

UNCLASSIFIED

AD 410395

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

410395

CATALOGED BY DDC

AS AD NO. \_\_\_\_\_

410395

TECHNICAL MANUSCRIPT 73

LIGHT INHIBITION  
OF UREDOSPORE GERMINATION  
IN *Puccinia graminis* var. *tritici*

JUNE 1963

UNITED STATES ARMY  
BIOLOGICAL LABORATORIES  
FORT DETRICK

NO OTS

U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY  
U.S. ARMY BIOLOGICAL LABORATORIES  
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 73

LIGHT INHIBITION  
OF UREDOSPORE GERMINATION  
IN PUCCINIA GRAMINIS VAR. TRITICI

The work reported here was performed under Project 4B11-01-004, "Anticrop Warfare Research." The Expenditure order was 2084. This material was originally submitted as manuscript 5188.

Curtis V. Givan  
K. R. Bromfield

Crops Division  
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1C022301A066

June 1963

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

Foreign announcement and dissemination of this document by DDC is limited.

The information in this document has not been cleared for release to the public.

#### ACKNOWLEDGMENT

The authors wish to express their appreciation to Drs. C. G. Schmitt, F. O. Smith, and C. H. Kingsolver for assistance in preparation of the manuscript.

#### ABSTRACT

Uredospores of Puccinia graminis var. tritici were germinated on one per cent water agar at 10° and 20°C under various light conditions. Short-period (two-hour) germination was inhibited by light intensities above 200 foot-candles; however, this was not a permanent and irreversible inhibition, but only a depression of the initial rate of germination, since germination percentage for longer incubation periods (6 to 8 hours) was virtually the same for spores germinating in darkness and in continuous light. Even for short incubation periods, light inhibition was reversible by a subsequent dark period. The decrease in germination rate due to light is temperature-sensitive, the degree of inhibition with a given light intensity being much greater at 10°C than at higher temperatures. Additional evidence for temperature sensitivity is that intensities too low to be inhibitory at 20°C strongly suppress germination at 10°C. The temperature sensitivity indicates the probable involvement of enzymatic as well as photochemical reactions in the light response.

CONTENTS

|                                     |    |
|-------------------------------------|----|
| Acknowledgments . . . . .           | 3  |
| Abstract. . . . .                   | 3  |
| I. INTRODUCTION . . . . .           | 5  |
| II. MATERIALS AND METHODS . . . . . | 6  |
| III. RESULTS . . . . .              | 7  |
| IV. DISCUSSION . . . . .            | 11 |
| Literature Cited . . . . .          | 12 |

FIGURES

|                                                                                                                                                                                            |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 1. Rate of Germination Under Continuous Light (400 f.c.) and Continuous Darkness, Based Upon Germination Percentages Observed After Two Hours' and Six Hours' Incubation at 20°C . . . . . | 9  |
| 2. Rate of Germination Under Continuous Light (400 f.c.) and Continuous Darkness, Based Upon Germination Percentages Observed After Two Hours' and Six Hours' Incubation at 10°C . . . . . | 10 |

TABLES

|                                                                                                                      |   |
|----------------------------------------------------------------------------------------------------------------------|---|
| I. Germination Percentage of Uredospores Incubated For Two Hours at 10° and 20°C Under Four Light Regimens . . . . . | 7 |
|----------------------------------------------------------------------------------------------------------------------|---|

## I. INTRODUCTION

The effects of light on the germination of uredospores of various rusts have been studied in the past by a number of investigators. Stock<sup>1</sup> and Dillon Weston<sup>2</sup> found that light was inhibitory to the germination of Puccinia graminis var. tritici uredospores. Wilson,<sup>3</sup> working with Uromyces phaseoli var. typica, reported a similar inhibition. Stroede<sup>4</sup> observed that light inhibited uredospore germination in Puccinia glumarum f. sp. tritici (= Puccinia striiformis), the inhibition occurring only at the higher temperatures investigated. McCracken and Burleigh,<sup>5</sup> however, found that light promoted uredospore germination in P. striiformis, promotion occurring at higher temperatures (13.5°C) but not at lower ones (2°C). Sharp et al.,<sup>6</sup> investigating the effects of various light intensities on uredospore germination in P. graminis var. tritici, found that intensities in excess of 300 foot-candles inhibited germination, the degree of inhibition being roughly proportional to intensity in the range of 300 to 1000 foot-candles. The conclusions of the latter investigators were based on per cent germination observed after two hours' incubation. Cochrane<sup>7</sup> observed that artificial light of quite high intensity (1250 f.c.) depressed both the rate and final level of germination in uredospores of Phragmidium mucronatum. An intensity of 200 foot-candles caused a slight depression of the initial germination rate, but had no effect on the final germination percentage.

Stock,<sup>1</sup> though presenting no actual germination figures, stated in his report that the initial inhibitory effects of light were overcome after several hours' incubation, since illuminated and nonilluminated spores germinated at similar levels after long incubation periods. Dillon Weston<sup>2</sup> did not report recovery during the illumination period, but noted that spores exposed to light for several hours were able to germinate when later placed in conditions of darkness or dim light. Thus, there were reports that light inhibition could be overcome both in darkness and in the light; however, since neither Dillon Weston nor Stock reported recovery both in darkness and in the light, and since neither investigator stated the light intensity employed, the relative rates of recovery in light and in darkness were not known. The principal aim of the present study was to re-examine the rates and ultimate levels of germination of spores incubated in darkness and in light of known intensity. In view of the reports of Stroede<sup>4</sup> and McCracken and Burleigh<sup>5</sup> that light responses in other rusts were temperature-sensitive, the present investigations were carried out with particular reference to the effects of temperature on the initial response of the uredospores to light and on the rate of recovery, both in darkness and in the light.

## II. MATERIALS AND METHODS

The experiments reported here fall into two major categories: (1) studies concerning rate of germination in continuous light, and (2) studies of dark reversibility of light inhibition. Both studies were carried out at 10°C and 20°C in order to determine the effects of temperature on inhibition and upon recovery. Uredospores of race 56, Puccinia graminis var. tritici (Eriks. & E. Henn.) Guyot were used in all cases. These spores were grown on seedlings of Baart wheat (C. I. 1697) at 20-30°C, collected with a cyclone collecting device, and stored in screw-cap vials at 4°C until used. Maximum age of spores was 60 days.

For the studies of germination rate in continuous light, uredospores were incubated in continuous darkness and in continuous light at 10° and 20°C for periods of two and six hours on one per cent water agar. In a few instances longer incubation periods were also employed. The germination percentage was then determined after the two- and six-hour incubation periods. To investigate reversibility by darkness, spores were incubated for a two-hour period at 10° and 20°C under four light-dark regimens: two hours' continuous darkness, two hours' continuous light, one hour of light followed by an hour of darkness, and one hour of darkness followed by an hour of light.

The light intensity employed in these studies was approximately 400 foot-candles at the surface of the agar as measured with a Weston illumination meter. A bank of eighteen fluorescent tubes (General Electric, Standard Warm White) served as the light source. All experiments were repeated on several different days, and each day's experiment was done in quadruplicate, two hundred spores on each of four plates being counted for each light or dark treatment.

### III. RESULTS

The results of a typical experiment showing rates of germination in continuous light and in darkness at 20°C are shown in Figure 1. Light decreases the initial rate of germination with respect to that of spores incubated in the dark. However, after the first two hours, the rate of germination of the dark controls drops off rapidly, whereas that of the spores incubated in the light remains at a more or less constant rate for the entire six-hour period. This means that the inhibition produced by light is much less pronounced after six hours than after two hours of incubation; thus, the long-term depression of germination is far less than would be indicated by determining the inhibition after two hours' incubation alone, as was done by Sharp *et al.*<sup>6</sup> In a few experiments incubation periods in excess of six hours were employed; after eight to ten hours, the germination level of spores incubated in the light very closely approximated that of spores germinating in continuous darkness. These results entirely corroborate the earlier findings of Stock.<sup>1</sup>

The results of five dark reversibility experiments are shown in Table I. As in experiments of the previous type, the highest level of germination occurred when spores were incubated in the dark. Also as previously shown, the germination of spores incubated in continuous light for two hours was considerably depressed. Inhibition was invariably observed when light was supplied for half the incubation period (either the first or second hour following inoculation); but in either case the depression was far less than that produced by two hours of continuous light, indicating that recovery in the dark proceeds much more rapidly than in the light, at least for short incubation periods.

TABLE I. GERMINATION PERCENTAGE OF UREDOSPORES INCUBATED FOR TWO HOURS AT 10° AND 20°C UNDER FOUR LIGHT REGIMENS

| Experiment | Incubation Temperature, °C | Germination Percentage |                              |                              |            |
|------------|----------------------------|------------------------|------------------------------|------------------------------|------------|
|            |                            | 2 hr. light            | 1 hr. light, then 1 hr. dark | 1 hr. dark, then 1 hr. light | 2 hr. dark |
| 1          | 20                         | 7                      | 35                           | 30                           | 43         |
| 2          | 20                         | 64                     | 72                           | 73                           | 78         |
| 3          | 20                         | 31                     | 63                           | 55                           | 64         |
| 4          | 10                         | 5                      | 12                           | 12                           | 21         |
| 5          | 10                         | 2                      | 30                           | 44                           | 58         |

The effects of temperature on the germination of spores subjected to continuous illumination are shown in Figures 1 and 2. At 10°C (Figure 2) the initial depression of the germination rate was much more marked than at 20°C (Figure 1), the percentage germination in the former case being virtually zero after two hours' incubation under 400 foot-candles of light. (At 15°C depression was greater than at 20°C, but less than at 10°C). Subsequently however, the rate of germination at 10°C increased rapidly, and after six to eight hours, the germination percentage closely approximated that of the controls, indicating that the recovery rate following the initially strong inhibition is comparable to that observed at 20°C. Spores incubated in the dark at 10°C displayed a rate of germination similar to that observed at 20°C (an observation also made earlier by Stock). Increased light sensitivity at 10°C was also indicated in experiments with a reduced light intensity of 200 f.c. Such an intensity markedly lowered the germination of spores incubated at 10°C but did not affect that of spores incubated at 20°C. When the spores were given four light regimens at 10°C (see Table I) relatively high germination levels were obtained except where continuous light was supplied, showing that dark reversibility of photoinhibition occurs readily at this temperature just as at 20°C.

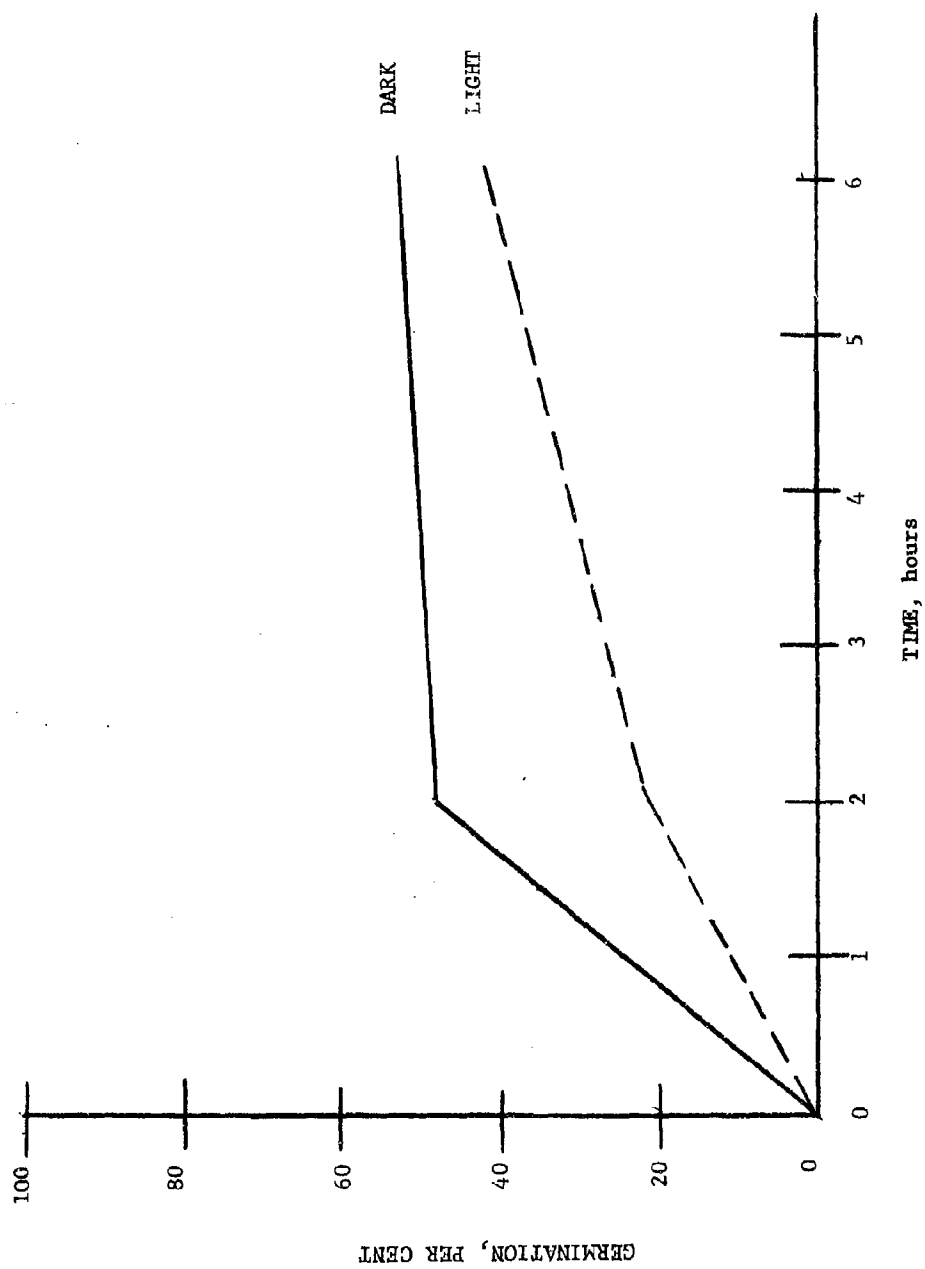


Figure 1. Rate of Germination under Continuous Light (400f.c.) and Continuous Darkness, Based upon Germination Percentages Observed after Two Hours' and Six Hours' Incubation at 20°C.

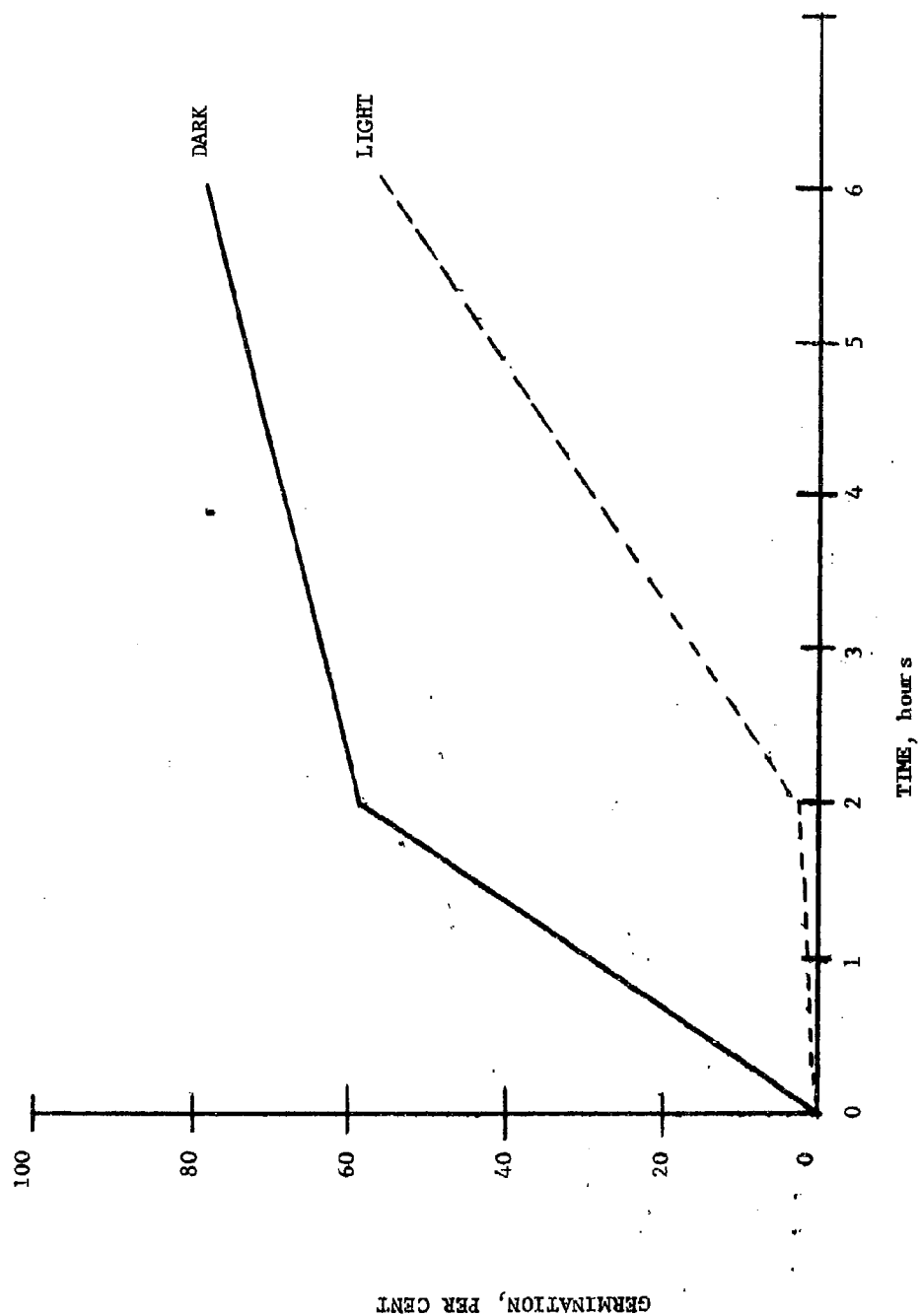


Figure 2. Rate of Germination Under Continuous Light (400 f.c.) and Continuous Darkness, Based Upon Germination Percentages Observed After Two Hours' and Six Hours' Incubation at 10°C.

#### IV. DISCUSSION

It may be concluded from the data reported here that the light-induced reduction of uredospore germination in *P. graminis* var. *tritici* is not an absolute inhibition, but rather a depression of the germination rate during the period immediately following placement of the spores on a substratum favorable for germination, as Stock<sup>1</sup> reported. Spores incubated in the dark germinate rapidly at first but more slowly as time progresses. Those incubated in the light, however, show a rate of germination that is initially low but persists for several hours, and in some cases actually increases. As a result, after relatively long incubation periods, there is not the large difference in germination observed when spores have been incubated for only a short time.

Furthermore, it is clear that light inhibition is readily reversible by subsequent exposure to darkness, as indicated by Dillon Weston,<sup>2</sup> since spores incubated under one hour of light followed by an hour of darkness show considerably better germination than those incubated in continuous light for two hours. Thus, recovery is much more rapid in the dark than in the light (at least for short incubation periods), and it occurs rapidly at both 10° and 20°C.

The evidence for the disappearance of photoinhibition in darkness and in light and for temperature sensitivity of the response allows some insight into the mechanism of the phenomenon. The temperature-sensitive nature of the response strongly suggests that enzymatic as well as photochemical reactions are involved. Therefore, it appears likely that an initial photochemical reaction is followed by a series of enzymatic processes that in some way depress the initial germination rate. This may be due to the production of a metabolic inhibitor that initially lowers the germination rate but subsequently breaks down. The difference in recovery rates in darkness and in light may be accounted for by assuming the following: the formation of the inhibitory substance proceeds rapidly when the spores are placed in the light, the rate of inhibitor formation remaining constant throughout the incubation period for any given light intensity. However, there is a delay in the initiation of the reactions that break down the inhibitor. Thus, in the early stages of the incubation period, there is an accumulation of inhibitor within the spores. In continuous light, the reactions breaking down the inhibitor eventually move rapidly enough to deplete the endogenous concentration of the inhibitor, so that germination can proceed. Transfer of the spores to darkness causes production of inhibitor to cease, so that the concentration becomes reduced quite rapidly with a concomitant increase in the germination rate.

The severity of the inhibition at lower temperatures may be accounted for by assuming either that enzymatic reactions having a low temperature optimum are involved in the production of the inhibitor, or alternatively, that enzymes having a relatively high temperature optimum are responsible for its breakdown. The rapid rate of dark recovery at 10°C tends to argue against the latter alternative. It must, however, be emphasized that there is at present no direct physiological or biochemical evidence for the occurrence of such an inhibitor, and the inhibitory effects of light may not involve inhibitor formation.

LITERATURE CITED

1. Stock, F. "Untersuchungen über Keimung und Keimschlauchwachstum der Uredosporen einiger Getreideroste," *Phytopathol. Z.* 3:231-279, 1931.
2. Dillon Weston, W. A. R. "The reaction of disease organisms to certain wave lengths in the visible and invisible spectrum," *Phytopathol. Z.* 4:229-246, 1932.
3. Wilson, E. M. "Aspartic and glutamic acid as self-inhibitors of uredospore germination," *Phytopathology* 48:595-600, 1958.
4. Stroede, W. "Über den Einfluss von Temperatur und Licht auf die Keimung der Uredosporen von Puccinia glumarum f. sp. tritici (Schmidt) Erikss. et Hen.," *Phytopathol. Z.* 5:613-624, 1933.
5. McCracken, F. I., and Burleigh, J. R. "Influence of light and temperature on in vitro germination of Puccinia striiformis uredospores," *Phytopathology* 52:742, 1962.
6. Sharp, E. L.; Schmitt, C. G.; Staley, J. M.; and Kingsolver, C. H. "Some critical factors involved in establishment of Puccinia graminis var. tritici," *Phytopathology* 48:469-474, 1958.
7. Cochrane, V. W. "The effect of artificial light on germination of urediospores of Phragmidium mucronatum (Fr.) Schlecht. *Phytopathology* 35:458-462, 1945.