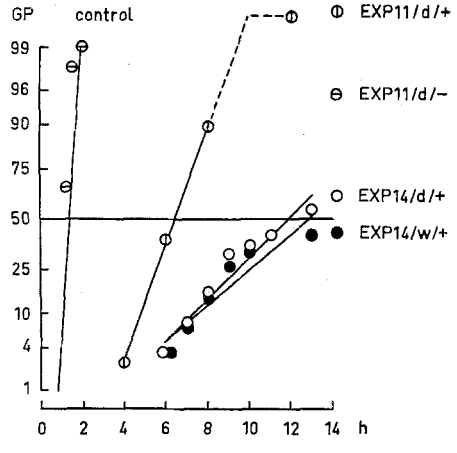

FIG. 4. Light inhibition of germinating spores. Spores have been produced in light and hydrated during spore storage.

Lichtremming van kiemende sporen. De sporen zijn geproduceerd in het licht en gehydrateerd gedurende de sporebewaring.

GP = Germination percentage / *Kiempercentage*
d = Dry conditions during spore production / *Sporeproduktie in droog milieu*
h = Hours / *Uren*
w = Wet conditions during spore production / *Sporevorming in nat milieu*
\+ = Hydrated / *Gehydrateerd*
− = Non-hydrated / *Niet gehydrateerd*

Most remarkable is the result obtained with non-hydrated spores produced in darkness under dry conditions. In Table 5 the germination percentage after two hours has been given as 37. This figure was the average value of four experiments with germination percentages of 16.6, 28.8, 39 and 80.4 respectively. These values are significantly different from each other. Fig. 5 shows, that in the beginning the germination in these four experiments was as fast as in the uninhibited controls. When a certain level was reached, the germination rate decreased sharply, remaining constant until the terminal germination percentage was reached.

This behaviour can be explained by the hypothesis that the population of the spore sample is composed out of three components:

A – a sub-population produced soon after the beginning of darkness but which has still profited from the preceding light treatment,
B – a sub-population produced in darkness showing light-sensitivity,
C – an inert sub-population which has died or which is too weak to germinate.

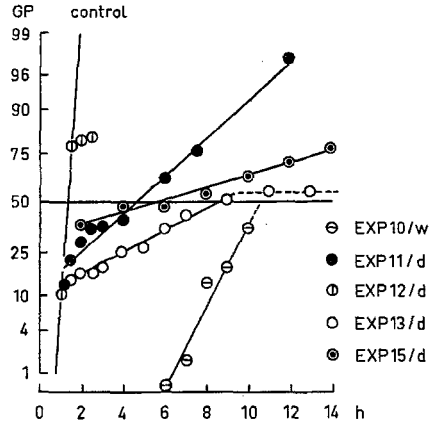

FIG. 5. Light inhibition of germinating spores produced in darkness and not hydrated.
Lichtremming van kiemende sporen geproduceerd in duisternis en niet gehydrateerd.

d = Dry conditions during spore production / *Sporeproduktie in droog milieu*
EXP = Experiment / *Proef*
GP = Germination percentage / *Kiempercentage*
h = Hours / *Uren*
w = Wet conditions during spore production / *Sporeproduktie in droog milieu*

TABLE 5. The influence of various treatments during the spore production period and the spore storage period on the germination percentage after 2.0 hours (experiments 09 to 15).

De invloed van verschillende behandelingen gedurende de sporeproduktieperiode en de sporebewaarperiode op het kiempercentage na 2,0 uur (proef 9 tot 15).

Phase 1 Spore production		Phase 2 Spore storage[1]	Phase 3 Spore germination	
			Light intensity in lux 40,000	0
Light	Dry	–	98 b	98 b
		+	0	99 a
	Wet	–	98 b	98 b
		+	0	99 a
Dark	Dry	–	37[3] d	99 a
		+	0	99 a
	Wet	–	0	87 c
		+	2[2] e	97 b

[1] + = Hydrated
– = Non-hydrated
[2] 18,000 lux
[3] This figure is the average of 4 experiments, yielding the following germination percentages: 16.6, 28.8, 39 and 80.4; see explanation in text.

TABLE 6. Estimated characteristics of the fast (A), slow (B) and inert (C) sub-populations of samples consisting of unhydrated spores produced in the dark.

Geschatte kenmerken van de snelle (A), trage (B) en inerte (C) sub-populaties van monsters bestaande uit ongehydrateerde, in het donker gevormde sporen.

Phase 1 Spore production		Phase 3 Spore germination					Experiment
Humidity	Duration in days	Sub-population in % of spore sample			Sub-population B		
		A	B	C	Germination time	Germination rate	
Wet	2	0	100	0	10.6	0.55	10
Dry	1	80	?	?	–	–	12
	2	40	40	20	9.1	0.23	15
	2	30	68	2	6.3	0.39	11
	3	15	40	45	5.5	0.52	13

This hypothesis was tested graphically (Fig. 6) after estimating the relative numbers of each sub-population (Table 6). It was assumed that these relative numbers remained constant throughout the experiment. For each sub-population the regression line of the relative germination percentage on time has been drawn together with the computed and observed germination percentages of the total population. The good fit of computed to observed values is in favour of the hypothesis.

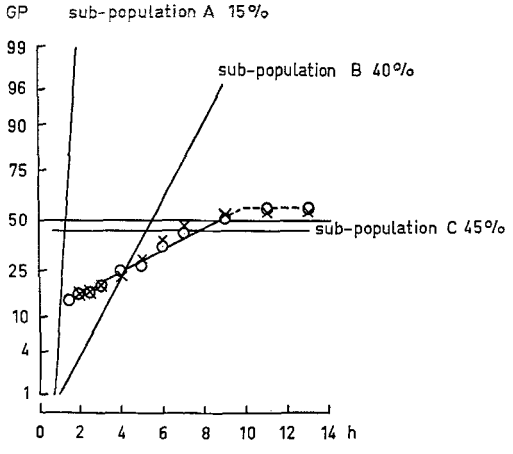

FIG. 6. Light inhibition of germinating spores produced in darkness at low humidity and not subsequently hydrated, experiment 13; observed and computed data. The computed data were based on the specifications in Table 6.

Lichtremming van kiemende sporen, geproduceerd in duisternis bij lage luchtvochtigheid en niet nadien gehydrateerd, proef 13; waargenomen en berekende gegevens. De berekende gegevens zijn gebaseerd op de specificaties van tabel 6.

GP = Germination percentage / *Kiempercentage*
h = Hours / *Uren*
o = Observed data / *Waargenomen gegevens*
× = Computed data / *Berekende gegevens*

The probit line of experiment 10 in Fig. 5 gives additional support to this hypothesis. The spores were produced in darkness in a nearly saturated atmosphere. Being practically hydrated during the spore production period, they were light-sensitive and they consequently behaved like sub-population B.

The extent of sub-population B varies between experiments (Table 6) but its principle characters remain fairly constant. The germination time is prolonged with a factor 4.1 to 6.7 when the spores were formed at low humidity or 7.9 when formed at high humidity. The light-sensitivity due to spore formation in darkness is of the same order as the light-sensitivity of spores formed in the light and subsequently hydrated. This is confirmed by the slow-down factor of the germination rate which was 7.8 to 17.6 when spores formed in darkness under dry conditions and 7.4 when formed under wet conditions.

The extent of sub-population A as a percentage of the total spore population is inversely related to the duration of the spore production period (Table 6). This is an independent argument in favour of the above hypothesis, because the period of the A production is an unknown but constant period immediately after the onset of darkness and the period of the B-C production is a variable.

The effect of hydration on the light-sensitivity of germinating spores produced in the light has already been shown; that on spores produced in the dark remains to be demonstrated. In Table 7 and Fig. 7 the relevant data are shown for the total spore sample and for sub-population B. The data suggest that with hydration the germination time is longer and the germination rate is faster than without hydration. The effect of hydration on germination rate is somewhat unexpected.

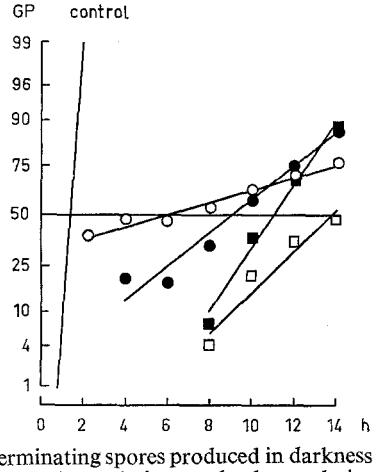

FIG. 7. Light inhibition of germinating spores produced in darkness at low humidity, with and without hydration, in total populations and sub-populations B. Experiment 15.
Lichtremming van kiemende sporen, geproduceerd in duisternis bij lage luchtvochtigheid met en zonder hydratatie, bij de totale populaties en bij de sub-populaties B. Proef 15.

GP = Germination percentage / *Kiempercentage*
h = Hours / *Uren*
○ = Total population, non-hydrated / *Totale populatie, niet gehydrateerd*
● = Sub-population B, non-hydrated / *Sub-populatie B, niet gehydrateerd*
□ = Total population, hydrated / *Totale populatie, gehydrateerd*
■ = Sub-population B, hydrated / *Sub-populatie B, gehydrateerd*

TABLE 7. The influence of hydration on the germination in light of spores, produced in darkness at low humidity.

De invloed van hydratatie op de kieming in licht van sporen, geproduceerd in duisternis bij lage luchtvochtigheid.

	Total spore sample		Sub-population B		Experiment
Hydration[1]	–	+	–	+	
Germination time	4.6	8.3	6.3	8.3	11
	5.9	13.7	9.1	11.1	15
Germination rate	0.26	0.52	0.39	0.52	11
	0.08	0.28	0.23	0.43	15

[1] – = Non-hydrated
+ = Hydrated

The reversibility of light-inhibition

In the foregoing paragraph it was shown that light-inhibition of germinating, light-sensitive spores is a transient phenomenon. Light-inhibition can, however, lead to inertia or death of part of the spores. This is indicated by the terminal germination percentage in relation to the duration of the germination period (Table 8). The term "terminal germination percentage" is used here in the sense of the last germination percentage determined. Inertia of part of the inhibited spores is not a rule, but it occurs frequently. It is concluded that a variable number of light-sensitive spores is killed by light during germination.

TABLE 8. The effect of light during spore germination on light-sensitive spores as shown by the terminal germination percentage and the time at which it has been determined.

De invloed van licht tijdens de sporekieming op lichtgevoelige sporen, aangetoond met het terminale kiempercentage en de tijd waarop dit bepaald is.

Phase 1 Spore production		Phase 2 Spore storage[1]	Phase 3 Spore germination in 40,000 lux	
			Terminal germination percentage	Time in hours
Light	Dry	+	100	12
		+	55	13
	Wet	+	84	40
Dark	Dry	+	96	12
		+	54	24
		–	98	12
		–	54	22[2]
		–	81	24
	Wet	–	95	32

[1] + = Hydrated
– = Non-hydrated
[2] 51 after 9 hours

The foregoing does not mean that light-inhibition is irreversible. On the contrary, when inhibited spores are placed in darkness they germinate rapidly, as is shown in Table 9. Spores, which were light-sensitive because they had been produced in darkness, were exposed to light of 40,000 lux during germination. At regular intervals samples were placed in darkness and examined after two

hours. The rise of the germination percentage during two hours of darkness was always bigger than that after two more hours light. The conclusion is that light-inhibition of light-sensitive, germinating spores is largely reversible. Attention is drawn to the fact, that after two hours of exposure to light the light-inhibition is no longer fully reversible. Prolonged exposure to light of germinating, light-sensitive spores leads to inertia or death of part of the spores.

TABLE 9. The influence of 2 hours darkness on the germination of light-inhibited spores after various exposure times (experiment 15).
De invloed van 2 uur duisternis op de kieming van door licht geremde sporen na verschillende belichtingstijden (proef 15).

Period in hours of germination in light of 40,000 lux i	Germination percentage at the end of this period GP_i	Germination percentage after 2 additional hours of darkness GP_{id}
00	–	99
02	39	99
04	48	99
06	47	88
08	54	83
10	64	87
12	71	82

Only the light-sensitive B-population is responsible for the reversibility of the light-inhibition. It is interesting to estimate the germination rate of the ungerminated part of sub-population B after transfer to darkness.

The following calculations are needed:
T = total number of spores in sample = 500,
A = number of spores in sub-population A = 200,
B = number of spores in sub-population B,
C = number of spores in sub-population C,
GP = germination percentage,
t = total, germinated and ungerminated, of sub-population,
$+$ = germinated part of sub-population,
$-$ = ungerminated part of sub-population,
i = sampling time index,
id = index of sampling time + 2 hours darkness.

$B{+}_i = T{+}_i - A{+}_i = T{+}_i - 198.$
$B{-}_i = Bt_i - B{+}_i = Bt_i - (T{+}_i - 198).$
$B{+}_{id} = T{+}_{id} - A{+}_{id} = T{+}_{id} - 198.$
$GP(B{-}_i)_{id} = 100.\ (B{+}_{id} - B{+}_i) \,/\, B{-}_i.$

The germination percentage of the ungerminated part of sub-population B after two hours darkness, $GP(B{-}_i)_{id}$, can be calculated. The germination percentage of the inhibited and ungerminated part of sub-population B at the onset of darkness, $GP(B{-}_i)_i$, can be assigned an arbitrary value near 0, for example 1. The probit lines connecting $GP(B{-}_i)_i$ with $GP(B{-}_i)_{id}$ are shown in Fig. 8. Their slopes are estimates for the growth rate of the ungerminated part of sub-population B when exposed to darkness. Fig. 8 suggests that the inhibited

part of sub-population *B* germinates as soon as the inhibiting factor is removed at a germination rate which is more or less independent from the duration of the light exposure.

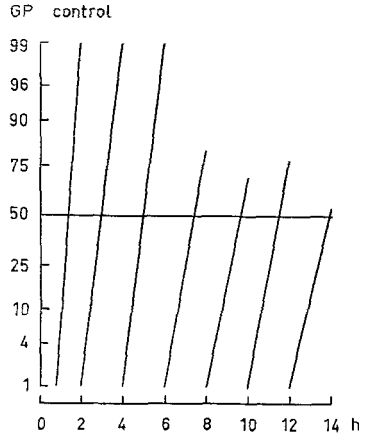

FIG. 8. The reversibility of light inhibition by darkness in germinating spores, produced in darkness at low humidity and not subsequently hydrated. Experiment 15 (explanation in text).

De omkeerbaarheid van lichtremming door duisternis bij kiemende sporen, geproduceerd in duisternis bij lage luchtvochtigheid en niet nadien gehydrateerd. Proef 15 (verklaring in tekst).

GP = Germination percentage / *Kiempercentage*
h = Hours / *Uren*

Admittedly, the foregoing considerations are theoretical, because a number of unproved assumptions has been made. For example, it was assumed that the magnitudes of the various sub-populations were independent from the duration of the germination period in the light. But Table 9 indicates that sub-population *C* is 1% at a duration shorter than 6 hours and increases to at least 10% at a duration of 14 hours. The results of the calculations must be regarded as approximations only. Nevertheless, the main point emerges clearly: most light-inhibited spores germinate at a high rate when placed in darkness after light exposure periods up to some 12 hours.

The stochastic approach to germination problems

Attention is drawn to the stochastic concept of germination per unit of time. The germination rate is a measure for the standard deviation σ of the hypothetical normal curve representing the variation of germination in time. Numerically the germination rate is the inverse of σ. The area under the normal curve can be used as a measure for the number of spores *N* belonging to the population described by that particular normal curve. The germination time stands for the average value μ of the normal distribution; μ and σ can be computed or estimated graphically from the probit lines. If the indexes *a*, *b* and *c* are attributed to the sub-populations *A*, *B* and *C* the three sub-populations are fully characterized by the values N_a, N_b, N_c; μ_a, μ_b, μ_c, and σ_a, σ_b, σ_c.

In Fig. 9 the result of experiment 13 has been analyzed. Spores were produced in darkness at low humidity and they were unhydrated. Fig. 9 has been simpli-

fied by omitting sub-population C to which all ungerminated spores belong. At the end of the experiment N_c was 44; no information on μ_c and σ_c was available. The areas under the curves A and B are proportional to N_a and N_b. The reference value $Y = 1.0$ has been chosen to represent the number of spores germinating at time μ_a. The curve S represents the integrated curve $\int_{x=0}^{t} y_t \cdot dt$, where y_t is the germination percentage of the total spore sample at time t. Curve S has been derived from curve A and B. The good fit of computed to observed data suggests that detailed testing of the stochastic approach to germination problems deserves attention.

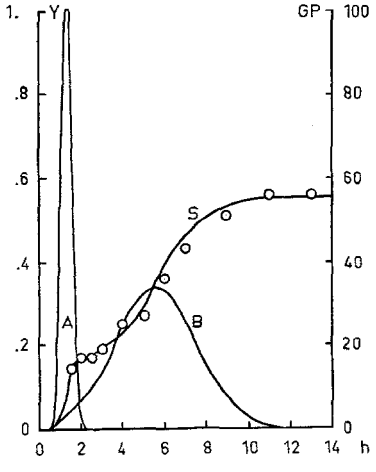

FIG. 9. Germination per unit of time as a stochastic variable. Experiment 13, spores produced in darkness at low humidity, without subsequent hydration. See explanation in text.
Kieming per tijdseenheid als stochastische variabele. Proef 13, sporen geproduceerd in duisternis bij lage luchtvochtigheid zonder daarop volgende hydratatie. Zie verklaring in de tekst.

GP = Germination percentage / *Kiempercentage*
h = Hours / *Uren*
Y = Reference value for the number of spores germinating at time h
Referentiewaarde voor het aantal sporen dat kiemt op tijdstip h

DISCUSSION

In an effort to avoid ambiguity one is forced to use such terms as germination percentage, germination rate, etc. For each of these terms a working definition, admittedly arbitrary, has been given.

Only SHARP (1965) gives a working definition of *germination*. This definition is not always easy to use and at times the results depend on the personality, the mood and the fatigue of the researcher. This difficulty can be partly overcome by the use of statistical methods. The use of a definition, albeit a poor one, is much better than its omission because without working definition results cannot be compared between authors. Once germination is defined, the meaning of *germination percentage* is clear when specifications are given of the spores which are and which are not included in the counting.

The *terminal germination* is the maximum value of the germination percent-

age found at the end of a particular time series. A high terminal germination (nearly 100%) in control experiments is a desirable experimental asset. Strict control of the environmental conditions during the spore production period and the use of a homogeneous spore sample, without immature or over-mature spores, may lead to a high terminal germination. In yellow rust *(P. striiformis)* this ideal is difficult to attain. Some of the obscurities in the results of GIVAN & BROMFIELD (1964 a,b), using brown rust *(P. recondita)* or black rust *(P. graminis)*, may be due to low terminal germination as a result of inhomogeneity of their spore samples.

Taking the germination percentage as a criterium the germinating spores experience 7,600 lux as darkness. GIVAN & BROMFIELD state that 165 ft-c is ineffective, whereas 275 ft-c gives ca. 50% inhibition. Since 165 ft-c measured with a flat light meter equals ca. 3,300 lux as measured with a spherical light meter, the threshold value for light-sensitivity is somewhere between 3,000 and 8,000 lux (spherical). The threshold value for light-sensitivity as measured by germ tube growth is definitely lower: 3,000 lux in Table 2. The threshold value varies between experiments and it possibly depends on the pre-treatment of the spores and also on the type of light used. It is worth noting that the threshold value for light-sensitivity as measured by the phototropic reaction of germinating spores is ca. 2 lux (GETTKANDT, 1954). In the final experiments a high light intensity was used (40,000 lux spherical) but apparently spores were not severely damaged when exposed during a short period ($\leqslant$ 4 hours). After long exposures there is certainly some damage done (Table 9).

The *germination rate* is usually taken to mean the increase of the germination percentage per unit of time computed from two arbitrarily chosen moments in the germination process. The resulting figures can be used for comparison between treatments within one experiment but not for comparison between experiments and still less between authors. The reason is that the figures thus obtained are no essential parameters of the germination process. Working from the hypothesis that the number of germinating spores per unit of time has an approximately normal distribution, the germination rate as defined in this paper is a parameter of the germination process which is independent from the moments at which the germination percentages are determined.

From the sowing of the wheat plants on which the rust was grown until the end of a germination experiment the temperature was kept at ca. 20 °C, which is near the middle of the relatively flat temperature optimum of spore germination and germ tube growth (CHESTER, 1946). The effect on germination of other temperatures during the phases spore production, storage and germination has not been studied but this effect has been found in all three wheat rusts (a.o. TOLLENAAR & HOUSTON, 1966).

GIVAN & BROMFIELD (1964a) stated, that light has no detectable effect on germination of non-hydrated spores. This is not quite true. A significant light inhibition was found with non-hydrated spores produced in constant darkness. Apparently treatment during the spore production period exerts great influence upon the future reaction of germinating spores to light. In the experiments reported above, inhomogeneity of spore samples produced in darkness caused certain difficulties. In yellow rust spores produced in darkness also show light-sensitivity (SCHRÖDER & HASSEBRAUK, 1965: 635).

Hydration during storage greatly increases light-sensitivity in brown rust,

in agreement with GIVAN & BROMFIELD (1964a). This is also true for black rust (GIVAN & BROMFIELD, 1964b) but the reverse seems to hold true for yellow rust (SCHRÖDER & HASSEBRAUK, 1964). Fig. 7 suggests that the effect of hydration on germination time may differ from that on germination rate, the former being retarded and the latter speeded up.

Spores produced in dry darkness and hydrated have a longer germination time than those unhydrated. This probably holds true even when only sub-population *B* is considered. The effects of darkness during production and of hydration during storage are in some way additive. Spores produced in wet darkness are probably hydrated during the spore production period. Spores produced in wet light behave as non-hydrated. One must suppose that the leaf surface and the spores were so much heated by the incident light and heat radiation that the humidity at leaf and spore surface was below saturation and within the range where the effect of hydration on germinating spores decreases sharply with a small decrease in relative humidity (SHARP, 1965). The hydration effects mentioned here may offer a partial explanation of the remark made by SCHRÖDER & HASSEBRAUK (1964: 634) that the observations on the effect of high relative humidity during spore production on the germination rate of yellow rust spores are contradictory.

The results of GIVAN & BROMFIELD on reversibility of light-inhibition are partly confirmed. Fig. 8 suggests that rapid germination of sub-population *B* starts immediately after being put into darkness, nearly independent of the duration of previous exposure to light. A new feature is the decrease of the germination percentage after two hours' darkness with increasing length of the preceding light-exposure (Fig. 8, Table 9). Apparently, part of the light-inhibited spores (sub-population *B*) is killed after exposure times of six hours or longer. Consequently, the reversibility of the light-inhibition is only a partial one. This finding was only possible because of a more prolonged and intense inhibition and a higher germination percentage than previously published. The light-sensitivity induced during spore formation in darkness cannot be fitted into the theory of the enzymatic inhibitor proposed by GIVAN & BROMFIELD. Since spores produced in darkness germinate rapidly in darkness, lack of nutritional substances offers no explanation. A new theory cannot be given to cover all the facts known at present.

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SAMENVATTING

De proeven bestonden uit drie fasen: in fase 1 werden de sporen gevormd onder verschillende omstandigheden van licht en luchtvochtigheid, in fase 2 werden de sporen bewaard eveneens onder verschillende omstandigheden van licht en luchtvochtigheid en in fase 3 kiemden de sporen bij verschillende licht-intensiteiten en belichtingstijden. Alle proeven verliepen bij temperaturen dicht bij het optimum voor sporulatie en sporekieming.

In fase 1 werd een homogeen sporenmonster verkregen door alleen rijpe sporen te oogsten die gedurende een bepaalde korte periode onder geconditioneerde omstandigheden van licht, temperatuur en relatieve vochtigheid waren gevormd (fig. 1). Tijdens fase 2 vond eventueel hydratatie van de sporen (een nacht bewaren bij 100% rv.) plaats. In fase 3 kiemden de sporen bij geconditioneerde temperaturen op wateragar (fig. 2). Met een sedimentatietoren werd gezorgd voor een regelmatige verdeling van de sporen in de gewenste dichtheid. Vijfhonderd vrijliggende sporen per petrischaal werden als gekiemd of ongekiemd geklassificeerd en geteld.

Een spore werd als gekiemd beschouwd wanneer de kiembuis langer was dan de kleinste sporediameter. Als vrijliggende sporen werden die sporen gerekend, die door een afstand van tenminste 1 sporediameter van andere sporen gescheiden waren. Het kiempercentage is het aantal gekiemde sporen uitgedrukt als percentage van het aantal getelde sporen. Niet alle sporen kiemen op hetzelfde tijdstip. De frequentieverdeling van de kiemingstijdstippen uitgezet tegen de tijd kan worden beschouwd als een normaalverdeling. De kiemtijd is dan het gemiddelde tijdstip van kieming in uren, gerekend vanaf het tijdstip van sporensedimentatie. In de grafieken is niet het kiempercentage zelf, maar de probit van het kiempercentage uitgezet tegen de tijd. De regressielijn van probit kiempercentage op de tijd wordt probitlijn genoemd. De kiemsnelheid is de tangens van de hellingshoek van de probitlijn in probit-eenheden per uur.

In tabel 1 zijn de proefomstandigheden gespecificeerd. Gemeten naar het kiempercentage wordt een lichtintensiteit van 7.600 lux door de sporen als donker ervaren; 3.000 lux geeft echter al een remmende werking op de kiembuisgroei (tabel 2). Sporen gevormd in duisternis zijn lichtgevoeliger dan sporen gevormd in licht (tabel 3), zulks onafhankelijk van de ouderdom der sporenhoopjes. Hydratatie maakt sporen sterk lichtgevoelig. Sporen gevormd in duisternis bij lage luchtvochtigheid en ongehydrateerd zijn ten dele lichtgevoelig (tabel 5). De kieming in donker verloopt, onafhankelijk van de voorbehandeling, snel en volledig (fig. 3). De kieming van sporen gevormd in licht en ongehydrateerd is zelfs bij hoge lichtintensiteit niet vertraagd (fig. 4). Tabel 8 laat zien dat de kieming van lichtgevoelige sporen in licht niet alleen vertraagd, maar soms ook gedeeltelijk verhinderd wordt; blijkbaar wordt een deel der sporen beschadigd.

Het afwijkende gedrag van sporen gevormd in duisternis bij lage luchtvochtigheid en ongehydrateerd wordt verklaard door het sporenmonster opgebouwd te denken uit drie sub-populaties: A. lichtongevoelig, direct na invallen van de duisternis gevormd en nog van de voorafgaande lichtperiode geprofiteerd hebbend; B. lichtgevoelig; C. inert of dood.

Grafische toetsing na schatting van de groottes van de sub-populaties bevestigt deze veronderstelling (tabel 6, fig. 6). Een nadere aanwijzing vormt de inverse relatie tussen de tijdsduur van de sporeproduktieperiode en de grootte van de sub-populatie A. Hydratatie van de sporen gevormd in duisternis bij lage luchtvochtigheid heeft in het geval van sub-populatie B tot gevolg een verlenging van de kiemtijd en verhoging van de kiemsnelheid (tabel 7, fig. 7). Na vier uur kieming bij hoge lichtintensiteit neemt sub-populatie C toe ten koste van B. Het resterende deel van sub-populatie B ontkiemt snel zodra het licht wordt weggenomen, waarbij de kiemsnelheid tamelijk onafhankelijk is van de lengte van de voorafgaande belichtingstijd (fig. 8, tabel 9). De lichtremming is

dus gedeeltelijk reversibel. Fig. 9 beeldt de stochastische conceptie van de kieming van de sub-populaties uit.

Nieuw is het inzicht, dat bij bruine roest van tarwe de omstandigheden van licht en luchtvochtigheid tijdens de sporevorming het kiemgedrag van uredosporen ten opzichte van licht sterk beïnvloeden.

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