STUDIES IN CEREAL DISEASES

VI

A Study of the Effect of Environmental Factors on the Variability of Physiologic Forms of Puccinia Graminis Tritici. Erikss. and Henn.

By
THORVALDUR JOHNSON
DOMINION RUST RESEARCH LABORATORY
WINNIPEG, MAN.

DIVISION OF BOTANY
DOMINION EXPERIMENTAL FARMS

H. T. GÜSSOW
DOMINION BOTANIST

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A Study of the Effect of Environmental Factors on the Variability of Physiologic Forms of *Puccinia Graminis Tritici.* Erikss. and Henn.*

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THORVALDUR JOHNSON

Dominion Rust Research Laboratory, Winnipeg, Man.

INTRODUCTION

The effect of the environment on rust development has, in the past, received less attention than many other phases of rust investigation. In the work reported in this paper an attempt was made to determine the effect of some of the more important factors of the environment on the development of *Puccinia graminis tritici* Erikss. and Henn.

This study falls naturally into two divisions. The first is concerned with the effect of environmental conditions on the development of the uredinial stage of wheat stem rust; the second deals with their effect on the formation and germination of the teliospores. In both these fields the rôle of environment has been made manifest, partly through observation, partly through controlled experiments. As the environment, however, is composed of several constituents the combined action of which brings about the ultimate result, it is only through controlled experiments that it is possible to reveal the part which each of these environmental factors plays in producing the total effect. Accurate control of some of these factors is difficult to obtain and sometimes entails considerable expense. Consequently, such control is not always available to investigators, and, in its absence, the influence of certain external conditions remains a matter of conjecture rather than of definite knowledge.

In dealing with the effect of environment on the development of stem rust, the only factor under absolute control was temperature. By varying temperature while other conditions remained relatively constant, it was possible to ascertain the part which that factor played in the development of the parasite on its host. Other external conditions, such as light, humidity and carbon-dioxide concentration, were under imperfect control, so that their relative importance can be stated with less accuracy.

The study on teliospore germination, which forms the second part of this paper, was undertaken chiefly to discover a practicable method of germinating teliospores formed in the greenhouse. Such teliospores had previously been found to be very erratic in their germination as might, perhaps, be expected, since they are often produced under conditions differing widely from the conditions found in nature. Two elements, especially, were taken into consideration: first, the conditions obtaining during the formation of the spores; second, the conditions to which the spores were exposed subsequent to their formation. From the results, it is evident, that both these elements are of importance as determiners of the germinability of the spores.

* Submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

PART I

Studies on the Effects of Environmental Factors on the Uredinial Development of Certain Physiologic Forms of *Puccinia Graminis Tritici.*

THE EFFECT OF TEMPERATURE ON UREDINIAL DEVELOPMENT WITH SPECIAL REFERENCE TO THE X-REACTION

It has long been recognized that temperature exerts a pronounced effect on the development of the uredinial stage of rusts. Much of the evidence relative to this effect, however, has been derived from observation rather than from controlled experiments. Gassner (11) states that in South America the various cereal rusts are affected differently by climatic conditions. *Puccinia graminis* Pers. was commonly found in early summer and autumn, while *Puccinia triticina Erikss.* and *Puccinia coronifera* Kleb. produced new infections at all seasons. This suggests, in the case of *Puccinia graminis,* that temperature, which varies with the seasons, may be, at least partially, instrumental in determining the spread of the rust. Stakman and Lambert (29), in a discussion on the relation of temperature to the occurrence of stem rust epidemics in the United States, concluded that " there has been a tendency for destructive epidemics to develop in warm growing seasons and for cool seasons to be comparatively free from rust."

While the investigation here described was in progress, Waterhouse (33) published experimental results which show that seasonal variations in climate at Sidney, Australia, exert a marked influence on the rust reactions of wheat, oats, and barley seedlings grown in the greenhouse. This was true especially for stem rust on seedlings of wheat varieties which exhibited the so-called X-reaction. In general, these varieties were resistant during the winter, susceptible during the summer, but showed the characteristic X-type of reaction in the spring and fall. This author concluded that " important differences occur in the reactions of hosts to rust attack in the plant house. These differences may be extreme. Complete susceptibility shown by a rust under summer conditions may change to complete resistance in the winter." It seems obvious that temperature and light are the environmental conditions mainly responsible for these changes.

Observations made by the writer in the course of determining physiologic forms of *P. graminis tritici* under greenhouse conditions likewise suggested that temperature had considerable influence on rust reactions. This was particularly the case with physiologic forms which produced the X-reaction on several varieties of durum wheat. The variability of the reactions on these varieties, from time to time, was so great as to cause considerable difficulty in determining the identities of these physiologic forms. Eventually, temperature-control apparatus was installed in two sections of the greenhouse, and an effort was made to determine the extent of these fluctuations under conditions of controlled temperature.

The work presented in this paper on the effect of temperature on uredinal development can not be considered more than a preliminary study. It demonstrates that temperature has a far-reaching effect, but it does not solve the undoubtedly complicated mechanism by which this effect is brought about. A further study along chemical or physiological lines must be made.

Methods

Apparatus for the control of temperature was installed in the greenhouses of the Dominion Rust Research Laboratory, at Winnipeg, about November 15, 1929. The apparatus was installed in two sections of the greenhouse and hence only two accurately controlled temperatures were available at one time. In selecting the two temperatures, it was decided to maintain one section of the greenhouse somewhat above and the other somewhat below the average greenhouse temperature. This temperature, which runs between 65° F. and 70° F., appears to be near the optimum for the development of both the wheat seedlings and the stem-rust organism. Thus the temperature ranges selected for the controlled sections of the greenhouse, namely, 55°—60° F., and 70°—75° F., were, respectively, below and above the assumed optimum. Some fluctuation occurred, but, for the most part, the temperature remained within the limits stated. A record was kept of the temperature variations by means of thermographs. Figure 1 shows one of these weekly records selected at random. The humidity of the air varied considerably in each section during different parts of the day, but, in general, the humidities of the two sections were comparable. Conditions of light did not differ appreciably in the two sections of the greenhouse except on very cold winter days when the glass of the section kept at the lower temperature became more heavily frosted, thus reducing the light somewhat. In both sections, during the period from November 15, 1929, to February 15, 1930, the daylight was supplemented by artificial light of equal intensity. Consequently, environmental conditions, other than temperature, were so nearly alike in the two sections that it seems safe to ascribe any differences in rust development to the influence of temperature.

The object primarily in view was to study the effect of these temperatures on the development of certain physiologic forms of wheat stem rust, which varied considerably from time to time on some of the differential hosts. These had previously been identified as forms which produced an X-reaction on certain hosts, and will, for the sake of brevity, be designated here as "X-forms." Other forms, owing to the variability of the host reactions, were still in the greenhouse awaiting identification. These also were included in the experiments.

In general, the procedure followed was to inoculate two sets of differential hosts by each physiologic form. Prior to inoculation all the host plants were grown under the same conditions at a temperature intermediate between those obtaining in the controlled sections. One of these sets was kept in each of the controlled sections from the time of inoculation until observations could be made on the rust reaction. Care was taken to place such sets in corresponding positions in the two greenhouses so that conditions, rather than temperature, might be as nearly equivalent as possible. The fact that incubation for the formation of uredinia required from three to five days longer in the cooler section was taken into consideration in making the observations on rust development. Hence, readings of the rust reactions were, as a rule, taken correspondingly later in the cooler section.

The symbols used in recording the rust reactions are those originally employed by Stakman and Levine (30). The arabic numerals "0" to "4" indicate the types of infection in order of increasing severity. An additional type is represented by "X". A description of these infection types is as follows:—

Type 0. Host immune. No uredinia are formed; hypersensitive flecks are frequently present. The reaction is then indicated by "0;".

Type 1. Host very resistant. Uredinia are minute, each being surrounded by a well defined necrotic area.

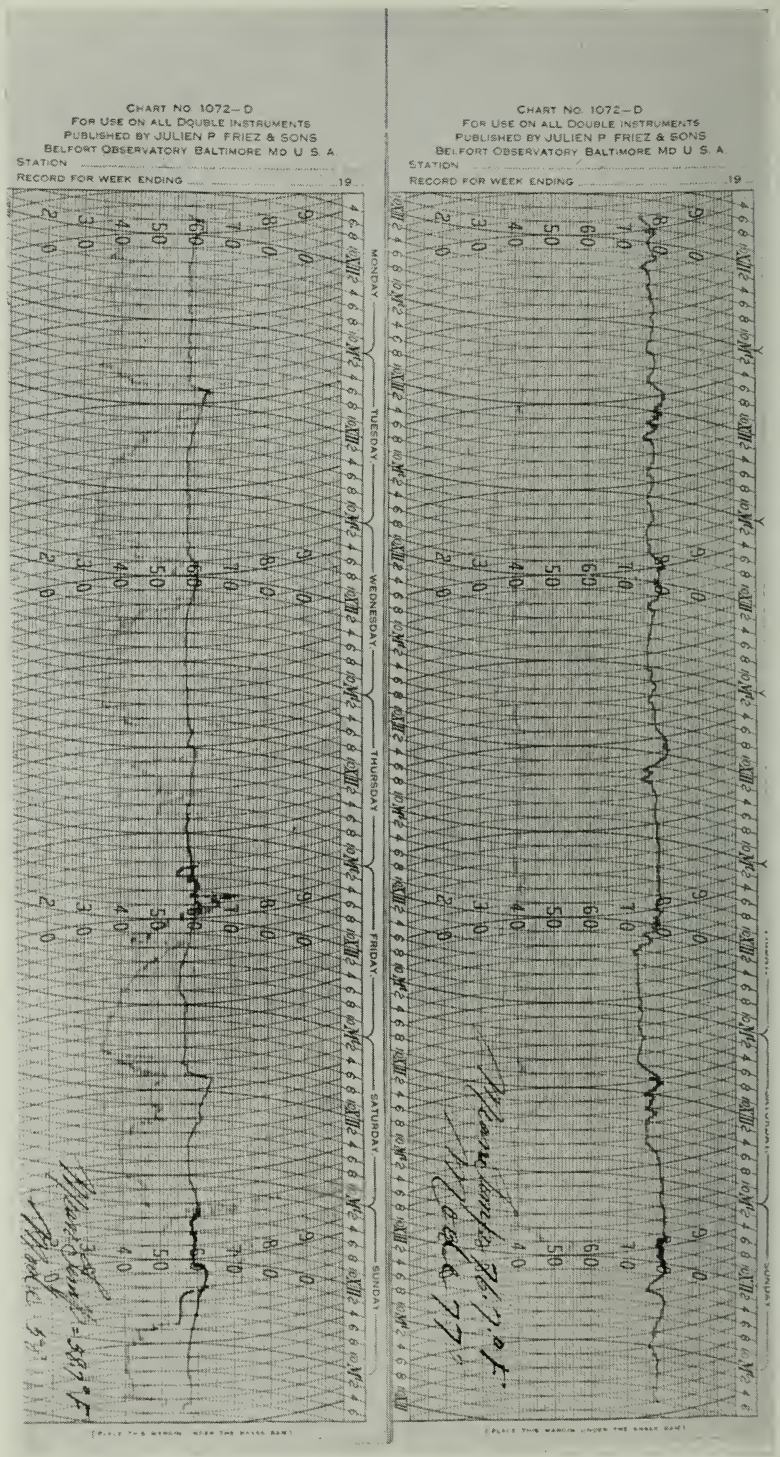

FIG. 1.—Charts showing thermographic records for the two greenhouse sections under temperature control during the week ending January 27, 1930.

Type 2. Host moderately resistant. Uredinia are small to medium in size, and are commonly found in islands of green tissue surrounded by necrotic halos.

Type 3. Host moderately susceptible. Uredinia are medium in size, and do not coalesce very frequently. Hypersensitiveness is absent, but the pustules may be surrounded by chlorotic areas.

Type 4. Host very susceptible. Uredinia are large and generally confluent. Hypersensitiveness is absent, but slight chlorosis may accompany pustules.

Type X. Host reaction heterogeneous. Uredinia on the same leaf vary in size; frequently all the types of infection are found occurring together on the same leaf.

The signs (=), (−), (±), (+) are used to indicate quantitative variations in the above types.

The hosts studied were selected from the twelve differential hosts employed by Stakman and Levine (30) for the determination of physiologic forms of *Puccinia graminis tritici.* A list of these hosts, including the source of each, will be found in Table 1.

TABLE 1.—Differential Hosts Used for the Determination of Physiologic Forms of *Puccinia graminis tritici*

Species of Triticum	Variety	Source
T. compactum	Little Club	C.I. 4066
T. vulgare	Marquis	Ottawa No. 15
T. vulgare	Marquis x Kanred	R.L. 226
T. vulgare	Kota	C.I. 5878
T. durum	Arnautka	(Selection from C.I. 1493 made at Dom. Rust Res. Lab.)
T. durum	Mindum	C.I. 5296
T. durum	Speltz Marz	C.I. 6236
T. durum	Kubanka	(Selection from Kubanka C.I. 2094 made at Dom. Rust Res. Lab.)
T. durum	Acme	C.I. 5284
T. monococcum	Einkorn	C.I. 2433
T. dicoccum	Vernal emmer	C.I. 3686
T. dicoccum	Khapli	C.I. 4013

Experiments on the Effect of Temperature on the Development of the X-Reaction on Durum Wheat Varieties

(*a*) From November 15, 1929, to February 15, 1930

The experiments described under this heading were carried out at the time of year when daylight is at its minimum, in both duration and intensity. After the middle of February, conditions of natural light improve rapidly, and, for this reason, experiments made after that date will be treated separately. From November 15 to February 15, the greenhouses were lighted artificially at night, for otherwise, rust development tends to be subnormal.

The differential varieties which most commonly show the X-reaction are Arnautka, Mindum, Speltz Marz, and Kubanka. The selection from Arnautka used in these experiments, however, shows this reaction but rarely. The other three varieties show a characteristic X-reaction when infected with certain physiologic forms. Other forms produce either a 4-type or a 1-type of reaction on these varieties. The experiments were designed to determine to what extent each of these three types of reaction, namely, X, 4, and 1, were capable of fluctuating through the influence of temperature. Lack of space prevents the tabulation of all the data gathered from these experiments. Typical results, however, are shown in Tables 2 to 9, and the results of all the experiments are

FIG. 2.—Reactions of Mindum seedlings to physiologic form 29. Left: leaves of plants kept at a mean temperature of 58.0°F. Right: leaves of plants kept at a mean temperature of 73.1°F.

FIG. 3.—Reactions of Speltz Marz seedlings to physiologic form 29. Left: leaves of plants kept at a mean temperature of 58.0°F. Right: leaves of plants kept at a mean temperature of 73·1°F.

summarized in Table 10. Ten of the 13 physiologic forms used are X-forms and were selected to determine the effect of temperature on the X-reaction. Two of the forms, namely, 11 and 17, produce a characteristic 4-type, and one, form 92, produces a 1- or 0;-type on these varieties.

An examination of Tables 2 to 6 will reveal that the reactions of Mindum, Speltz Marz, and Kubanka to the X-forms are influenced profoundly by temperature. Tables 7 and 8 show, on the other hand, that the 4-type of reaction is very little influenced by temperature, and the same holds true for the 1-type of reaction in Table 9. Table 10 summarizes the reactions of Mindum, Speltz Marz, and Kubanka. From a study of these tables it becomes apparent that none of these varieties produced a typical X-reaction at the two temperatures, 55°-60° F. and 70°-75° F., under the conditions of light that prevailed during the tests. At the lower temperature, the reaction was commonly 0; or X-; at the higher, it was commonly 4, and only very occasionally X. The only forms which tended to produce an X-reaction at the higher temperature were 48 and 86. Figures 2 and 3 show the characteristic reactions at the high and low temperatures of Mindum and Speltz Marz to form 29 and Figures 4 and 5 show the reactions of Speltz Marz and Kubanka to form 48. The mean temperatures at which these reactions were obtained will be found in Tables 2 and 5 respectively.

It is obvious from these results that the temperature which favours the formation of the X-reaction, under the conditions of light that prevailed, is somewhere between 60° F. and 75° F., and probably between 60° F. and 70° F. It seems also likely, although this is not so apparent, that there is some difference among the physiologic forms in the optimum temperature at which they produce the X-reaction. To demonstrate either, or both, of these facts it was necessary to alter the temperatures in the controlled sections of the greenhouse. Moreover, since the conditions of light changed as the winter progressed, and as it seemed possible that, at a later date, the temperatures used previously might not result in the same reactions, it became essential to work with three of four temperatures simultaneously. This was attempted in subsequent experiments because it was not considered safe to assume that the results obtained previously would hold under changed conditions of light.

It is apparent that the hosts which are capable of producing the X-reaction are more resistant to the X-forms at a low than at a high temperature. It had been observed, when such hosts were kept for two weeks or longer after inoculation in the low and then transferred to the high-temperature section, that no further increase in rust development took place. Either the host plant retained the resistance it had developed at the low temperature, or the fungous mycelium had been rendered so weak as to be incapable of recovering its virulence under congenial conditions.

Experiments were planned to determine how soon the host plant kept at a low temperature develops a resistance which can not be broken down by exposing it to a higher temperature; or, with reference to the fungus, to determine how short an exposure to a low temperature renders the fungus incapable of recovering its virulence when transferred to a higher temperature. Form 89 and form 48 were selected as characteristic X-forms. Speltz Marz seedlings, in seven pots, were inoculated by form 89 and Mindum seedlings by form 48. After inoculation, six of these pots were placed in the cool section while one was kept in the warm section as a check. One pot was removed from the cool to the warm section after intervals of 4, 6, 8, 10 and 12 days following inoculation. Notes taken 14 days after inoculation are shown in Tables 11 and 12. A second examination one week later revealed no perceptible further development. The first of these tables shows that form 89 is practically incapable of any further development on Speltz Marz

FIG. 4.—Reactions of Speltz Marz seedlings to physiologic form 48. Left: leaves of plants kept at a mean temperature of 58.0°F. Right: leaves of plants kept at a mean temperature of 73·6°F.

FIG. 5.—Reactions of Kubanka seedlings to physiologic form 48. Left: leaves of plants kept at a mean temperature of 58·0°F. Right: leaves of plants kept at a mean temperature of 73·6°F.

after a period of ten days at a temperature of about 57° F; the second table shows that the same holds true for form 48 on Mindum after eight days at the low temperature.

A single experiment was carried out to determine to what extent Kubanka seedlings infected by an X-form at a high temperature were capable of developing resistance when transferred to a low temperature. The procedure was the reverse of that described for the two previous experiments. Kubanka seedlings inoculated by form 87 were kept in the warm section at a mean temperature of 76·8° F. and transferred after 4, 5, 6, 8, and 10 days to the cool section, where the mean temperature was 59·7° F. Table 13 shows the results. There was no apparent difference in the rust reactions when notes were taken fifteen days after inoculation. Even the plants kept at the high temperature for only four days showed the same type of infection as those kept at that temperature for the whole period of fifteen days. In other words, the host plants were incapable of developing resistance to the fungus after the latter had been allowed four days of development at a congenial temperature, during which time it presumably had been able to establish itself thoroughly in the host tissues. This suggests that the first four days or less, after inoculation, constitute the critical period during which the subsequent relationship of host and fungus is in a large measure determined. It should be stated, however, that this generalization is applicable only to the peculiar host-parasite complex under discussion; for the same host variety infected by a physiologic form which does not produce an X-reaction will show no visible difference in its reactions at high and low temperatures.

TABLE 2.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 29 at High and Low Temperatures

	Physiologic form 29			
	Test No. 1		Test No. 2	
Mean temperature during test	73·1° F.	58·0° F.	74·0° F.	58·3° F.
Marquis	———	———	7 (4)− / 10	9 (2) + (3) + c / 10
Kanred	0 / 11	0 / 11	0 / 10	0 / 11
Arnautka	10 (3) + / 10	9 (3) − 1 (X) / 11 11	10 (4) / 10	11 (3), (4)− / 11
Mindum	8(4)− / 11	2(X)−; 2 / 6	4(3)+2(X)+ / 6 6	9(X)− / 9
Speltz Marz	9(3)+ / 11	9(X)= / 10	5(3)+ 5(X)+ / 10 10	7(X)− 4(; & 1) / 12 12
Kubanka	9(3)± / 11	4(X)−; 3 / 10	6(4)−1(X)+ / 9 9	7(X)1(3) / 9 9
Date of inoculation	29–11–29	29–11–29	27–12–29	27–12–29
Date of marking	14–12–29	14–12–29	10 –1–30	14– 1–30

Explanatory Note.—The reaction types are enclosed in parentheses. The figures preceding the reaction types indicate the number of leaves infected, *e.g.*, 8(4)− shows that 8 out of 11 leaves were infected and the reaction type was (4)−. A semi-colon indicates necrotic flecks; the figure following a semi-colon represents the number of leaves on which flecks appeared.

TABLE 3.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 32 at High and Low Temperatures

	Physiologic form 32			
	Test No. 1		Test No. 2	
Mean temperature during test	75·8° F.	58·8° F.	75·8° F.	60·1° F.
Marquis	11(4) 13	6(4) 11	———	———
Kanred	13(4) 13	13(3)+ 14	12(3)+ 13	12(3)± 13
Arnautka	13(4)+ 13	14(4) 15	10(3)+ 10	9(X) 1(3)± 10 10
Mindum	8(4)+ 9	1(X)−; 5 12	5(3)2(X) 8 8	0; 10 10
Speltz Marz	18(4)+ 18	1(X)−; 11 12	7(X) 6(3) 13 13	1(; & 1); 12 13
Kubanka	14(4) 14	6(X)=; 8 15	6(3)±3(X) 9 9	1(; & 1); 10 11
Einkorn	4(3)+ 7	4(3) 4	17(3)c 20	16(3) 20
Vernal	1(1) 5	0; 4 7	3(; & 1); 5 9	1(; & 1); 8 9
Date of inoculation	15–1–30	15–1–30	24–1–30	24–1–30
Date of marking	1–2–30	1–2–30	6–2–30	9–2–30

TABLE 4.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 38 at High and Low Temperatures

	Physiologic form 38			
	Test No. 1		Test No. 2	
Mean temperature during test	71·5° F.	58·1° F.	73·6° F.	58·0° F.
Marquis	5(2)/6	5(2)+/8	6(2)+/12	8(2)/9
Kanred	6(4)/9	8(3)/9	10(3)+/11	7(3)±/9
Mindum	5(4)+/7	3(X)/4	6(4)−1(X)+/8 8	0; 9/11
Speltz Marz	7(4)1(X)/9 9	5(X)3(3)±/9 9	8(4)− 3(X)+/11 11	1(X); 9/12
Kubanka	8(4)+/11	10(X)/10	8(4)1(X)/9 9	1(X)=; 8/10
Vernal	0; 7/8	0; 9/9	———	———
Date of inoculation	18–11–29	18–11–29	4–12–29	4–12–29
Date of marking	3–12–29	3–12–29	16–12–29	23–12–29

TABLE 5.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 48 at High and Low Temperatures

	Physiologic form 48			
	Test No. 1		Test No. 2	
Mean temperature during test	73·6° F.	58·1° F.	73·6° F.	58·0° F.
Marquis	5(2)/7	7(2)/11	7(2)/8	8(1)/8
Kanred	———	———	0/10	0/10
Arnautka	9(4)+/10	9(4)—/10	11(4)+/11	11(4)/11
Mindum	4(4)+1(X)+/7 7	0; 3/6	7(X)1(3)+/8 8	0; 6/7
Speltz Marz	9(4)+/10	2(3)− 1(X)−;4/10 10	6(X) 5(4)−/11 11	0; 7/10
Kubanka	11(4)+/11	5(X)−; 5/12	11(3)±/11	0; 10/11
Date of inoculation	21–11–29	21–11–29	4–12–29	4–12–29
Date of marking	3–12–29	3–12–29	16–12–29	23–12–29

TABLE 6.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 86 at High and Low Temperatures

——	Physiologic form 86	
Mean temperature during test	75·8° F.	60·1° F.
Marquis	8(3)± c 1(4)− / 10 10	8(3)+ / 11
Kanred	13(3)+ / 14	12(3) / 13
Kota	3(3)−; 1 / 8	3(; & 1); 6 / 10
Arnautka	10(3), (4)− / 10	11(3)+ / 12
Mindum	8(3)± 6(X) / 15 15	0; 10 / 11
Speltz Marz	10(X) 1(4)− / 11 11	2(; & 1); 9 / 12
Kubanka	17(3)± / 17	3(X) 5(; & 1); 3 / 12 12
Vernal	7(3)+ / 8	10(3)± / 10
Date of inoculation	24–1–30	24–1–30
Date of marking	6–2–30	9–2–30

TABLE 7.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 11 at High and Low Temperatures

——	Physiologic form 11	
Mean temperature during test	75·6° F.	59·7° F.
Marquis	7(4) — 14	12(3)+ — 14
Kanred	14(4)− — 15	10(3)+ — 14
Kota	12(3), (4)− — 13	9(3), (4) — 11
Arnautka	12(4)+ — 13	10(4) — 10
Mindum	12(4)+ — 13	10(4)− — 11
Speltz Marz	8(4) — 11	12(4)− — 12
Einkorn	4(3)+ — 5	8(3)+ — 12
Vernal	0; 3 — 5	0; 4 — 7
Date of inoculation	15–1–30	19–1–30
Date of marking	27–1–30	5–2–30

TABLE 8.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 17*a* at High and Low Temperatures

——	Physiologic form 17*a*	
Mean temperature during test	74·0° F.	58·3° F.
Marquis	8(3)+(4) / 12 — 1(3)± / 12	11(2) / 12
Kanred	0 / 10	0 / 12
Arnautka	7(4) / 9	10(4)– / 10
Mindum	6(4)– / 7	11(3)± / 15
Speltz Marz	10(4)– / 11	8(4)– / 10
Kubanka	10(4)– / 11	10(3)± / 10
Date of inoculation	27–12–29	27–12–29
Date of marking	10–1–30	14–1–30

TABLE 9.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 92 at High and Low Temperatures

——	Physiologic form 92	
Mean temperature during test	73·6° F.	56·9° F.
Marquis	6(4) 1(X) — — 9 9	6(X) — 8
Kanred	0 — 10	0 — 9
Kota	1(X)−; 1 — 10	1(X)−; 5 — 8
Arnautka	0; 10 —— 11	4(1); 4 — 8
Mindum	0; 7 — 9	1(1); 5 — 10
Speltz Marz	0; 2 — 10	5(1); 5 — 10
Kubanka	10(4)− — 11	10(3)+ — 10
Einkorn	10(1) — 12	7(1) — 7
Date of innoculation	5–12–29	5–12–29
Date of marking	17–12–29	21–12–29

TABLE 10—Mean Reactions of Mindum, Speltz Marz and Kubanka Seedlings to twelve Physiologic Forms of *Puccinia graminis tritici* at 55-60° F. and 70-75° F., from Nov. 15, 1929, to Feb. 15, 1930

Physiologic form	11				29						30						32					
Host variety	Mnd		SpM		Mnd		SpM		Kub		Mnd		SpM		Kub		Mnd		SpM		Kub	
Temperature (°F.)	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75
1st test	4 −	4+	4−	4	0;	4−	X−	3±	X−	3±	0;	4−	0;	4−	0;	4−	0;	4−	0;	4+	0;	4
2nd test	.....	.....	.....	.....	X−	3+	X−	3+	X	4−	.....	.....	.....	.....	.....	.....	0;	3	0;	X	0;	3±

Physiologic form	38						48						86						87					
Host variety	Mnd		SpM		Kub		Mnd		SpM		Kub		Mnd		SpM		Kub		Mnd		SpM		Kub	
Temperature (°F.)	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75
1st test	X	4	X	4	X	4+	0;	4+	0;	4+	X−	4+	0;	3±	0;	X	0;	3±	0;	4	X=	4	0;	4−
2nd test	0;	4−	0;	4−	0;	4−	0;	X	0;	X	0;	3±	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

Physiologic form	89						90						94						17					
Host variety	Mnd		SpM		Kub		Mnd		SpM		Kub		Mnd		SpM		Kub		Mnd		SpM		Kub	
Temperature (°F.)	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75	55 to 60	70 to 75
1st test	0;	4	0;	4	0;	4	0;	4	0;	4	.....	.....	X−	3+	X−	3+	X−	3	3±	4−	4−	4−	3±	4−
2nd test	0; X=	4	0;	4	X−	4	0;	4 +	0;	4+	0; X−	4+												

TABLE 11.—The Reactions of Speltz Marz Seedlings to Form 89, When Kept at a Mean Temperature of 56·8° F. for Four, Six, Eight, Ten and Twelve Days After Inoculation Before Removal to a High Temperature (74·8°F.)

Pot No.	Number of days at 56·8° F.	Number of days at 74·8° F.	Rust reaction
1	..	14	11 (4) 2(X)+ / 13 13
2	14	..	0; 13 / 14
3	4	10	8(3)+ 4(X) / 12 12
4	6	8	1(3)+ 9(X); 1 / 11 11
5	8	6	10(X)−; 4 / 15
6	10	4	4(X)=; 9 / 13
7	12	2	5(X)=; 7 / 13

TABLE 12.—The Reactions of Mindum Seedlings to form 48, When Kept at a Mean Temperature of 56·8°F. for Four, Six, Eight, Ten, and Twelve Days After Inoculation Before Removal to a High Temperature (74·8°F.).

Pot No.	Number of days at 56·8°F.	Number of days at 74·8°F.	Rust reaction
1	..	14	1(3)+6(X) / 8 8
2	14	..	1(; & 1); 3 / 8
3	4	10	3(X)−; 2 / 7
4	6	8	2(X)=; 5 / 10
5	8	6	0; 5 / 8
6	10	4	0; 7 / 7
7	12	2	0; 7 / 9

TABLE 13.—The Reactions of Kubanka Seedlings to Form 87, When Kept at a Mean Temperature of 76·8°F. for Four, Five, Six, Eight, and Ten Days After Inoculation Before Removal to a Low Temperature (59·7°F.).

Pot No.	Number of days at 76·8°F.	Number of days at 59·7°F.	Rust Reaction
1	15	..	12(4) / 12
2	..	15	9(X)= 1(; & 1); 2 / 13 13
3	4	11	6(3), (4)− 3(X)+ / 10 10
4	5	10	11(3), (4)− / 11
5	6	9	12(3), (4)− 2(X)+ / 16 16
6	8	7	18(4) / 18
7	10	5	11(4) / 11

(b) From February 20 to March 24, 1930.

The object of this second series of experiments was twofold: first, to determine to what extent the increased intensity and duration of daylight affected the X-reaction under the conditions of temperature which prevailed in previous experiments; second, to determine if all the X-forms used in the earlier experiments behaved similarly with respect to temperature. To accomplish the first of these objects, two sections of the greenhouse were maintained at the temperatures used in previous experiments. It was planned to compare the reactions of the differential hosts, particularly, Mindum, Speltz Marz, and Kubanka, with those obtained at the same temperatures during mid-winter, on the assumption that light was the only environmental variable which had changed. The second object was accomplished by bringing two other sections of the greenhouse under temperature control, thus making four sections available for a study of the effects of temperature on rust reactions. The temperature of the two additional sections was controlled by manipulation of the ventilators and the heating system. The temperature was observed and recorded hourly by attendants in the greenhouse from 7 a.m., to 8 p.m., and a thermographic record was kept. The control was resonably good except for occasional fluctuations at night. The temperatures at which it was sought to maintain the four greenhouse sections were respectively, 59° F., 64° F., 70° F., and 78° F. The reactions of differential hosts to ten X-forms were studied at the three lower temperatures, and their reactions to three forms were recorded at all four temepratures. Nine of these forms were the same ones which had been used in the earlier series of experiments. The tenth form, 76, was substituted for form 94 which was not available when this series of tests was begun.

Results typical of these tests will be found in Tables 14 to 18. A summary comprising the mean reactions of Mindum, Speltz Marz, and Kubanka is given

in Table 19. A study of these tables reveals that Mindum, Speltz Marz, and Kubanka produce a weak X-reaction to the majority of these forms at a temperature of 59° F., at which, during the middle of winter, the reactions were almost without exception 0; and 1. Figure 6 shows the reaction of Mindum to form 86 at four different temperatures. Since the intensity of light was the only factor in the environment which had changed appreciably during the interval between the first and second series of experiments, it must necessarily be held responsible for this difference in host reaction. This view is supported by experiments (unpublished data by Dr. Margaret Newton) in which plants of Mindum, Speltz Marz, and Kubanka, infected by X-forms were submitted to different light intensities. These plants invariably produced a weaker X-reaction at lower light intensities.

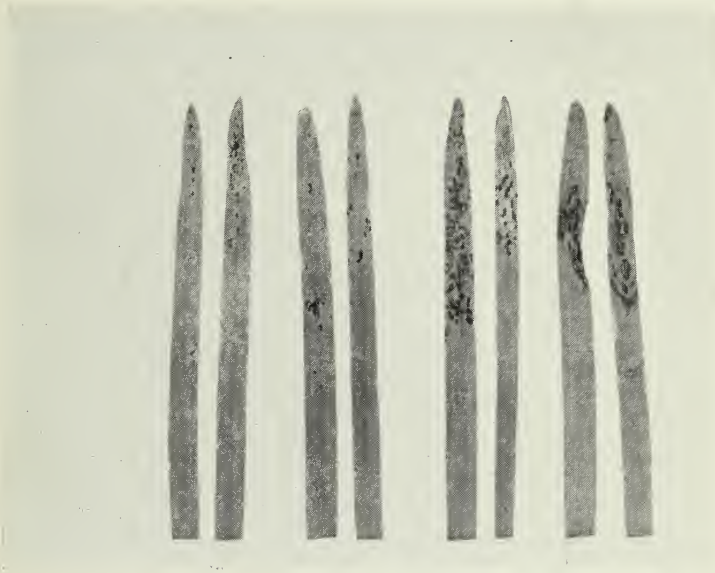

FIG. 6.—Reactions of Mindum seedlings to physiologic form 86 at four different temperatures. Left to right, leaves of plants kept at mean temperatures of 59·4°F., 66·3°F., 72·3°F., and 78·0°F.

Judging from a comparison of the results of the second series of experiments with those of the first series, it appears that an increase in light intensity tends to permit the development of the X-reaction at lower temperatures. In other words, when light intensity is high, the X-reaction can develop on a host which is immune at the same temperature under low light intensity.

Table 19 reveals that the varieties Mindum, Speltz Marz, and Kubanka do not produce identical reactions at the same temperature. In general, Mindum produces the lightest rust reaction, Kubanka the heaviest, while Speltz Marz occupies an intermediate position. This is most apparent at low temperatures; at progressively higher temperatures, the difference diminishes until it disappears in the temperature range where all three varieties produce a 4-type of infection.

An attempt was made to classify the ten X-forms used in the second series of experiments on the basis of the reaction of Mindum at the four temperatures employed. Previous observations had led the writer to the conclusion that the X-forms differ in their behaviour towards temperature, that is, they differ in the temperature ranges at which they are capable of showing the X-reaction on certain hosts. This classification, which is expressed graphically in Figure 7,

was based mainly on the second series of experiments, because these tests were performed, for the most part, simultaneously. These forms are arranged in the figure in the order of decreasing capacity to produce X-reactions at low temperatures. In other words, the forms which develop X-reactions at the lowest temperatures, are placed first, while those which require a higher temperature to produce X-reactions are placed last. The forms studied are rather similar in respect to the temperature ranges at which they produce the X-reaction. There is, however, a rather striking difference between the behaviour of forms 29 and 90 in comparison with form 48. The latter begins to produce an X-reaction at the temperature where form 90 has passed through the range in which it forms an X-type and begins to produce a 4-type of reaction.

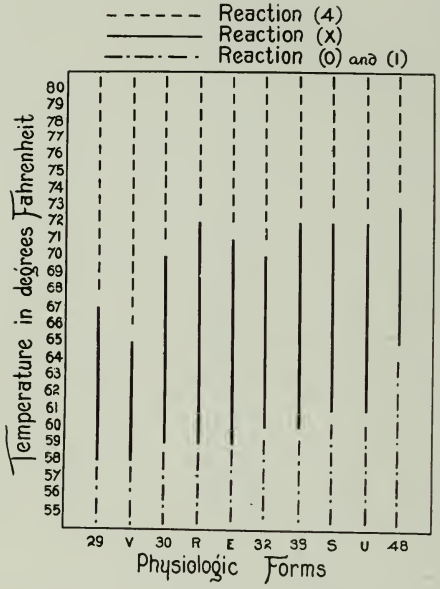

Fig. 7.—A diagrammatic representation of the behaviour of ten physiologic forms towards temperature, based on the reactions of the variety Mindum at several temperatures. The solid lines represent the temperature ranges at which the X-reaction is produced.

This result has an important bearing on the selection of a suitable temperature for the identification of physiologic forms. It is obvious from Figure 7 that there is only one temperature at which all these forms can be recognized as X-forms, namely 65° F. This, of course, does not imply that 65° F., is the proper temperature for the identification of physiologic forms at all seasons of the year, for a further increase in light intensity will probably result in shifting the range for X-formation downwards in the scale of temperature, so that, in mid-summer, for instance, when the light intensity is very high, the optimum temperature for the detection of an X-reaction may be considerably lower. This agrees with the results obtained by Waterhouse (33), who found that certain physiologic forms of *Puccinia graminis tritici* produced a 0;-type of reaction in the winter, an X-type in the spring and fall, and a 4-type in the summer.

The Effect of Temperature on the X-reactions of Other Host Varieties

Apart from Mindum, Speltz Marz, and Kubanka, several other differential hosts are capable of producing the X-reaction. Marquis, Kota, Acme, and Vernal emmer have all been observed to react in this manner when infected by certain physiologic forms. Since these, with the exception of Acme, are not closely related to the aforementioned durum varieties, it is of interest to discover how far the behaviour of their X-reactions to temperature parallels that of the X-type of reaction in the durums. The few tests made, indicate that the X-reactions of Kota and Vernal, at least, respond to the influence of temperature, although, in the case of Vernal, to a lesser extent than the X-reactions of the durums. Table 20 shows the reaction of Kota to form 91 at temperatures of 72° F., and 59° F., respectively. Table 21 shows the reaction of Vernal to form 82 at approximately the same temperatures, and Table 22, its reactions to form 85 at four different temperatures. (See also Fig. 8.) It is evident that temperature has markedly less effect on the X-reaction of Vernal than on the reactions of the durum varieties which invariably lose their X-type and become completely susceptible at 78° F.

FIG. 8.—Reactions of Vernal emmer to physiologic form 85 at four different temperatures. Left to right, leaves of plants kept at mean temperatures of 59·4°F., 66·3°F., 72·3°F., and 78·0°F.

Table 8 shows that Marquis is capable of reacting in a similar manner. The physiologic form by which Marquis was infected, temporarily given the number 17*a*, was previously identified as form 17. At a temperature of 74° F., the infection on Marquis was characteristic of form 17, namely, a 4-type; but at 58° F., Marquis produced a typical 2-type reaction. Subsequent tests proved that this discrepancy was not due to contamination.

The Effect of Temperature on the X-reactions of Older Plants

The preceding work has been concerned with the effect of environmental factors on the X-reactions of seedlings. Certain questions came to mind while this work was in progress. Is this type of rust reaction characteristic of seedlings alone, or does it occur on plants in later developmental stages? In what manner does the rust reaction of the latter respond to temperature when the plants are infected by X-forms? The following experiments were carried out to clear up these points.

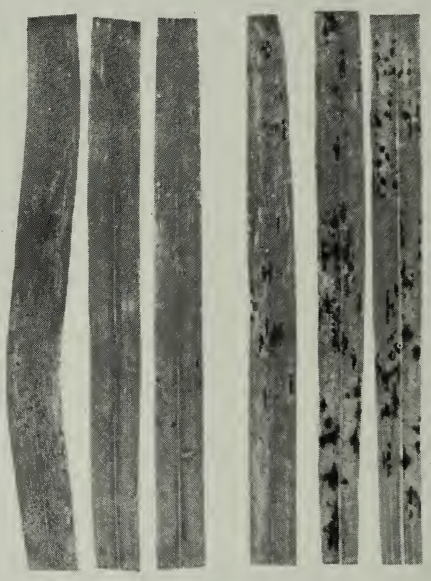

FIG. 9.—Reactions of leaves of Kubanka plants infected by physiologic form 86 after heading. Left: leaves of plants kept at a mean temperature of 59·8°F.; the uppermost leaf of stem at the left; the second leaf in the middle; the third leaf at the right. Right: leaves of plants kept at a mean temperature of 78·0°F. The leaves are arranged in the same order as those on the left.

Mindum and Speltz Marz plants were grown in six-inch flower pots until the plants reached the four leaf stage. At this stage they were inoculated by three physiologic forms to which the seedlings of these varieties produce a typical X-reaction. Mindum plants inoculated by forms 89 and 87, and Speltz Marz plants inoculated by form 29 were kept at mean temperatures of 75·6° F., and 57·8° F., respectively, until notes were taken on rust development. Table 23 shows the reactions of the four leaves of each plant at the two temperatures. The plants kept at the lower temperature were all immune; those at the higher temperature, either susceptible or heterogeneous in reaction. From these facts, it is evident that the rust reactions of plants at this stage of development correspond rather closely with those of seedlings in their relation to temperature.

The differences in reaction between the first, second, third, and fourth leaves of each plant are not very striking, although at the high temperature, the lower leaves appear to be slightly more susceptible than the upper ones.

Later, Mindum and Kubanka plants were infected by physiologic form 86 about two weeks after the emergence of the ears. This form produces an X-reaction on the seedlings of these varieties at normal temperatures. After inoculation, these plants were kept at mean temperatures of 60° F., and 78° F., for a period of three weeks. Only the uppermost three leaves of each plant were in a condition to be infected, the lower leaves having withered by the

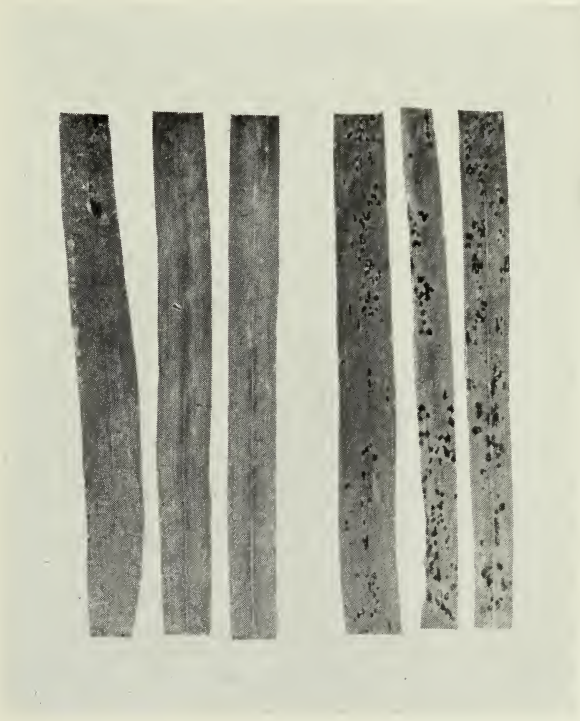

FIG. 10.—Reactions of leaves of Mindum plants infected by physiologic form 86 after heading. Left: leaves of plants kept at a mean temperature of 59·8°F.; the uppermost leaf of stem at the left; the second leaf in the middle; the third leaf at the right. Right: leaves of plants kept at a mean temperature of 78·0°F. The leaves are arranged in the same order as those on the left.

time of innoculation. Table 24 shows the reactions of these plants at the above-mentioned temperatures, and Figures 9, and 10 show the types of infection. A comparison of these reactions, with those of the same varieties in the seedling stage obtained simultaneously (Table 15 and Fig. 6) shows that there is very little difference in the temperature responses of the rust reactions of seedlings and fully developed plants. The latter, however, are slightly less susceptible than the former at 78° F. The results obtained at the lower temperature with seedlings and mature plants are in close agreement.

This comparison shows that the capacity of the host-parasite complex to respond to temperature is not a transitory phenomenon characteristic only of a certain developmental stage of the plant, but persists throughout its entire life.

TABLE 14.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 32 at Different Temperatures

——	Physiologic form 32		
Mean temperature during test	71·2°F.	64·4° F.	59·3° F.
Marquis	7(3), (4)−c / 9	9(3), (4)−c / 9	5(3)±c / 7
Kanred	14(4)− / 14	7(3)+ / 10	10(3)+ / 11
Arnautka	12(4)− / 12	12(4)− / 13	12(4)− / 13
Mindum	6(4)− / 10 3(X)+ / 10 1(X) / 10	7(X) / 9 1(X)+ / 9	1(; & 1); 9 / 12
Speltz Marz	6(4)− / 10 3(X)+ / 10	6(4)− / 13 6(X)+ / 13	6(X) / 11 3(X)= / 11
Kubanka	10(4) / 12	10(3), (4)− / 13 1(X)+ / 13 1 (X) / 13	
Date of inoculation	20–2–30	20–2–30	20–2–30
Date of marking.	5–3–30	5 3–30	9–3–30

TABLE 15.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 86 at Different Temperatures

——	Physiologic form 86			
Mean temperature during test	78·0° F.	72·3° F.	66·3° F.	59·4° F.
Kanred	11(4)+ / 13	9 (4) / 10	9(4)− / 9	11(3)+ / 11
Arnatuka	8(4)+ / 8	9(4) / 9	9(4)+ / 10	10(4)+ / 10
Mindum	9(4)+ / 10	5(X)+ / 9 4(3, 4) / 9	9(X) / 10	12(X)− / 12
Speltz Marz	11(4)+ / 11	7(X)+ / 10 3(3), (4)− / 10	9(X) / 11	10(X)− / 10
Kubanka	16(4)+ / 16	12(4) / 13	11(X)+ / 11	12(X) / 13
Vernal	10(4) / 10	11(4) / 13	9(4) / 9	10(4) / 10
Date of inoculation	4–3–30	4–3–30	4–3–30	4–3–30
Date of marking	16–3–30	17–3–30	20–3–30	22–3–30

TABLE 16.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 76 at Different Temperatures

——	Physiologic form 76			
Mean temperature during test	78·0° F.	71·0° F.	66·3° F.	59·4 °F.
Kanred	0 12	3(3)+ 11	1(3)+ 12	0 12
Arnautka	10(4)− 10	9(4)− 9	8(3)+ 9	6(3)+ 9
Mindum	11(4)− 11	4(X)+ 4(4)− 9 9	6(X) 2(4)− 9 9	7(X)− 1(X) 10 10
Speltz Marz	10(4)− 10	8(X)+ 2(4)− 11 11	6(X) 1(3)+ 9 9	5(X) 2(3)+ 9 9
Kubanka	10(4)− 10	12(4)− 12	7(X)+ 3(4)− 10 10	10(X) 11
Einkorn	13(3)± 15	11(3)+ 11	13(3)+ 15	13(3)+ 15
Vernal	8(X)− 1(1); 1 10 10	6(X)− 3(1); 2 12 12	13(X)−; 1 15	8(X)− 1(X); 3 12 12
Date of inoculation	6–3–30	6–3–30	6–3–30	6–3–30
Date of marking	20–3–30	21–3–30	22–3–30	24–3–30

TABLE 17.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 29 at Different Temperatures

—	Physiologic form 29						
	Test No. 1			Test No. 2			
Mean temperature during test	71·2° F.	64·4° F.	59·3° F.	78·0° F.	72·3° F.	66·3° F.	59·4° F.
Kanred	0/10	0/11	0/11	1(3)+/12	0/12	0/11	1(4)−/13
Arnautka	9(4)+/9	10(4)+/10	10(4)−/11	11(4)+/12	12(4)+/13	11(4)/11	10(4)/11
Mindum	——	——	——	10(4)+/11	7(4)1(X)/8 8	7(X)+2(4)/9 9	8(X)+ 2(4)−/10 10
Speltz Marz	9(4)/11	7(X)+ 2(4)/10 10	7(X)2(X)=/9 9	12(4)+/12	13(4)/13	6(X)+1(4)−/7 7	9(X)+ 5(4)−/14 14
Kubanka	——	——	——	13(4)+/14	9(4)+/9	13(4)+/13	14(4)/14
Date of inoculation	20–230	20–2–30	20–2–30	3–3–30	3–3–30	3–3–30	3–3–30
Date of marking	5–3–30	5–3–30	9–3–30	16–3–30	17–3–30	20–3–30	21–3–30

TABLE 18.—Reactions of Wheat Varieties in the Seedling Stage to Physiologic Form 48 at Different Temperatures

——	Physiologic form 48		
Mean temperature during test	71·8° F.	64·3° F.	58·9° F.
Marquis	4(1)+ / 7	6(1) / 8	6(1) / 8
Kanred	0 / 12	0 / 8	0; 6 / 9
Arnautka	5(4)+ / 5	5(4) / 5	6(4)− / 6
Mindum	4(X) / 4	0; 8 / 9	0; 7 / 8
Speltz Marz	5(X) / 5	3(X)− / 10 4(; & 1); 3 / 10	1(X)=; 6 / 8
Kubanka	9(4)− / 13	8(X) / 8	1(3)3(X)=2(; & 1); 3 / 9 9 9
Date of inoculation	24–2–30	24–2–30	24–2–30
Date of marking	9–3–30	9–3–30	12–3–30

TABLE 19.—Mean Reactions of Mindum, Speltz Marz and Kubanka Seedlings to Ten Physiologic Forms of *Puccinia graminis tritici* at Several Temperatures during the Period from February 20 to March 24, 1930

Physiologic form	29				30			32			38			48		
Temperature (°F.)	78	72	66	59	72	64	59	71	64	59	72	64	59	72	64	59
Mindum reaction	4+	4	X+	X+	4+	X+	X−	4−	X	0;	X	X	0;	X	0;	0;
Speltz Marz reaction	4+	4	X+	X+	4−	4−	X	4−	X+	X	X+	X	0;	X	0;	0;
Kubanka reaction	4+	4+	4+	4	4−	4−	X	4	4−		4−	X	X−	4−	X	0;

Physiologic form	86				87			89			90			76			
Temperature (°F.)	78	72	66	59	72	64	59	72	64	59	72	64	59	78	71	66	59
Mindum reaction	4+	X+	X	X−	X+	X−	0;	X+	X−	0;	4−	4−	X	4−	X+	X	X−
Speltz Marz reaction	4+	X+	X	X−	X+	X−	0;	X+	X−	X=	4+	4−	X	4−	X+	X	X
Kubanka reaction	4+	4	X+	X	4	4−	X−	4−	X	X−	4+	4	X	4−	4−	X+	X

TABLE 20.—Reactions of Kota and Vernal Emmer to Physiologic Form 91 at High and Low Temperatures

——	Physiologic form 91			
——	Test No. 1		Test No. 2	
Mean temperature during test	71·9° F.	58·9° F.	71·9° F.	58·5° F.
Kota	$\frac{12(3)}{21} \pm \frac{2(X)}{21}$	$\frac{6(X)}{23} = \frac{2(3)}{23} = ; 7$	$\frac{4(3)}{10} \pm \frac{1(X)}{-10}$	$\frac{3(X)}{8} \frac{1(1)}{8}$
Vernal	—	—	$\frac{4(X)}{6} \frac{1(3)}{6} -$	$\frac{4(X)}{9}$
Date of inoculation	26–11–29	26–11–29	18–12–29	18–12–29
Date of marking	11–12–29	11–12–29	4– 1–30	6– 1–30

TABLE 21.—Reactions of Vernal Emmer to Physiologic Form 82 at High and Low Temperatures

——	Physiologic form 82	
Mean temperature during test	73·1° F.	58·0° F.
Vernal	$\frac{9(X)}{16} \frac{5(3)}{16} -$	$\frac{4(X)}{19} - \frac{3(X)}{19} = \frac{3(1); 4}{19}$
Date of inoculation	29–11–29	29–11–29
Date of marking	16–12–29	16–12–29

TABLE 22.—Reactions of Vernal Emmer to Physiologic Form 85 at Four Different Temperatures

——	Physiologic form 85			
Mean temperature during test	78·0° F.	72·3° F.	66·3° F.	59·4° F.
Vernal	$\frac{7(X)}{10}$	$\frac{6(X)}{12} \frac{1(; \& 1)}{12}$	$\frac{2(X)}{9} \frac{4(X)}{9} - ; 1$	$\frac{6(X)}{14} = ; 8$
Date of inoculation	4–3–30	4–3–30	4–3–30	4–3–30
Date of marking	16–3–30	17–3–30	20–3–30	22–3–30

TABLE 23.—Reactions of Mindum and Speltz Marz in the Four-Leaf Stage to X-Forms at High and Low Temperatures. Mindum Infected by Forms 89 and 87, Speltz Marz by Form 29

——	Physiologic form	1st leaf	2nd leaf	3rd leaf	4th leaf
Mean temperature during test 75·6° F.	89	2(X) = 2(1) 5 5	2(X); 3 5	3(X) − 1(1); 1 5 5	1(X) − 2(1); 1 5 5
Date of inoculation 14–1–30	87	3 (3) 6	4(X) 1(3) 6 6	5(X) − ; 1 6	5(X) − ; 1 6
Date of marking 27–1–30	29	3(4) 2(3) 5 5	2(4) 2(X) 5 5	3(X) + 1(4) 5 5	3(X) + 1(X) 5 5
Mean temperature during test 57·8° F.	89	0; 6 6	0; 4 6	0; 5 6	0; 5 6
Date of inoculation 14–1–30	87	2(; & 1); 4 6	0; 5 6	0; 5 6	0; 3 6
Date of marking 30–1–30	29	0; 2 4	0; 3 4	0; 4 4	0; 4 4

TABLE 24.—Reactions of the Three Uppermost Leaves of Mindum and Kubanka After Heading, to Form 86 at High and Low Temperatures

——	Host	Uppermost leaf	2nd leaf from top	3rd leaf from top
Mean temperature during experiment 78° F.	Mindum	3(X) 3	3(X) + 3	3(X) + 3
	Kubanka	4(X) + 4	4(3), (4) 4	3(4) 4
Mean temperature during experiment 59·8° F.	Mindum	1(; & 1); 4 5	1(; & 1); 4 5	2(; & 1); 3 5
	Kubanka	3(; & 1); 1 4	4(; & 1) 4	3(X) − 4

Studies on the morphology and chemical composition of Mindum and Speltz Marz seedlings grown at high and low temperatures

The question of the nature of host resistance and susceptibility at low and high temperatures is one which cannot be overlooked. In this connection a chemical study of seedlings grown under different conditions of temperature might conceivably produce a clue to the nature of this type of resistance. The possibility of distinguishable morphological differences, too, should not be overlooked. In the present work no extensive investigation could be undertaken along either of these lines; but, since it was thought possible, although not probable, that there might be a correlation between some easily ascertainable morphological or chemical character and host resistance, a few attempts were

made to discover structural or chemical differences in plants grown at high and low temperatures. As no such differences were found, these experiments will be considered briefly.

An attempt was first made to determine if there were any morphological differences between Speltz Marz seedlings grown at 58° F. and 75° F., such as, differences in thickness of cell walls. Sections were made of Speltz Marz leaves about 21 days after the planting of the seed. These were prepared by means of the paraffin method, stained with various stains, and examined. No structural differences of any kind could be observed. The staining of the tissue elements by safranin, eosin, methyl blue, and methylene blue was identical in sections of leaves grown at both temperatures.

A few microchemical tests were made, according to methods outlined by Harvey (14) in an attempt to discover chemical differences in Mindum and Speltz Marz seedlings grown at high and low temperatures. Dickson and Holbert (5) state that "wheat seedlings grown at low temperatures (8° C.) are high in soluble, carbohydrate building-substances, principally dextrin, sucrose, and hexoses, and have cellulose cell walls, with well lignified walls in the cortical cells of the colorhiza and coleoptile. The wheat seedings grown at high temperatures (16° C.-24° C.) are relatively low in these building-substances and have a high pentosan content. The cell walls of the protective sheath tissues in the high-temperature seedlings are largely intermediate pentosan-yielding substances."

This suggested that similar differences might be found in the leaves of Mindum and Speltz seedlings grown at low and high temperatures. Microchemical tests were made with seedlings grown for 21 days at 58° F. and 75° F. respectively. These tests were chiefly concerned with comparisons of the cellulose reactions of these seedlings and comparisons of their reactions for the presence of the pectic compounds.

The following tests were used to detect the presence of cellulose: (1) the hydro-cellulose reaction (I_2KI solution and 75 per cent H_2SO_4); (2) the crystallization of cellulose by means of 2 per cent KOH, copper oxide ammonia, and a subsequent treatment with 20 per cent ammonia. Several repetitions of these tests failed to reveal any differences between seedlings grown at the two different temperatures. Both high and low-temperature sedlings contained cellulose in abundance.

Several tests were made for the detection of pectic substances. Free-hand sections of leaf tissue were stained by methylene blue and ruthenium red without any differences being observed in the staining properties of the high and low-temperature leaves. The staining reactions were positive in both cases.

Although these tests indicated the presence of both cellulose and pectic substances in leaves grown under dissimilar temperature conditions, they are merely qualitative tests and give no indication of any quantitative differences that may exist. They are, moreoever, concerned chiefly with cell-wall composition, and reveal no protoplasmic differences, which, after all, are more likely to account for the diversity in host reaction under different conditions of temperature.

The nature of protoplasmic resistance to rust has never been established. Ward (32) and others have suggested that the host plant may produce toxic substances which inhibit the development of the fungus and thereby bring about resistance. Possibly the host varieties which are resistant under certain conditions of temperature and susceptible under others, develop, at a low temperature, substances which are toxic to certain physiologic forms of the rust organism. An attempt was made to determine if this was the case.

When infected by physiologic form 32, at approximately 75° F. Speltz Marz produces a 4-type of reaction; at 58° F. the reaction is 0;. It was thought possible that Speltz Marz leaves kept at the lower temperature for 17 days might

have produced substances toxic to the germ tubes of spores of form 32, as the mycelium of this form apparently cannot develop in the host tissues under these temperature conditions. To test this possibility, two leaves of Speltz Marz from the warm greenhouse and two from the cool greenhouse were crushed separately in a mortor. The juices thus obtained were mixed with sterile distilled water in the proportion of one part of leaf extract to three parts of water. The diluted leaf extracts were then placed in Syracuse watch glasses, and urediniospores of form 32 were dusted on the surfaces. There was no appreciable difference in spore germination when counts were made on the following day. Between 80 and 90 per cent of the spores germinated in both leaf extracts. Although this experiment does not demonstrate the existence of any toxic principle in the extract of resistant leaves, it does not, on the other hand, prove its absence, because of the great physiological difference between the development of germ tubes from spores and the development of mycelia in the plant tissues. The former can take place in a medium entirely lacking in nutrients, such as distilled water; the latter is dependent on the presence of suitable nutrients, and perhaps on other conditions not at present understood.

THE EFFECT OF THE CARBON DIOXIDE CONCENTRATION OF THE AIR ON THE DEVELOPMENT OF THE X-REACTION

The concentration of atmospheric carbon dioxide has long been known to exert a great influence on the development of green plants; and because rust development is considerably dependent on the condition of the host, it is natural to expect that the concentration of carbon dioxide in the air might have some effect on rust development.

Gassner (12) and Gassner and Straib (13) have investigated the effects of different atmospheric carbon-dioxide concentrations on the development of several graminaceous rusts, including *Puccinia graminis tritici*. They found that an increase in the carbon-dioxide concentration was accompanied by an increased rust development up to a certain point. An increase beyond this optimum led to a decrease in rust development, and eventually, at very high concentrations, caused a complete cessation of pustule formation. The optimum concentration of carbon dioxide for *P. graminis tritici* was found to lie between 0·3 and 0·75 per cent, and the upper limit of toleration was stated to be about six per cent. The stimulation due to increased carbon dioxide was expressed mainly in a shortening of the incubation period and in increase in pustule development.

The work cited above suggested a similar series of experiments to determine the effect of increased carbon-dioxide supply on the development of the X-reaction which is characteristic of certain physiologic forms of wheat stem rust. As shown elsewhere in this paper, this type of reaction is more easily influenced by temperature than a resistant or a susceptible type. Hence it was thought likely that any influence of carbon dioxide on rust development, would be most easily detected by means of this type of rust reaction.

The technique described by Gassner and Straib (13) was followed with slight modifications. Wheat seedlings were inoculated by a physiologic form which normally produces an X-reaction on the leaves of the wheat variety used. These were placed in bell jars of known capacity. The carbon-dioxide concentration required was obtained from a weighed amount of sodium carbonate calculated to produce the requisite amount of carbon dioxide. The evolution of the gas was secured by dissolving the sodium carbonate in water together with sodium bisulphate in excess of the amount required to complete the reaction. The details of a typical experiment are as follows: A small

pot containing ten inoculated seedlings was placed on a clean sheet of glass under a bell jar of five and one-half litres capacity. The point of contact between the bell jar and the glass plate was made air tight by means of vaseline. A small beaker containing weighed quantities of sodium carbonate and sodium bisulphate was placed in the bell jar beside the pot. At the moment when these were covered by the bell jar, a quantity of distilled water was poured into the beaker, causing an immediate evolution of carbon dioxide. The carbon dioxide supply was renewed, in this manner, every morning for about 15 days. Although this procedure did not ensure a perfectly uniform supply of gas, it served the purpose of comparing the effect of different concentrations on the rust development.

It was planned to test the effect of six different carbon-dioxide concentrations on the development of form 90 on the seedling leaves of Speltz Marz at a temperature of about 60° F. This form was selected because its reaction on this host is easily affected by temperature. During December, 1929, and January, 1930, its types of rust reaction on Speltz Marz were 0; at 55-60° F. and 4 at 75-80° F. It was thought that if the experiment were performed at the lower temperature, carbon dioxide in increasing quantities might influence rust development and produce an X or even heavier reaction.

Six bell jars of five and one-half litre capacity were used, one jar for each concentration of carbon dioxide. One pot of Speltz Marz seedlings was placed in each bell jar, after inoculation, when the leaves were about two inches long. The supply of carbon dioxide was renewed daily for about 15 days, as described above. The amounts of sodium carbonate (anhydrous) required to produce the desired concentrations are given in Table 25.

TABLE 25.—Carbon-dioxide Concentrations Used, and Amounts of Sodium Carbonate Required to Obtain Each Concentration

Bell jar No.	CO_2 Concentration in per cent	Amount Na_2CO_3 used per day
		gms.
1	0·03	
2	0·15	0·0391
3	0·75	0·1955
4	1·50	0·3910
5	3·00	0·7820
6	4·50	1·1730

In addition to the six pots of Speltz Marz kept at different carbon-dioxide concentrations in the bell jars, one pot was kept uncovered on the greenhouse bench as a check, as the humidity in the bell jars was undoubtedly different from that in the greenhouse. The rust reactions of the seedlings in these seven pots at the end of 15 days are shown in Table 26.

TABLE 26.—Reactions of Speltz Marz Seedlings to Form 90 When Kept at Different Concentrations of Carbon Dioxide for Fifteen Days after Inoculation

Concentration of carbon dioxide in per cent	Rust reaction of seedlings
0·03 (in the open)	4/8 (X) 4/8 (; & 1)
0·03 (in bell jar)	9/9 (X)
0·15 "	10/11 (X) ; 1
0·75 "	10/11 (X) 1/11 (; & 1)
1·50 "	8/11 (X)— 2/11 (; & 1); 1
3·00 "	13/18 (X)— ; 5
4·50 "	12/12 (X)—

The results of this experiment are not very conclusive. There is no marked stimulation of rust development at any of the carbon-dioxide concentrations used. There is, on the other hand, a slight retardation at the three highest concentrations. It is possible that light acted as a limiting factor, as the experiment was performed during the period from February 4 to 19, when light was still rather poor. This does not, however, seem probable, because under the same conditions of light, but at a higher temperature, the reaction of Speltz Marz to this physiologic form was invariably of the susceptible type 4. Temperature (the mean for the period of the experiment was 61·1° F.) may have been the limiting factor which prevented the development of the rust reaction beyond the X-type. It should be noted, however, that in periods of sunshine, the temperature inside the bell jars rose from 4 to 6 degrees Fahrenheit higher than that of the greenhouse.

This experiment was repeated, with the difference, however, that the variety Mindum was substituted for Speltz Marz, and form 48 substituted for form 90. Daylight had improved considerably in the interval and the temperature of the greenhouse had been raised to 67° F., which was a favourable temperature for the development of the X-reaction. Table 27 shows a record of the reactions of Mindum at the different carbon-dioxide concentrations. A photograph of host reactions obtained at each concentration is shown in Figure 11.

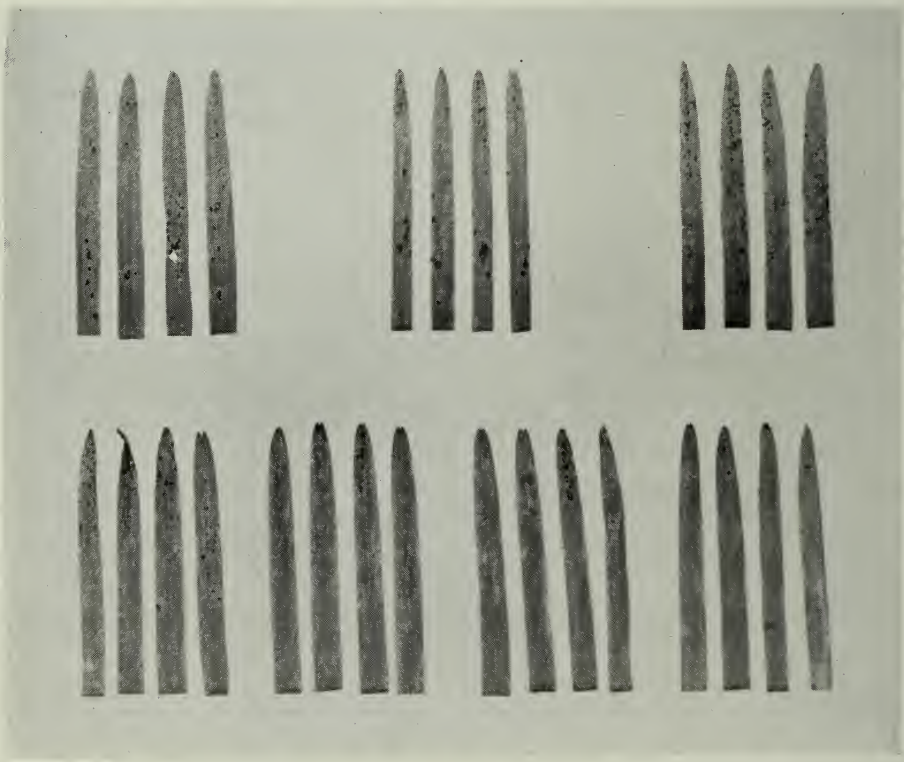

Fig. 11.—Reactions of Mindum seedlings to physiologic form 48 at six different concentrations of carbon dioxide. Upper row, left to right: check plants kept in the open (CO_2 conc. 0.03 per cent); check plants kept under bell jar (CO_2 conc. 0.03 per cent); plants kept at 0.15 per cent CO_2 concentration. Lower row, left to right: plants kept at 0.75 per cent CO_2 concentration; plants kept at 1.50 per cent CO_2 concentration; plants kept at 3.0 per cent CO_2 concentration; plants kept at 4.5 per cent CO_2 concentration.

TABLE 27.—Reactions of Mindum Seedlings to Form 48, When Kept at Different Concentrations of Carbon Dioxide for Twelve Days after Inoculation

Concentration of carbon dioxide in per cent	Rust reactions of seedlings
0·03 (in the open)	8/10 (X) 1/10 (3)± 1/10 (1)
0·03 (in bell jar)	8/8 (X)
0·15 "	9/11 (X) 2/11 (X)−
0·75 "	10/10 (X)
1·50 "	3/9 (X) 3/9 (X)= ; 3
3·00 "	1/9 (X) 6/9 (X)= ; 2
4·50 "	7/9 (X)= ; 2

The results of the second experiment agree on most points with those obtained in the first. Here, again, there is no marked increase in rust development due to the increase in carbon-dioxide concentration. There is, on the contrary, a notable decrease at concentrations of 1·5 per cent and above. The pustules at these higher concentrations are minute and hypersensitive flecks are abundant on the leaves.

It was noted when the leaves were examined 12 days after inoculation, that all the pustules on leaves covered by the bell jars contained teliospores, while no teliospores were present on the check plants which were uncovered. The teliospore formation was evidently not due to increased carbon dioxide, as all the pustules on the plants in the bell jar of the lowest concentration (0·03 per cent) had produced teliospores. This phenomenon is apparently related to the higher humidity or the higher temperature inside the bell jars. It is possible that the formation of teliospores was due to the increased humidity, as Smith (26) has shown that high atmospheric humidity favours teliospore production in *Puccinia asparagi* D.C.

The results of these experiments agree with the results obtained by Gassner and Straib on the effect of carbon dioxide on the development on *Puccinia graminis tritici* with the exception that these authors reported some stimulation at concentrations of 0·30 and 0·75 per cent carbon dioxide. At concentrations of 1·5 per cent and above, they observed the same detrimental effect on rust development as is shown in Table 27. They likewise remarked on the abundant hypersensitive areas on leaves in the higher concentrations.

There seems to be no simple explanation for the lack of stimulation of rust development through increase in carbon-dioxide concentration, unless temperature can be considered the limiting factor in both experiments. Light, certainly, was not the limiting factor in the second experiment and very probably not in the first one. If these tests can be considered representative of the effect of carbon dioxide it must be conceded that variations in this environmental factor are of less importance in rust investigations than fluctuations in temperature or in light intensity.

THE EFFECT OF MINERAL STARVATION OF THE HOST ON THE DEVELOPMENT OF THE X-REACTION

Ever since the pioneer work of Liebig on soil fertility, before the middle of the last century, it has been known that plants are dependent on certain chemical nutrients. If any of the essential minerals are withheld from the plant, considerable disturbances are brought about in its metabolism. As rusts are obligate parasites, it seems logical to expect that changes brought about in the green plant through mineral starvation may also have considerable effect on rust development. This idea was tested by Ward (32), who investigated the effect of mineral starvation on the development of *Puccinia dispersa*, Erikss. (*Puccinia bromina*, Erikss.) on species of Bromus. He concluded that "lack of minerals

in no way secured immunity from infection, though seedlings deficient in phosphorus or nitrogen, tended to show retardation of infection." He claimed, however, that, on plants well supplied with mineral salts, greater quantities of fungous mycelium and consequently of urediniospores were produced than on those grown in a solution deficient in minerals. Similar conclusions were reached by Stakman (27), for *Puccinia graminis* Pers., and Raines (25) for *Puccinia sorghi* Schw.

Since the completion of the work reported in this paper Hassebrauk (15) has stated that certain mineral nutrients produced considerable effects on the development of *Puccinia triticina,* Erikss., on the seedlings of certain wheat varieties. This effect was most noticeable on varieties which were moderately susceptible, but very slight on varieties highly resistant or completely susceptible. Moderately susceptible varieties were more resistant when grown on nutrient solutions deficient in nitrogenous salts, and, conversely, were more susceptible when these salts were supplied in excess. Deficiency in salts containing calcium increased susceptability to rust, while an abundant supply of these salts increased the resistance of the host.

Owing to the fact that the X-reaction appears to be more sensitive to environmental conditions than other rust reactions, it was decided to determine the effect of mineral starvation of the host upon its development. It was planned to grow seedlings of varieties, which normally show this reaction, in nutrient solutions, some of which were deficient in certain mineral salts. The basic nutrient solution used in these tests was Shive's which contains KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. Five solutions were made up as follows:—

1. 1 gm. $Ca(NO_3)_2$
   0·25 gm. $MgSO_4$
   0·25 gm. KH_2PO_4
   trace of iron
   } in 1,000 ccs. of distilled water

2. 0·25 gm. $MgSO_4$
   0·25 gm. KH_2PO_4
   trace of iron
   } in 1,000 ccs. of distilled water

3. 1 gm. $Ca(NO_3)_2$
   0·25 gm. KH_2PO_4
   trace of iron
   } in 1,000 ccs. of distilled water

4. 1 gm. $Ca(NO_3)_2$
   0·25 gm. $MgSO_4$
   trace of iron
   } in 1,000 ccs. of distilled water

5. 1 gm. $Ca(NO_3)_2$
   0·25 gm. $MgSO_4$
   0·25 gm. KH_2PO_4
   } in 1,000 ccs. of distilled water

In the above solutions, No. 1 contains all the essential elements; No. 2 lacks Ca $(NO_3)_2$, No. 3 lacks $MgSO_4$, No. 4 lacks KH_2PO_4; and No. 5 lacks iron.

Experiment No. 1.—For this experiment were selected the wheat variety Mindum, and form 38 which normally produces an X-reaction on this host.

The Mindum seed was germinated in blotting paper, moistened with distilled water. After germination the endosperm of each seed was removed and the young seedlings were transferred to glass jars containing about 775 ccs. of solution. Five sedlings were transferred to each jar, the roots being passed down into the solution through holes in a paraffin covering in the neck of the jar. To protect the roots from light, the jars were placed in a box which was then filled with sand, so that the sand came up to the top of the jars. In addition to the five solutions mentioned above, a jar of distilled water containing five seedlings was used as a check. After inoculation, the seedlings in the six jars were placed in a greenhouse maintained at a temperature slightly

above 60° F., where normally an X-reaction was produced on Mindum by this physiologic form. Sixteen days after inoculation, observations were made on the rust reactions and the condition of the seedlings. These observations are included in Table 28.

TABLE 28.—Experiment No. 1—Rust Reactions on Seedling Leaves of Mindum Grown in Nutrient Solutions and Infected by Physiologic Form 38.

Solution	Rust reaction	Condition of seedlings
1	1/5 (X)	One plant dead; four plants healthy
2	1/5 (3)+ ; 1	All plants chlorotic
3	1/5 (3)	All plants chlorotic, but less so than in 2
4	1/5 (3)± ; 1	All plants healthy
5	3/5 (X)	Four plants healthy
Distilled water	1/5 (4)	Three plants dead and one dying, plant bearing pustule cholorotic

An examination of Table 28 will show that it is practically impossible to draw any conclusions from Experiment No. 1, owing to the fewness of the infections. A second experiment, a duplicate of the first with the exception that the seedlings were grown in sand cultures, met a similar fate. Owing to these failures, a somewhat different procedure was adopted in succeeding experiments.

Experiment No. 3.—The same solutions were used as in Experiment No. 1, with this difference, that the Mindum seedlings were grown in purified quartz sand in four-inch flower pots, the bottoms of which had previously been corked anad sealed with pariffin to prevent leaking. The seed was planted directly in the sand moistened with the solutions. To prevent too great a concentration of the nutrient solutions, the pots were replenished with measured amounts of distilled water for two successive days and with their respective solutions every third day. Owing to the fact that the endosperm was not removed from these seedlings, a longer interval, than in Experiment No. 1, was allowed between germination and inoculation. Physiologic form 38 was used, as in Experiment No. 1, and the seedlings were inoculated when between three and four inches high. After inoculation, the plants were kept under conditions similar to those described for Experiment No. 1. Observations made 16 days after inoculation are recorded in Table 29.

TABLE 29.—Experiment No. 3—Rust Reactions on Seedling Leaves of Mindum Grown in Nutrient Solutions and Infected by Physiologic Form 38

Solution	Rust reaction	Condition of seedlings
1	4/11 (X)− ; 3	All plants fairly healthy
2	1/10 (3)−	All leaves withered
3	4/8 (X)− 1/8 (3)−; 1	All plants healthy
4	4/9 (X)− 1/9 (3)−	All plants fairly healthy, leaves slightly chlorotic
5	1/10 (3)−; 4	All plants healthy
Distilled water	1/13 (X) ; 1	Four leaves withered, the rest chlorotic

The data recorded in Table 29 do not indicate that there is any wide deparature from the characteristic Mindum reactions to form 38 at this temperature (about 60° F.). The chief deviation from normal is expressed in the fewness of the infections on leaves growing in solutions deficient in mineral salts. Infection took place on only one of the ten leaves growing in the nitrate-deficient solution No. 2; and only two of thirteen leaves growing in distilled water became infected or showed visible signs of infection.

Experiment No. 4.—The temperature at which the first three experiments were carried out was somewhat below the optimum temperature for the development of a normal X-reaction. The fourth experiment was identical with experiment No. 3, except that the seedlings were grown at a temperature above the optimum for the development of the X-reaction (about 75° F). The normal Mindum reaction to form 38 at this temperature, as shown elsewhere in this paper, is about 4. The object in view was to determine if mineral starvation at this temperature led to any abnormality in rust reaction. Observation made 15 days after inoculation are recorded in Table 30.

TABLE 30.—Experiment No. 4—Rust Reactions on Seedling Leaves of Mindum Grown in Nutrient Solutions at a Temperature of About 75° F., and Infected by Physiologic Form 38

Solution	Rust reaction	Condition of seedlings
1	7/8 (4)	Leaves all healthy
2	5/9 (4)	Leaves all healthy
3	6/10 (3)	Leaves fairly healthy
4	8/10 (3)±	Leaves rather chlorotic
5	9/10 (4)	Leaves all healthy
Distilled water	1/6 (3)	Leaves all small; 2 leaves deep green; 2 leaves chlorotic; 2 leaves dead

The results of experiment No. 4 do not indicate that mineral starvation produces any appreciable change from the normal in rust reaction. If there is any influence, it seems rather to lead to a reduction in the number of infections on plants starved of minerals, and perhaps, as originally pointed out by Ward, to a reduction in the size of pustule and number of urediniospores produced. The type of infection remains much the same on plants starved of minerals as on healthy plants.

DISCUSSION

Of the four environmental factors which probably determine rust development, in so far as it is determined by external conditions, namely, temperature, light, humidity, and carbon-dioxide concentration, only the first and the last named were studied under controlled conditions. The evidence on the effect of light is of a more or less indirect nature. The effect of atmospheric humidity was not studied. It has been demonstrated that fluctuations in temperature produce very striking effects on the development of certain physiologic forms of the stem-rust organism on some host varieties. Fluctuations in light intensity may produce an equally marked effect, although this has not been proved. Variations in the concentration or carbon dioxide apparently are less effective.

What is the nature of this response to temperature? This question arises naturally as a result of this work. Is it a direct response of the parasite or a direct response of the host? It is apparently not the former, as the same physiologic form on a different host shows no response whatever to temperature, except for a slight lengthening of its incubation period at lower temperatures. That is, if it were a direct response of the fungus, the response takes place when the parasite is growing on one host but not when it is growing on another. As this does not seem possible, the inevitable conclusion is that the response is not a direct one on the part of the fungus.

If it is not a direct response on the part of the fungus, is it a direct response on the part of the host? This is equally improbable, because the same host variety shows this response when infected by certain physiologic forms but not when infected by others. That is, it is only when such a host is infected by the so-called "X-forms," that any difference can be detected in its ultimate

rust reaction at high and low temperatures. When the host is infected by physiologic forms to which it is normally susceptible, there is no difference in its reactions at different temperatures. The same holds true when it is infected by forms to which it is normally resistant. The idea of a direct response of the host to temperature, must, then, apparently be dismissed.

There seems to be only one other alternative. The effect of temperature is registered on the "host-parasite complex." This expression can, perhaps, be defined as a system composed of two elements; the host, and its parasite. When the host is susceptible to the parasite under all conditions permitting its existence, there obtains, apparently, a sort of symbiosis between the two, which ceases only with the death of the host. The tissues of the host seem to tolerate the fungus without any apparent effort of resistance. On the other hand, when the host is resistant to the fungus under all environmental conditions, its tissues are in some way antagonistic to the fungus and do not tolerate its growth under any conditions.

An entirely different situation prevails when the normal interaction of host and parasite results in the X-reaction. Then, certain changes of the environment bring about an interaction of host and fungus which results in the former becoming completely susceptible. Conversely, other environmental changes result in complete resistance. Figure 12 represents graphically the results on the host reaction when temperature and light are variables in the environment.

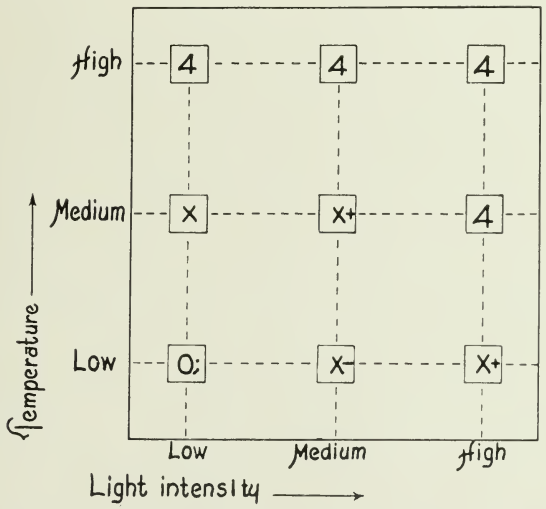

FIG. 12.—A graphic representation of the effects of temperature and light intensity on the development of the X-reaction on Mindum, Speltz, Marz, and Kubanka when both temperature and light intensity are variables.

The present investigation does not appear to throw much new light on the question of rust resistance. This subject has been discussed in the past by many investigators. Two main theories have been advanced; first, that resistance is due to starvation of the fungus, second, that it results from the development, in the host, of toxic substances which inhibit the growth of the parasite and ultimately bring about its death.

The first of these theories postulates that starvation of the fungus results from a lack of the proper nutrients in the host tissues. The work of Dickson and Holbert (5, 6) indicates strongly that the resistance of wheat seedlings to *Gibberella saubinettii* (Mont.) Sacc. at low temperatures may be explained in

this manner. Susceptibility to the same organism at high temperatures appears to be due to the presence of the nutrients required by the fungus. In other words, the metabolism of the plant is so affected by temperature that substances which support the growth of the fungus are produced at one temperature but not at another. If this explanation is to be applied to the resistance of Mindum, Speltz Marz and Kubanka to the X-forms at low temperatures and their susceptibility at high temperatures, it must be assumed that the metabolism of these varieties varies greatly with temperature changes while that of other closely related varieties, such as Arnautka, and Acme, does not. The last mentioned varieties show no response to temperature fluctuations, as measured by their rust reactions. In the absence of conclusive studies on the chemical nature of high and low-temperature seedlings of these varieties, it is impossible to say how far their metabolism differs.

Other chemical differences have been reported in wheat plants grown at high and low temperatures in addition to the differences in carbohydrates found by Dickson and his co-workers. Hurd-Karrer (17) states that considerable differences occur in the acidity of the cell sap in leaves of wheat plants grown at high and low temperatures. The acidity is higher at high temperatures. It does not, however, seem probable that there is any relation between this factor and rust resistance, as she reports in another paper (16) that there is no correlation between acidity changes during the growth period of wheat and rust resistance.

The other theory, that rust resistance is due to the capacity of the host to produce antitoxins which interact with toxins secreted by the fungus, meets with more general support among rust investigators. Wellensiek (35), who favours the starvation theory, however, points out that the existence of toxins and antitoxins in plants has never been proved. According to this theory, a certain host variety has the capacity, when attacked by some physiologic forms, to produce toxic substances which result in resistance to the parasite being developed. If this theory is applied to cases where a host is resistant to a physiologic form at a low temperature and susceptible at a higher temperature, it is evident that the host can only produce its antitoxins at certain temperatures.

There is no positive evidence as to which of these two theories applies to the cases under discussion. The data thus far accumulated are inadequate for the establishment of any generalization in this respect.

The practical significance of this phenomenon, apart from its importance in the identification of physiologic forms, lies in the fact that many of the wheat varieties now commonly grown may vary in their rust resistance in accordance with weather conditions. In this connection, it is important that new wheat varieties distributed among farmers should possess complete resistance, as varieties which vary in their resistance according to climatic conditions may be resistant under one set of weather conditions, and susceptible under another.

PART II

Studies on the Effects of Environmental Factors on the Formation and Germination of Teliospores of Certain Physiologic Forms of *Puccinia Graminis Tritici.*

TELIOSPORE FORMATION IN PHYSIOLOGIC FORMS OF *PUCCINIA GRAMINIS TRITICI*

Review of Literature on the Production of Teliospores

One of the many interesting phenomena in the life history of a rust is the formation of the teliospores, which commonly takes place in the sori that previously had borne urediniospores. Many investigators have studied the process of teliospore formation in different rust species, and some have advanced theories as to the causes of the cessation of urediniospore formation and the development of teliospores. Some workers have held the opinion that this change from urediniospore to teliospore production was initiated by environmental factors, such as, changes in climatic conditions; others have suggested that the essential factors operative in this process were changes in the metabolism of the host, *e.g.*, the gradual dying of the host. Still others have found that the change from uredinial to telial production has taken place, at a certain more or less definite time, irrespective of any apparent metabolic changes in the host or changes in the environment. These conclusions need not be considered contradictory, as they are based on studies of different species of rust growing on diverse host species.

The first theory, namely, that teliospore formation was initiated by climatic conditions, was advanced by Iwanoff (18) as a result of his study of the development of teliospores at different altitudes in Switzerland. He showed that in several species (*Puccinia Pimpinellae* (Strs.) Link., *Puccinia Violae* (Schum.) D.C., *Puccinia Celakowskyana* Bub., *Puccinia Galii* Schw.) the teliospores develop at different times according to whether the rust is found in the lowlands (at Bern 520 meters above sea level) or in alpine regions (at 2,700 meters above sea). In the lowlands, the uredinial generation persisted much longer than in the alpine regions. At high altitudes, accompanied by low temperatures, the uredinial generation was much repressed and teliospores were developed earlier. He considered a cool temperature the most important factor in bringing about the early formation of telia.

Morgenthaler (21), who repeated some of this work, arrived at somewhat different conclusions. He infected plants of *Veratrum album* L. with *Uromyces Veratri* (D.C.) Schr. f. sp. *Homogynes*. Some of the plants he kept at low altitudes (at Bern); others he transferred to higher altitudes. The results of this experiment were in agreement with Iwanoff's work, in so far as the uredinial generation was markedly shortened at the higher altitude. He noted, however, that the transported plants appeared to have suffered from the change of climate. The leaves turned prematurely brown and frequently died back from the tips. On this dead area the first telia were commonly found. In general, his conclusion was that the production of the uredinial and telial stages depend upon the condition of the host plant, or of that part of it which bears the fungus. He considered that the dying of the host, either through age or a diseased condition, brings

about the early formation of teliospores. A similar conclusion was reached by Eremeyeva and Karakulin (8) from a study of sunflower rust, *Puccinia helianthi* Schw.

This view was likewise held by Magnus (22), who considered it generally applicable to the production of teliospores in the rusts. It differs from Iwanoff's viewpoint mainly in that it ascribes the formation of teliospores to the indirect, instead of the direct, influence of external factors.

Waters (34), who studied teliospore formation in ten species of rusts, not including *Puccinia graminis*, states that "all the rusts studied are directly dependent upon the photosynthetic activity of the host. Any single factor or set of factors, such as light, temperature and moisture or as in the case of climate, a complex of these factors may so influence and do influence the metabolism of the host, that the fungus reacts by changing from the uredinial to the telial generation."

Gassner (10) states, concerning the development of teliospores of the cereal rusts, that teliospores develop when the host plant reaches a certain stage of maturity, and that telial development is independent of external conditions.

In recent years some investigators have stated that, in certain rusts, teliospores are formed after a more or less definite interval following infection by urediniospores. This has especially been found to be the case in some of the physiologic forms of graminaceous rusts. Bailey (1), in a discussion of the physiologic forms of *Puccinia graminis avenae* Erikss. and Henn., states that "forms 3 and 4 are very definitely characterized by the rapidity with which they form teliospores. In infections with form 4, teliospores have been found to form within 20 to 40 days following inoculation, depending on environmental conditions. With form 3 the interval was usually but slightly longer; while it was very much longer with the other forms when they formed teliospores at all." A similar statement is made by Parson (24) for *Puccinia coronata avenae* (Corda) Erikss. and Henn. In this rust, there was likewise a tendency for some of the physiologic forms to produce teliospores earlier than others. This tendency seemed to be inherent in the form and not due to the effect of certain hosts or environmental conditions.

It would probably be unwise to attempt to generalize from the findings of these investigators. That environmental conditions play a part in the development of teliospores of most rusts cannot be doubted. It is probable, however, that this effect is indirect rather than direct, or in other words, that the environment acts on the fungus through the medium of the host rather than directly. It may also be pointed out that the inter-relationships of host and fungus are so various and complex that it is not surprising to find wide discrepancies between the reactions of the various host-parasite complexes to environmental conditions.

Observations on Teliospore Formation in the Physiologic Forms of *Puccinia graminis tritici*

TELIOSPORE FORMATION ON SEEDLING LEAVES OF WHEAT PLANTS

During the course of the routine work of identifying physiologic forms of *Puccinia graminis tritici*, it was frequently observed that certain forms tended to produce teliospores on the infected seedling leaves of wheat plants. In the summer of 1928, certain physiologic forms were compared with respect to the tendency to form teliospores on leaves of seedlings grown in the greenhouse. No experiments were planned exclusively for this purpose, but notes were taken on the telial formation on hosts which were being tested for their rust reactions to several physiologic forms. Table 31 shows the results. It will be observed that only four of the thirteen physiologic forms produced teliospores on the seedling leaves, namely, forms 14, 33, 48, and 53. In form 53, teliospores were

TABLE 31.—The Formation of Teliospores of Physiologic Forms of *Puccinia graminis tritici* on Seedling Leaves

Host variety	Physiologic form																	
	9	14		15	16	27	33		38	48		49	50	52	53		57
	Interval in days, between inoculation and the formation of																
		first telia	complete telia				first telia	complete telia		first telia	complete telia				first telia	complete telia	
Ruby	No teliospores formed	.....	17	No teliospores formed	No. teliospores formed	No teliospores formed	15	19	No teliospores formed	16	19	No teliospores formed	No teliospores formed	No teliospores formed	.....	13	No teliospores formed
Ceres		17	.....				.....	.....		.....	.....				11	14	
N.D. 1656		.....	17				.....	.....		.....	.....				.....	.....	
Sevier x Dicklow		12	.....				.....	19		.....	14				11	14	
Webster		17	.....				15	19		14	19				.....	11	
Axminster		.....	17				15	19		.....	19				11	14	
Pelissier		.....	.....				19	.....		.....	.....				11	19	
Monad		.....	.....				.....	.....		14	.....				11	19	
Parker's		17	.....				.....	.....		.....	14				11	14	

visible within a day or two after the uredinia had broken out. The other nine forms failed to produce teliospores, no doubt, owing to the early death of the leaves, none of which lasted more than about 21 days. It was decided in later experiments, to compare telial formation among the different physiologic forms on the infected stems of growing wheat plants rather than on the seedling leaves.

In the above experiment there seemed to be no relation between the type of rust reaction of the host and the rapidity of teliospore formation. That is, telia were produced at about the same rate on resistant and susceptible host plants. This does not agree with the observations of Parker (23) who states that telia of crown rust of oats are formed more rapidly on resistant than on susceptible plants.

TELIOSPORE FORMATION ON STEMS OF WHEAT PLANTS

In the latter part of the winter of 1928-29, fifteen physiologic forms were compared as to the rate of teliospore formation on infected stems of several varieties of wheat plants grown in the greenhouse. This work is summarized in Table 32. The observations were made during the course of testing these varieties for their rust resistance. The plants were not all infected at the same time, as would have been desirable, but were inoculated at intervals during the months of January, February, and March. Inoculations were generally made shortly after the plants headed. The varieties on which observations were taken were for the most part susceptible or moderately resistant. No consistent differences were found in the rate of teliospore formation on resistant and susceptible plants; and on no one variety was teliospore formation appreciably more rapid than on others. Observations were most commonly made on the variety Garnet which was infected by fourteen of the fifteen forms.

The plants infected by each form were carefully examined for a sign of the earliest telia, and subsequently, at intervals, until telial formation was complete. Frequently the plants matured or died before telial development was finished, especially those infected by forms which produce teliospores at a slow rate. Forms 15, 17, 19 and 49 failed to complete telial development on any of the varieties infected.

By comparing the averages of the number of days required to produce the earliest telia, these physiologic forms can be arranged in order of rapidity of telial formation (Table 32).

TABLE 32.—Fifteen Physiologic Forms of *Puccinia graminis tritici* Arranged in Order of Rapidity of Teliospore Formation

Physiologic forms	Average number of days between inoculation and formation of first telia	Physiologic forms	Average number of days between inoculation and formation of first telia
53 and 48	16·0	34 and 21	31·0
33	19·0	52 and 19	32·0
14	24·0	9	33·0
49	27·5	15	34·0
38	28·0	50	36·0
36	29·5	17	47·5

It will appear from a comparison of Tables 31 and 32 that forms 14, 33, 48 and 53, develop telia later on the stems of full grown plants (Table 32), than on the seedling leaves (Table 31). In the latter case, however, the telia were produced in warm weather, in midsummer, while in the former, they were formed in late winter.

The Formation of Teliospores at Controlled Temperatures

The observations hitherto described were made on infected wheat plants growing in greenhouses subject to considerable fluctuations in temperature. When temperature control apparatus was installed in two sections of the greenhouse, it was thought desirable to determine the effect of temperature on the formation of teliospores. Two main objects were kept in mind: first, to determine the effects of different temperatures on the rate of teliospore formation; and, second, to determine the effects of the temperature at which the teliospores are formed on their germinability. Only the first of these will be discussed in the present section of this paper.

Two temperatures were chosen for this experiment, one somewhat above the ordinary greenhouse temperature, 70°-75° F., the other somewhat below it, 55°-60° F. These temperatures were fairly well maintained throughout the course of the experiment. On the other hand, no attempt was made to control the air humidity, which fluctuated considerably in both sections of the greenhouse. The per cent relative humidity, however, did not differ greatly in the two sections. Conditions of light were likewise much the same in the two sections; daylight was supplemented by artificial light at night, as the work was carried out during the winter months, when daylight is at a minimum. Consequently, conditions other than temperature were so much alike that any differences which might occur in rust development might be considered attributable to the difference in temperature.

The telial development of eighteen physiologic forms was compared at the two temperatures stated above. Several wheat plants of a susceptible variety were infected by each form soon after they headed out. These were grown in six-inch flower pots containing five or six plants per pot. The procedure followed was to inoculate the plants in two pots by a physiologic form, after which the pots were placed in corresponding positions in the high and low-temperature sections of the greenhouse. After the formation of uredinia observations were made every second day until telial development was complete. The data gathered by these observations is summarized in Table 33.

It was known from work done previously that physiologic forms of wheat stem rust differ greatly in the rate at which they form teliospores, or, in other words, they differ in the length of their respective uredinial period. One of the objects of the work now under discussion was to determine if temperature affected the length of the uredinial stage of the different forms. It is evident from a study of Table 33 that the first telia of each form were consistently formed sooner at the higher temperature, and, likewise, that telial production was always completed earlier at that temperature. Hence, it is obvious that high temperature hastens the development of telia, and, one might infer, shortens the uredinial stage. If the uredinial stage is considered as the period from inoculation to the production of the first telia or even to complete telial development, such an inference is entirely justified. If one, however, considers the uredinial generation to be the period from the formation of uredinia to the formation of telia the situation is somewhat altered. The reason for this is that the incubation period for the formation of uredinia was found to be about five days longer at the lower than at the higher temperature; or about twelve and seven days respectively. Consequently, the uredinial stage begins about five days later at 55°-60°F., than at 70°-75°F. If this is taken into consideration, and if the date of formation of the earliest telia is arbitrarily selected as the end of the uredinial stage, then there is very little difference to be found between the lengths of the uredinial stages at the high and low temperatures. As stated above, the uredinia were formed approximately five days earlier at the high than at the low temperature. The average time for the formation of the earliest telia was only 6·8 days earlier at the high temperature, a difference which can

TABLE 33.—The Rate of Teliospore Formation of Physiologic Forms of *Puccinia graminis tritici* at High Temperatures (70°–75° F.) and Low Temperatures (55°–60° F.)

Physiologic form	Temperature	Host variety	Date of inoculation	Date of development at first telia	Date of full telial development	Number of days to form first telia	Number of days to complete telial development
9	70°–75° F.	Garnet	8–11–29	3–12–29	18–12–29	25	40
9	55°–60° F.	Garnet	8–11–29	no infection			
9	70°–75° F.	Marquis	25–11–29	27–12–29	—	32	
9	55°–60° F.	Marquis	25–11–29	31–12–29	—	26	
14	70°–75° F.	Garnet	8–11–29	26–11–29	29–11–29	18	21
14	55°–60° F.	Garnet	8–11–29	29–11–29	11–12–29	21	33
30	70°–75° F.	Garnet	20–12–29	10– 1–30	—	21	
30	55°–60° F.	Garnet	20–12–29	14– 1–30	11– 2–30	25	53
33	70°–75° F.	Garnet	8–11–29	25–11–29	27–11–29	17	19
33	55°–60° F.	Garnet	8–11–29	1–12–29	13–12–29	23	35
48	70°–75° F.	Garnet	8–11–29	26–11–29	29–11–29	18	21
48	55°–60° F.	Carnet	8–11–29	29–11–29	7–12–29	21	29
53	70°–75° F.	Garnet	8–11–29	26–11–29	29–11–29	18	21
53	55°–60° F.	Garnet	8–11–29	3–12–29	11–12–29	25	33
56	70°–75° F.	Marquis	5–12–29	24–12–29	—	19	
56	56°–60° F.	Marquis	5–12–29	31–12–29	—	26	
56	70°–75° F.	L. Club	19–12–29	8– 1–30	—	20	
56	55°–60° F.	L. Club	19–12–29	14– 1–30	—	26	
78	70°–75° F.	Marquis	2–12–29	—	—	—	
78	55°–60° F.	Marquis	2–12–29	4– 1–30	—‡	33	64+
79	70°–75° F.	Marquis	25–11–29	13–12–29	27–12–29	18	32
79	55°–60° F.	Marquis	25–11–29	20–12–29	9– 1–30	25	45
80	70°–75° F.	Garnet	7–12–29	10– 1–30	—	34	
80	55°–60° F.	Garnet	7–12–29	12– 1–30	—	36	
81	70°–75° F.	Garnet	7–12–29	24–12–29	14– 1–30	17	38
81	55°–60° F.	Garnet	7–12–29	6– 1–30	22– 1–30	30	46
83	70°–75° F.	Marquis	25–11–29	11–12–29	27–12–29	16	32
83	55°–60° F.	Marquis	25–11–29	22–12–29	2– 1–30	27	38
85	70°–75° F.	Garnet	14–11–29	18–12–29	—	34	
85	55°–60° F.	Garnet	14–11–29	27–12–29	—	43	
86	70°–75° F.	Garnet	7–12–29	24–12–29	8– 1–30	17	32
86	55°–60° F.	Garnet	7–12–29	31–12–29	28– 1–30	24	52
91	70°–75° F.	Garnet	19–11–29	9–12–29	18–12–29	20	29
91	55°–60° F.	Garnet	19–11–29	15–12–29	2– 1–30	26	44
92	70°–75° F.	Garnet	14–11–29	—	11–12–29	—	27
92	55°–60° F.	Garnet	14–11–29	7–12–29	29–12–29	23	45
92	70°–75° F.	Marquis	9–12–29	24–12–29	10– 1–30	15	32
92	55°–60° F.	Marquis	9–12–29	2– 1–30	18– 1–30	24	40
92	70°–75° F.	Garnet	24–12–29	5– 1–30	10– 1–30	12	17
92	55°–60° F.	Garnet	24–12–29	10– 1–30	24– 1–30	17	31
93	70°–75° F.	L. Club	11–12–29	2– 1–30	22– 1–30	22	42
93	55°–60° F.	L. Club	11–12–29	8– 1–30	—	28	
95	70°–75° F.	L. Club	11–12–29	27–12–29	2– 1–30	16	22
95	55°–60° F.	L. Club	11–12–29	6– 1–30	—	26	

‡ Plants ripe on Feb. 4 (64 days after inoculation) with most of the rust still in the uredinial stage.

hardly be considered significant. The change from uredinia to telia is, however, a gradual process, and it is difficult to select any point at which the uredinial stage can be said to terminate and the telial stage to begin. It would perhaps be more satisfactory to consider the uredinial stage as the period from the formation of uredinia to the completion of telial formation. If this is done, a more significant different is found between the lengths of the uredinial stages at the two temperatures. Only ten of the eighteen physiologic forms in Table 33 are available for such a comparison. The incubation periods, of seven and twelve days respectively, must be taken into consideration to determine when the uredinial stages begin at the two temperatures. By subtracting seven and twelve respectively from the figures for complete telial development (last column of Table 33), a comparison can be made of the intervals between the formation of uredinia and complete telial formation for ten of the physiologic forms at the two temperatures. The comparison is shown in Table 34.

TABLE 34.—The Intervals, in Days, Between the Formation of Uredinia and Complete Telial Formation for Ten Physiologic Forms, at 55°–60° F. and 70°–75° F.

Temperature	70°–75° F.	55°–60° F.
Physiologic form 14	14	21
do 33	12	23
do 48	14	17
do 53	14	21
do 79	25	33
do 81	31	34
do 83	25	26
do 86	25	40
do 91	22	32
do 92	20	33
do 92	25	28
do 92	10	19
Mean	19·7	27·2

On the basis of the data in Table 34, it appears that the uredinial stage is considerably longer at a low than at a high temperature.

A comparison of the rate of telial formation at the two temperatures leads to a similar conclusion, namely, that the rate of telial formation is more rapid at the higher temperature. The time required for telial formation for each physiologic form can be defined as the time between the formation of the earliest telia and complete telial formation. Figures for this comparison are available for the ten physiologic forms included in Table 34. The average interval, for these forms, between the production of the earliest telia and complete telial development was 9·8 days at the high temperature and 14·8 days at the low. These figures appear to leave no doubt that temperature has a considerable effect on the rapidity of teliospore formation.

The material presented in Tables 31, 32, and 33 shows that there are wide differences between the rates of telial formation of certain physiologic forms. Each form appears to produce teliospores after a more or less definite period of uredinial development. One of the objects of the present investigation was to find out, if possible, to what extent this apparent periodicity is influenced by environmental conditions. Unfortunately, the only external condition studied was temperature. It is possible that conditions of light, air humidity, and carbon-dioxide concentration may have an equally marked effect. While it is evident from the work presented above that certain physiologic forms have inherent tendencies to produce teliospores after rather definite periods in the uredinial stage, there is also evidence that these periods can be shortened or lengthened through the action of temperature.

As it has been shown that temperature exerts an influence on the development of teliospores, it is important to decide whether the fungus is influenced directly by that factor, or indirectly through the medium of the host. The rate of telial formation on different parts of the wheat plant will throw some light on this question. The plants were infected, as previously stated, soon after heading. Care was taken to infect all parts of the plant from the lower part of the stem to the neck, including the leaves. At the time when data were being taken on the formation of the telia, a record was made of their development on the different regions of the plant. If the effect of temperature on the fungus were direct, it might be expected that the rate of telial production on different parts of the plant would not vary greatly. It was found, on the contrary, that the teliospores were formed at very different rates on different parts of the plant. In fact, the wheat plant can be divided into regions according to the order of telial formation. In general, the telia were formed on the plant parts in the following order: (1) nodes, (2) regions immediately below and above the nodes, (3) the lower stem (two first internodes) and the neck, (4) the middle internodes, (5) the leaves and the sheath of the uppermost leaf. It is apparent from this order of telial formation that the physiology and morphology of the part of the plant bearing the fungus have a marked effect on the formation of teliospores. Most of the plant parts bearing the earliest telia are either those which pass early into a state of lignification, or those which contain a large proportion of vascular tissue. The most succulent parts of the plant, including the leaves and especially the uppermost leaf sheath are invariably the last to bear teliospores. The earlier maturing of the plant tissues at the higher temperature has undoubtedly a pronounced effect in bringing about early telial production. Hence it seems that the influence of temperature on telial development is indirect and traceable through its effect on the host plant. Gassner's statement that teliospores develop independently of external conditions when the host plant reaches a certain stage of maturity cannot, however, be accepted at its face value, because some of the physiologic forms failed to produce teliospores, except in minute quantities, even when the host plant ripened.

As a result of this work, it seems warrantable to conclude that physiologic forms of *Puccinia graminis tritici* differ widely in their rates of teliospore formation, and, consequently, in the respective lengths of their uredinial stage. In spite of these inherent differences, the rate of teliospore production can be affected by environment, or, at least, by temperature acting through the medium of the host plant. To what extent other environmental conditions are similarly effective can only be determined by further experimental work.

THE EFFECT OF THE ENVIRONMENT ON THE GERMINABILITY OF TELIOSPORES OF *PUCCINIA GRAMINIS TRITICI*

Introduction

The teliospores of *Puccinia graminis*, like those of many other rusts, are formed in the late summer and autumn and, after overwintering, germinate in the following spring. Thus their period of dormancy seems to coincide with the winter season. The dormancy period does not, however, necessarily coincide with the winter season, as was shown by Eriksson and Henning who, according to Zimmerman (36), succeeded in germinating teliospores of *Puccinia graminis* at various times during the winter. Teliospores on barley germinated as early as December 12; on wheat, December 23; on oats, March 28; and on rye, April 6. Klebahn (19) also succeeded in germinating teliospores of *P. graminis* as early as January 10 by a process of alternate wetting and drying of the spores.

Waterhouse (33), in Australia, found that teliospores of stem rust formed on wheat in December, began to germinate after the middle of March and continued to produce sporidia at intervals, until maximum germination was reached in September.

These statements show that the period of dormancy in teliospores is not necessarily of the same length as the winter season, although in nature, the two undoubtedly coincide.

Preliminary Experiments

The writer made an attempt to determine how early teliospores of *Puccinia graminis tritici* formed in nature would germinate. On October 3, 1929, plants of *Hordeum jubatum* and *Agropyron tenerum*, bearing telia, were collected. The teliospores were probably formed early in September, about one month before the test was begun. It was planned in the subsequent treatment of these spores, to imitate as closely as possible the conditions to which teliospores are exposed in nature, with the exception of greatly shortening the period of freezing. The method adopted consisted in freezing the spores for a short period and then spraying them with tap water, after which the spores were wetted and dried alternately. The straws bearing the spores were frozen at -5° C., in blocks of ice in a frigidaire, from October 3 to October 26, a period of 23 days. They were then mounted on a wooden frame and sprayed with tap water the temperature of which varied from 8° to 10° C. This spraying lasted for 18 days, from October 26 to November 13. On the latter date, the spores were laid out to dry in the laboratory until November 30. On December 1, 2, and 3, they were examined for germination. The spores of *Hordeum jubatum* germinated slightly, those on *Agropyron tenerum*, abundantly. Barberry plants inoculated by spores from both these samples became heavily infected.

On December 4, *Agropyron tenerum* bearing teliospores was dug up from under the snow and brought into the laboratory. Straws bearing telia were soaked in water for a few hours and examined daily for germination from December 5 to December 9. No germination took place during this period. The spores, in a moist condition, were then suspended over a microscope slide in a petri dish from December 9 to December 19, and examined for germination on the latter date. Abundant germination had taken place in the interval. It should be pointed out that these spores had not been dried, and hence, their germination can not be attributed to the influence of alternate wetting and drying.

The above-mentioned method of freezing followed by alternate wetting and drying likewise resulted in the germination of teliospores of two physiologic forms of wheat stem rust, namely, forms 53 and 36, which had been formed in the greenhouse. The teliospores of form 53 were formed early in April, those of form 36, early in June, 1929. After formation, they were kept out-of-doors until August 28 when they were frozen at -5° C. in blocks of ice in a fridigaire. On October 3, they were removed and sprayed with tap water until October 21. As no germination was observed after spraying, the spores were allowed to dry in the laboratory. On December 4, the teliospores of both physiologic forms germinated well. In this case the drying of the spores appeared to be essential as they had failed to germinate, before freezing, after freezing, and after spraying.

The erratic germination, in previous years, of teliospores formed in the greenhouse had suggested that there might be some relation between the conditions of their formation and the germinability of the spores. This is also suggested by Eriksson (9) who believes that only teliospores formed late in autumn are capable of germinating in the following spring. If this is true, the temperature at which the spores are formed may be an important factor in determining their germinability. It was thought worth while to take this possibility into consideration in experiments which were being planned.

Experiments to Determine the Minimal Dormancy Period of Teliospores Formed in the Greenhouse

METHODS

The methods employed in the following series of experiments may be described briefly as follows. Wheat plants of susceptible varieties were inoculated by a number of physiologic forms of wheat stem rust shortly after the emergence of the heads. The plants inoculated by each form were divided into two lots, one of which was placed in a greenhouse maintained at a relatively high temperature, 70-75° F., the other in a greenhouse kept at a lower temperature, 55-60° F. Notes were taken on the formation of the teliospores as described in the section of this paper dealing with that phase of the work. When telial formation was complete, the stems bearing the spores were cut up into short pieces and subjected to treatments described under the individual experiments.

The technique of examining teliospores for germination was relatively simple, and was devised to the end that a large number of samples could be examined in a minimum amount of time. Short straws bearing the teliospores were suspended over a microscope slide in a petri dish partially lined with wet blotting paper. The slide rested on wet blotting paper in the bottom of the dish, the wet straws were fixed to the top, directly above the slide, by pressing them with the fingers against the soaked lining of blotting paper. The straws very rarely fell from their position. The sporidia were shot off from the promycelia of the teliospores and could easily be detected on the slides beneath. A fine film of water, usually present on the slide, aided in the identification of the sporidia, which always germinated in a characteristic manner when moisture was present. In addition to this fact, it might be said that there was little danger of confusing the sporidia with other fungous spores, as the teliospores formed in the greenhouse were rarely overgrown with other fungi, and rarely did other spores appear on the slides.

When spores which had previously been dried were tested for germination, they were soaked from four to six hours in tap water before they were suspended in the petri dish over the slide. Examination was usually made on two successive days.

In recording the amount of germination, the terms "trace", "slight", "fair", "good", and "excellent" are used. The term "trace" denotes that only a few sporidia were found on the slide. "Slight" indicates that sporidia were thinly distributed on the slide beneath the telia. "Fair" signifies that the masses of sporidia on the slide were just visible to the naked eye. "Good" and "excellent" are terms describing abundant germination; the latter term indicates that the sporidia form thick, white lines on the slide, visible at a distance of several feet. For convenience the numerals 1, 2, 3, 4, and 5 are used in tabulating the results. The numeral 1 represents "trace" germination; 2, "slight"; 3, "fair"; 4, "good"; and 5, "excellent".

DETAILS OF EXPERIMENTS

The main object of the following five series of experiments was to determine the minimal dormancy period of teliospores of *Puccinia graminis tritici* formed in the greenhouse. Other objects were kept in view at the same time, such as: the effect of temperature while the teliospores were forming on the germinability of the spores; the effect of freezing; the effect of alternate wetting and drying. The effect of alternate freezing and thawing was not taken into consideration in planning these experiments, as this had been investigated in previous years without any positive results.

Series A.—The principle on which this series of experiments was planned was that already mentioned, namely, of simulating the conditions to which teliospores are exposed in nature. The spores were subjected to various periods of freezing, followed by spraying with tap water and alternate wetting and drying. The shortest period of freezing in this series was six days. Following the freezing, the spores were sprayed with the tap water at 5° to 6° C. for seven days, after which they were alternately wetted and dried. In the latter process, the straws bearing the spores were usually soaked for about six hours in tap water and then suspended in petri dishes above microscope slides as previously described. In that position they were kept moist for two days, during which time the slides were examined daily for the presence of sporidia. After this, the spores were dried in the laboratory for two days at a temperature of from 20° to 25° C. and a very low atmospheric humidity. This process of alternate wetting and drying was repeated several times in each set of experiments. Spore germination was always carried out at 60°-65° F.

The results of this series of experiments are tabulated under Series A. 1, and Series A. 2, Tables 36 and 37. The only difference in detail between the two experiments consists in the periods of freezing, which were respectively six and fourteen days. There is no appreciable difference in the results of the two experiments. In both, germination began after the first period of drying and reached a maximum, at least with the spores formed at the lower temperature, after the third drying. In both, there was a striking difference in the germination of spores formed at high and low temperatures in favour of the latter. If there was any difference between the germination of teliospores frozen out of doors, at –20° to –35° C., and that of spores frozen in the frigidaire, at -5° C., it was expressed in the slightly more abundant germination of the spores frozen at -5° C.

TABLE 35.—Dates of Formation and Germination of Teliospores in Series A

——	Physiologic form	Host variety	Date first telia formed	Date of full telial formation	Date of first germination
Teliospores formed at 70–75° F.....	48	Garnet	26–11–29	29–11–29	5–2–30
	14	Garnet	26–11–29	29–11–29	1–2–30
	33	Garnet	26–11–29	27–11–29	28–1–30
	53	Garnet	26–11–29	29–11–29	28–1–30
Teliospores formed at 55–60° F.....	48	Garnet	29–11–29	11–12–29	24–1–30
	14	Garnet	29–11–29	13–12–29	24–1–30
	33	Garnet	1–12–29	13–12–29	24–1–30
	53	Garnet	3–12–29	11–12–29	28–1–30

TABLE 36.—Series A.-1—The Germination of Teliospores of Four Physiologic Forms of *Puccinia graminis tritici* Formed at Mean Temperatures of 58° F. and 72·5° F.

Physiologic form	Temperature during telial formation	Period of freezing	Period of spraying	Germination following 1st drying (5 days) Jan. 23	Jan. 24	2nd drying (2 days) Jan. 27	Jan. 28	3rd drying (2 days) Jan. 31	Feb. 1	4th drying (3 days) Feb. 5	Feb. 6	5th drying (3 days) Feb. 10	Feb. 11	6th drying (2 days) Feb. 14	Feb. 15	7th drying (1 day) Feb. 17	Feb. 18
14	58·0° F.	Outside 6 days	7 days	0	0	0	3	1	3	0	2	0	0	2	3	0	2
14	72·5° F.	"	"	0	0	0	0	0	0	2	2	0	0	2	2	2	0
33	58·0° F.	"	"	0	0	0	4	5	4	3	4	1	2	2	2	3	1
33	72·5° F.	"	"	0	0	0	1	2	1	3	2	2	2	2	1	2	0
48	58·0° F.	"	"	0	2	0	2	3	4	3	3	0	1	2	2	2	1
48	72·5° F.	"	"	0	0	0	0	0	0	1	0	0	0	0	0	0	0
53	58·0° F.	"	"	0	0	0	2	0	3	2	3	0	0	3	3	0	0
53	72·5° F.	Frigidaire 6 days	"	0	0	0	1	0	1	0	1	0	0	0	0	0	0
14	58·0° F.	"	"	0	1	0	4	5	4	5	5	2	0	3	3	2	2
14	72·5° F.	"	"	0	0	0	0	0	1	0	2	0	0	2	2	0	0
33	58·0° F.	"	"	0	4	3	5	5	4	2	4	2	3	3	4	2	0
33	72·5° F.	"	"	0	0	0	2	2	3	3	4	2	3	2	3	3	2

TABLE 36.—Series A.-1—The Germination of Teliospores of Four Physiologic Forms of *Puccinia graminis tritici* Formed at Mean Temperatures of 58° F. and 72·5° F.—*Continued*

Physiologic form	Temperature during telial formation	Period of freezing	Period of spraying	Germination following 8th drying (2 days)		9th drying (2 days)		10th drying (2 days)		11th drying (2 days)		12th drying (2 days)		13th drying (2 days)		14th drying (2 days)
				Feb. 21	Feb. 22	Feb. 25	Feb. 26	Mar. 1	Mar. 2	Mar. 5	Mar. 6	Mar. 9	Mar. 10	Mar. 13	Mar. 14	Mar. 17
14	58·0° F.	Outside 6 days	7 days	2	2	3	4	3	3	4	4	4	3	0	3	4
14	72·5° F.	"	"	0	1	2	2	1	2	3	3	3	2	2	3	4
33	58·0° F.	"	"	2	3	2	2	3	2	4	3	4	3	4	3	5
33	72·5° F.	"	"	0	0	1	2	2	1	3	2	3	1	3	2	4
48	58·0° F.	"	"	0	0	0	2	2	0	3	2	1	0	3	2	4
48	72·5° F.	"	"	0	0	0	0	0	0	1	0	0	0	0	1	0
53	58·0° F.	"	"	0	1	2	1	0	0	3	2	0	0	2	1	1
53	72·5 F.	Frigidaire 6 days	"	0	0	0	0	0	0	0	0	0	1	0	0	0
14	58·0° F.	"	"	3	3	4	4	4	4	4	2	4	1	4	4	4
14	72·5° F.	"	"	0	0	2	2	1	2	3	2	4	1	3	2	3
33	58·0° F.	"	"	3	4	3	3	4	4	4	2	4	2	4	2	5
33	72·5° F.	"	"	2	2	2	0	2	2	3	2	3	2	4	3	4

TABLE 36.—Series A.-1—The Germination of Teliospores of Four Physiologic Forms of *Puccinia graminis tritici* Formed at Mean Temperatures of 58° F. and 72·5° F.—*Concluded*

Physiologic form	Temperature during telial formation	Period of freezing	Period of spraying	Germination following 15th drying (2 days) Mar. 20	16th drying (2 days) Mar. 23	17th drying (2 days) Mar. 26	18th drying (2 days) Mar. 29	19th drying (2 days) Apr. 1	20th drying (2 days) Apr. 4
14	58·0° F.	Outside 6 days	7 days	5	4	3	0	0	0
14	72·5° F.	"	"	4	2	3	0	0	0
33	58·0° F.	"	"	4	4	3	3	1	1
33	72·5° F.	"	"	4	3	2	3	0	0
48	58·0° F.	"	"	3	3	3	2	0	0
48	72·5° F.	"	"	0	2	2	0	0	0
53	58·0° F.	"	"	1	1	0	0	0	0
53	72·5° F.	Frigidaire 6 days	"	0	0	0	0	0	0
14	58·0° F.	"	"	5	3	2	4	3	0
14	72·5° F.	"	"	3	1	2	0	1	0
33	58·0° F.	"	"	5	3	3	4	3	0
33	72·5° F.	"	"	5	4	3	3	1	0

Explanation of symbols: 0 = no germination. 1 = trace germination
2 = slight germination 3 = fair germination
4 = good germination 5 = excellent germination

TABLE 37.—Series A. 2—The Germination of Teliospores of Three Physiologic Forms of *Puccinia graminis tritici* Formed at Mean Temperatures of 58° F. and 72·5° F.

				Germination following													
				1st drying (2 days)		2nd drying (2 days)		3rd drying (4 days)		4th drying (2 days)		5th drying (2 days)		6th drying (2 days)		7th drying (2 days)		8th drying (2 days)	
Physiologic form	Mean temp. during telial formation	Period of freezing	Period of spraying	Jan. 29	Jan. 30	Feb. 2	Feb. 3	Feb. 8	Feb. 9	Feb. 12	Feb. 13	Feb. 16	Feb. 17	Feb. 20	Feb. 21	Feb. 24	Feb. 25	Feb. 28	Mar. 1
14	58·0° F.	Outside 14 days	7 days	0	0	3	4	5	5	5	5	5	4	3	2	2	4	3	3
14	72·5° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	58·0° F.	"	"	0	0	1	3	3	5	5	5	4	3	5	1	2	4	4	4
33	72·5° F.	"	"	0	0	0	0	2	2	3	3	3	3	2	1	1	2	2	1
48	58·0° F.	"	"	0	1	3	2	4	3	4	4	3	3	0	0	0	0	1	1
48	72·5° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	58·0° F.	Frigidaire 14 days	"	0	1	3	4	5	5	5	5	5	5	5	2	3	5	4	3
33	72·5° F.	"	"	0	0	0	2	3	3	4	4	4	4	4	2	3	3	3	2
14	58·0° F.	"	"	0	2	0	3	5	5	5	5	5	5	5	4	3	4	4	4
14	72·5° F.	"	"	0	0	0	0	0	1	2	3	3	2	2	2	1	2	0	2

Explanation of symbols: 0 = no germination 1 = trace germination
2 = slight germination 3 = fair germination
4 = good germination 5 = excellent germination

The teliospores formed at the lower temperature, 55°-60° F., began to germinate from 42 to 48 days after telial formation was complete (*Vide* Table 35); those formed at the higher temperature, 70°-75° F., about 60 days after formation. The fact that the teliospores produced at the higher temperature, although formed more than two weeks earlier than those at the lower temperature, germinated more sparsely than the latter spores indicates that age is not an important factor in the germinability of teliospores.

The method of alternately wetting and drying the same samples of spores gave the writer an opportunity of determining the duration of their germinability. This was done by continuing the alternate wetting and drying until the spores ceased entirely to germinate. Table 36 (Series A. 1.) shows that several of the samples continued to germinate from January 24 to April 1, 1930, or, for a period of 68 days. If only the days on which actual germination took place are considered, this period is reduced to 31 days. The fact that some of the spores germinated after as many as 19 repetitions of wetting and drying is rather remarkable. Apparently these spores remained unaffected by the first 18 repetitions but responded to the nineteenth.

There is no way of telling whether or not the spores germinate in the order of their formation; that is, whether the earliest-formed spores germinate first. It is evident, however, that there is no strict relation in time between the formation of the spores and their germination. If such a relation existed, the duration of germination should correspond with the duration of formation of the spores. The low-temperature teliospores in Series A. 1 were formed during a period of less than two weeks. They continued to germinate during a period of 68 days, which included 31 days of actual germination.

These differences in response among teliospores in the same sori can only be explained by assuming physiological differences between teliospores formed under identical conditions. These physiological differences prevent the teliospores from responding simultaneously to such stimuli as alternate wetting and drying.

FIG. 13.—A comparison of the germination of teliospores formed at 55-60°F., with that of teliospores formed at 70-75°F. Series A.1.

No conclusion can be drawn from these experiments as to what portion of the stimulation to germinability is due respectively to freezing and alternate wetting and drying. Much of the stimulus is probably due to the latter, as the high-temperature teliospores do not arrive at maximum germination until after the eleventh drying (*cf.* Fig. 13). Freezing, certainly, does not stimulate the spores to immediate germination. All the samples tested in the five series of experiments were examined for germination for two successive days following freezing and spraying. No germination was ever observed. On the other hand, germination almost invariably began after the first drying. Furthermore, there was very little difference between the germination of the spores frozen for six days (Table 36) and those frozen for fourteen days (Table 37). In some of the later experiments, consideration was given to factors responsible for the stimulation.

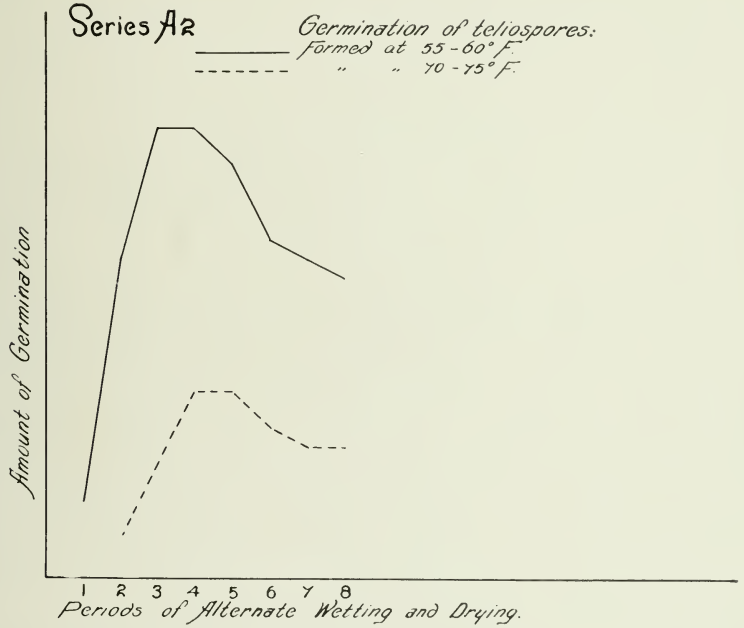

FIG. 14.—A comparison of the germination of teliospores formed at 55-60°F. with that of teliospores formed at 70-75°F. Series A.2.

Series B. The four physiologic forms used in Series A. are characterized by early and rapid teliospore formation. The experiments of Series B. were planned largely with the object of determining the success of this method of securing germination, when applied to teliospores of other forms some of which are characterized by slow telial formation. Unfortunately, little teliospore material of the latter forms was available, owing to the difficulty of inducing such forms to produce telia. Two of these forms, namely, 9 and 85, were included in Series B (Table 39).

The results of these experiments are so similar to those recorded for Series A. that no special discussion is necessary. The two physiologic forms, 9 and 85, characterized by slow telial production, germinated quite as well as the other more rapid teliospore-producing forms. The germination of the high and low-temperature teliospores is expressed graphically in Fig. 15.

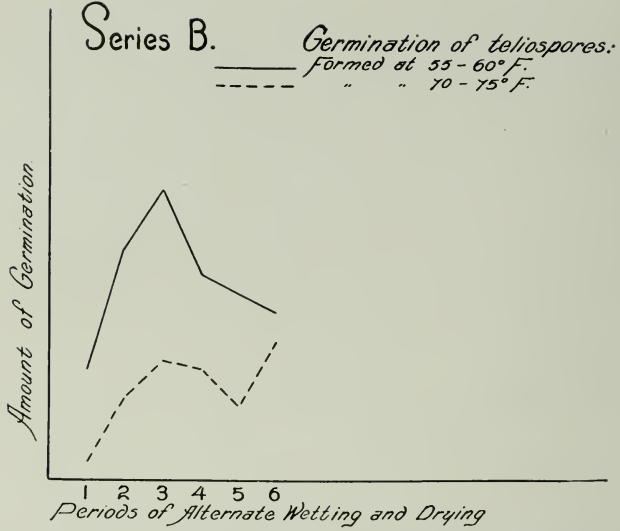

FIG. 15.—A comparison of the germination of teliospores formed at 55-60°F. with that of teliospores formed at 70-75°F. Series B.

TABLE 38.—Dates of Formation and Germination of Teliospores in Series B

—	Physiologic form	Host variety	Date first telia formed	Date of full telial formation	Date of first germination
Teliospores formed at 70–75°F.....	91	Marquis	9–12–29	18–12–29	9–2–30
	79	Marquis	13–12–29	27–12–29	14–2–30
	92	Garnet	5–12–29	9–12–29	17–2–30
	83	Marquis	11–12–29	27–12–29	17–2–30
	85	Garnet	18–12–29	.....	26–2–30
	9	Garnet	3–12–29	18–12–29	6–2–30
Teliospores formed at 55–60°F.....	91	Marquis	15–12–29	4– 1–30	6–2–30
	79	Marquis	20–12–29	9– 1–30	14–2–30
	92	Garnet	7–12–29	29–12–29	6–2–30
	83	Marquis	22–12–29	2– 1–30	14–2–30
	85	Garnet	27–12–29	.....	6–2–30

TABLE 39.—Series B.—The Germination of Teliospores of Eight Physiologic Forms of *P. graminis tritici* Formed at High and Low Temperatures

Physiologic form	Mean temp. during telial formation	Period of freezing	Period of spraying	Germination following: 1st drying (2 days) Feb. 5	Feb. 6	2nd drying (2 days) Feb. 9	Feb. 10	3rd drying (2 days) Feb. 13	Feb. 14	4th drying (2 days) Feb. 17	Feb. 18	5th drying (2 days) Feb. 21	Feb. 22	6th drying (2 days) Feb. 25	Feb. 26
		Outside													
91	73·9° F.	14 days	7 days	0	0	0	1	1	1	1	0	1	1	2	2
91	58·3° F.	"	"	0	2	3	4	5	4	3	2	2	3	2	2
79*	73·9° F.	"	"	0	0	0	0	0	1	1	0	0	0	0	2
79*	58·3° F.	"	"	0	0	0	0	0	1	1	0	1	1	0	0
92	73·9° F.	"	"	0	0	0	0	0	0	1	0	0	0	0	0
92	58·3° F.	"	"	0	3	3	3	4	3	2	2	1	2	2	2
83	73·9° F.	"	"	0	0	0	0	0	0	1	0	0	0	1	1
83	58·3° F.	"	"	0	0	0	0	0	2	0	0	0	0	0	2
85*	73·9° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	2
85	58·3° F.	"	"	0	2	4	4	4	4	3	0	2	2	0	0
		Frigidaire 21 days													
48	72·5° F.	"	"	0	0	0	0	1	2	1	1	0	1	0	2
48	58·0° F.	"	"	0	3	4	5	5	5	5	2	2	4	4	2
53	72·5° F.	"	"	0	0	0	2	2	2	1	1	1	1	0	1
53	58·0° F.	"	"	0	0	2	4	5	5	4	2	1	4	4	3
91	73·9° F.	"	14 days	0	0	1	2	2	3	3	1	1	2	3	3
91	58·3° F.	"	"	0	2	3	5	5	5	4	3	3	4	4	3
9	72·5° F.	"	21 days	0	2	4	4	2	4	3	0	0	3	2	2

*Very few telia on straws.

Series C. This series of experiments was planned chiefly to determine the relative effects of freezing and alternate wetting and drying on the germinability of teliospores. Telia of each of four physiologic forms were divided into five lots. One of these was not frozen but was sprayed for seven days, followed by alternate wetting and drying. A second lot was frozen for two days, sprayed for seven, and then alternately wetted and dried. The third lot was frozen for four days, sprayed for seven, and subsequently kept wet and dry alternately. The fourth lot was frozen for six days, but was otherwise treated like the second and third lots. A fifth lot was frozen for six days, but was afterwards kept constantly moist instead of being wetted and dried.

The results of these tests are shown in Tables 41, 42, and 43, and are expressed graphically in Fig. 16. Germination took place in all of the above-mentioned lots of teliospores. The spores frozen and subsequently wetted and dried germinated somewhat more consistently than the others. There was no very marked difference in the germination of spores frozen for two, four, and six days respectively. The unfrozen spores germinated somewhat more sparsely and less consistently. The spores frozen for six days and kept moist continually germinated poorly with the exception of two of the samples.

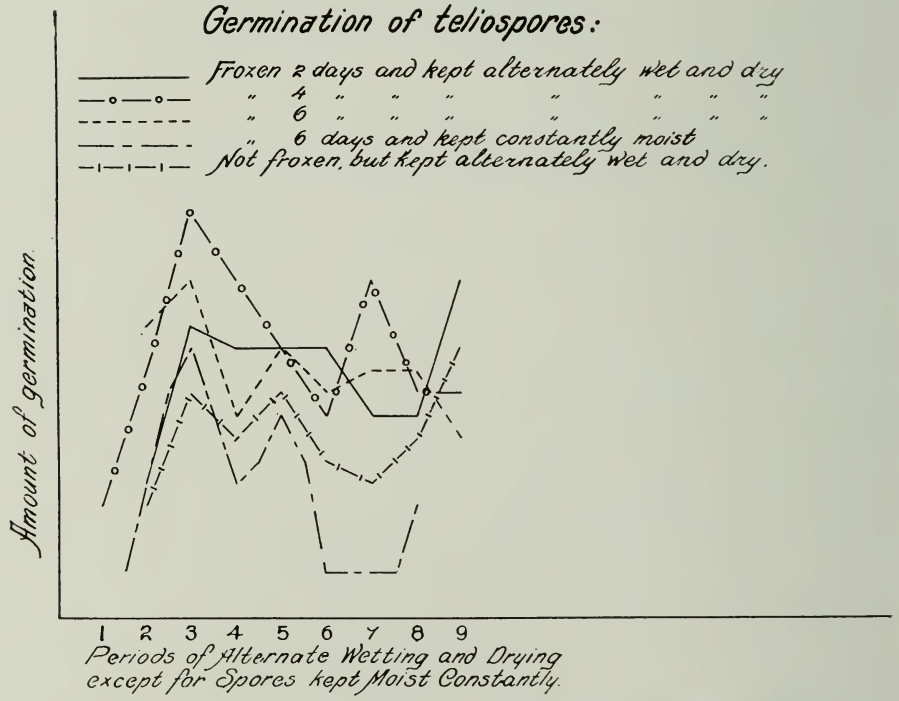

FIG. 16.—The effect of different treatments on teliospore germination. Series C.

If a comparison is made of the germination of the spores which were not frozen, but alternately wetted and dried with that of the spores which were frozen and kept continuously wet, it is evident that the former treatment resulted in better germination (*cf.* Fig. 16). It should also be pointed out that the germinability of the spores subjected to the latter treatment decreased gradually until they ceased to germinate, while the germinability of the spores given the former treatment was unimpaired at the time the experiment was discontinued.

It was unfortunate that these experiments did not include unfrozen spores kept constantly moist. Spores so treated would have been valuable for purposes of comparison with the spores which were frozen and subsequently kept moist. An experiment on the effect of citric acid on teliospore germination, which was carried on simultaneously with the experiments in Series C, contained one sample (untreated check) of teliospores of form 81, derived from the same telial material as used in these experiments. These spores had not been frozen, but were soaked in water and kept moist for 48 days. No germination took place during this period. Teliospores of the same form which had been frozen for twelve days, but otherwise treated in the same manner, germinated after being kept moist for 29 days.

From these experiments it appears that either freezing, or alternate wetting and drying has some stimulatory action on teliospore germination. The latter process, however, appears to be the more effective.

This series of experiments throws some light on the question of the length of the dormancy period of teliospores. Teliospores of the four physiologic forms, namely, 92, 86, 56, and 81, formed at the lower temperature, began to germinate respectively 26, 20, 30, and 37 days after teliospore development was finished (Table 40). Those formed at the higher temperature germinated rather sparsely, although formed somewhat earlier. Evidently the dormancy period of teliospores can be affected greatly by environmental conditions. In some of the above experiments the period of dormancy has actually been reduced to about one-tenth of the dormancy period of teliospores in nature, under the climatic conditions which prevail in Canada.

TABLE 40.—Dates of Formation and Germination of Teliospores in Series C

—	Physiologic form	Host variety	Date first telia formed	Date of full telial formation	Date of first germination
Teliospores formed at 70–75°F.....	92	Garnet	5- 1–30	10–1–30	24–2–30
	86	Garnet	24–12–29	8–1–30	19–2–30
	56	Marquis	24–12–29	6–1–30	25–2–30
	81	Garnet	24–12–29	14–1–30	25–2–30
Teliospores formed at 55–60°F.....	92	Garnet	10- 1–30	22–1–30	17–2–30
	86	Garnet	31–12–29	28–1–30	17–2–30
	56	Marquis	31–12–29	18–1–30	17–2–30
	81	Garnet	6- 1–30	22–1–30	18–2–30

TABLE 41.—Series C. 1—A Comparison of the Germination of Teliospores Frozen for Two Days and Unfrozen Teliospores of Four Physiologic Forms of *P. graminis tritici*

Physiologic form	Mean temperature during telial formation	Treatment before spraying	Period of spraying	Germination following 1st drying (2 days)		2nd drying (2 days)		3rd drying (2 days)		4th drying (2 days)		5th drying (2 days)		6th drying (2 days)		7th drying (2 days)		8th drying (2 days)		9th drying (2 days)	
				Feb. 14	Feb. 15	Feb. 18	Feb. 19	Feb. 22	Feb. 23	Feb. 26	Feb. 27	Mar. 2	Mar. 3	Mar. 6	Mar. 7	Mar. 10	Mar. 11	Mar. 14	Mar. 15	Mar. 18	Mar. 19
92	58·0° F.	Frozen 2 days	7 days	0	0	1	0	3	4	2	4	4	3	3	4	3	3	4	3	4	3
92	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	1	1	2	1	0	0	0
86	58·0° F.	"	"	0	0	3	3	4	4	3	3	2	2	1	2	1	0	0	0	2	2
86	74·3° F.	"	"	0	0	0	1	0	2	0	1	1	0	0	2	0	0	0	0	1	0
56	58·0° F.	"	"	0	0	0	0	2	1	1	2	2	2	2	3	1	3	3	0	5	3
56	74·3° F.	"	"	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1
81	58·0° F.	"	"	0	0	1	2	3	3	2	3	4	3	2	3	2	2	2	1	4	3
81	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	2
92	58·0° F.	at 6° C. 2 days	"	0	0	0	0	2	3	2	4	3	3	2	0	2	2	3	0	4	3
92	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	0	0	0
86	58·0° F.	"	"	0	0	3	1	4	4	1	2	2	1	0	0	0	0	1	0	2	1
86	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
56	58·0° F.	"	"	0	0	0	0	0	1	0	0	2	1	2	2	0	2	2	1	3	2
56	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	1	1	2	1	0	0	0	1	0
81	58·0° F.	"	"	0	0	0	2	2	2	0	2	3	3	3	3	1	2	2	2	3	3
81	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	2	2

TABLE 42.—Series C. 2—Germination of Teliospores of Four Physiologic Forms of *P. graminis tritici* Frozen for Four Days at −5° C. Followed by Spraying and Alternate Wetting and Drying

Physiologic form	Mean temp. during telial formation	Period of freezing	Period of spraying	Germination following																	
				1st drying (2 days)		2nd drying (2 days)		3rd drying (2 days)		4th drying (2 days)		5th drying (2 days)		6th drying (2 days)		7th drying (2 days)		8th drying (2 days)		9th drying (2 days)	
				Feb. 16	Feb. 17	Feb. 20	Feb. 21	Feb. 24	Feb. 25	Feb. 28	Mar. 1	Mar. 4	Mar. 5	Mar. 8	Mar. 9	Mar. 12	Mar. 13	Mar. 16	Mar. 17	Mar. 20	Mar 21
92	58·0° F.	4 days	7 days	0	2	2	3	4	5	3	5	4	5	4	2	5	4	2	2	0	2
92	74·3° F.	"	"	0	0	0	0	1	1	2	1	1	3	2	1	0	2	2	1	3	1
86	58·0° F.	"	"	0	2	3	4	4	5	3	3	2	3	2	1	2	4	2	2	3	1
86	74·3° F.	"	"	0	0	0	1	2	3	1	1	1	0	2	0	0	0	0	0	1	1
56	58·0° F.	"	"	0	1	2	1	2	4	2	3	2	2	2	2	2	3	3	1	2	0
56	74·3° F.	"	"	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0
81	58·0° F.	"	"	0	0	2	2	2	4	3	4	0	2	1	1	2	3	3	2	3	1
81	74·3° F.	"	"	0	0	0	0	0	3	1	1	1	1	0	0	0	2	2	0	0	0

Explanation of symbols: 0 = no germination 1 = trace germination
2 = slight germination 3 = fair germination
4 = good germination 5 = excellent germination

TABLE 43.—Series C 3—A Comparison of the Germination of Teliospores Frozen for Six Days and Alternately Wetted and Dried with Those Similarly Frozen but Kept Continually Moist

Physiologic form	Mean temp. during telial formation	Period of freezing	Period of spraying	Germination following: 1st drying (2 days)		2nd drying (2 days)		3rd drying (2 days)		4th drying (2 days)		5th drying (2 days)		6th drying (2 days)		7th drying (2 days)		8th drying (2 days)		9th drying (2 days) [1]	
				Feb. 18	Feb. 19	Feb. 22	Feb. 23	Feb. 26	Feb. 27	Mar. 2	Mar. 3	Mar. 6	Mar. 7	Mar. 10	Mar. 11	Mar. 14	Mar. 15	Mar. 18	Mar. 19	Mar. 22	Mar. 23
92	58·0° F.	6 days	7 days	0	0	0	3	1	3	4	5	3	4	4	4	3	2	5	3	3	3
92	74·3° F.	"	"	0	0	0	1	0	2	1	2	2	2	1	1	2	1	3	2	1	2
86	58·0° F.	"	"	0	0	4	5	3	4	0	0	2	2	1	0	3	1	2	2	0	1
86	74·3° F.	"	"	0	0	0	1	0	1	0	0	2	1	0	0	0	0	0	1	0	0
56	58·0° F.	"	"	0	0	0	2	3	3	1	0	2	3	1	2	2	0	2	1	0	1
56	74·3° F.	"	"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	58·0° F.	"	"	0	0	2	3	3	5	3	3	2	3	1	3	3	2	0	2	1	3
81	74·3° F.	"	"	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	1	1
			Physiologic form	Feb. 17	Feb. 19	Feb. 21	Feb. 23	Feb. 25	Feb. 27	Mar. 1	Mar. 3	Mar. 5	Mar. 7	Mar. 9	Mar. 11	Mar. 13	Mar. 16	Mar. 18	Mar. 20	Mar. 22	
Duplicates of above kept moist continually in petri dishes instead of alternately wetted and dried.			92	0	0	3	5	5	4	3	3	3	3	2	1	1	1	2	0	0	
			92	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	
			86	0	2	3	5	5	4	3	2	3	2	0	0	0	0	0	0	0	
			86	0	0	0	1	1	0	0	0	1	1	0	1	0	0	0	0	0	
			56	0	0	0	0	2	0	0	1	1	1	0	1	1	0	1	0	0	
			56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			81	0	0	0	0	0	1	0	1	2	1	0	0	0	1	2	0	0	
			81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE 44.—Series D—(A) Germination of teliospores of form 95 frozen at −5° C. for seven days, followed by spraying and alternate wetting and drying.
(B) Germination of teliospores of form 95 not frozen, but sprayed and alternately wetted and dried.
(C) Germination of teliospores of form 95 neither frozen nor wetted and dried, but kept continually moist.

A.

				Germination following						
				1st drying (2 days)		2nd drying (2 days)	3rd drying (2 days)	4th drying (2 days)	5th drying (2 days)	6th drying (2 days)
Physiologic form	Temperature during telial formation	Period of freezing	Period of spraying	Mar. 12	Mar. 13	Mar. 17	Mar. 20	Mar. 23	Mar. 26	Mar. 29
95	74·1° F.	7 days	7 days	0	1	2	3	4	5	4
	59·2° F.	"	"	0	2	5	5	5	5	5

B.

				Germination following						
				1st drying (2 days)		2nd drying (2 days)	3rd drying (2 days)	4th drying (2 days)	5th drying (2 days)	6th drying (2 days)
Physiologic form	Temperature during telial formation	Period of freezing	Period of spraying	Mar. 5	Mar. 6	Mar. 10	Mar. 14	Mar. 18	Mar. 22	Mar. 26
95	74·1° F.	no freezing	7 days	0	0	0	0	1	0	1
	59·2° F.	no freezing	"	0	0	1	4	3	1	2

C.

Two samples of teliospores formed at 74° F. and 59° F. were soaked in water for ten hours on February 21, 1930, and then kept moist continually in petri dishes. They were examined daily for germination from February 24 to April 4. No germination took place during this period.

Series D.—The chief object of these experiments was to determine whether teliospores formed in the greenhouse would germinate without any treatment other than that of being kept moist continuously. Physiologic form 95 was chosen for this experiment on account of the abundant supply of teliospores available. These spores were formed at temperatures of about 74° F. and 59° F. respectively. Telial development at the higher temperature was complete on January 15, that at the lower temperature on February 20.

On February 21, samples of teliospores formed at the two above-mentioned temperatures were soaked in water for about ten hours and subsequently kept moist in a petri dish at a temperature of about 65° F. Two other samples of the same telial material were sprayed for seven days without any previous freezing, and subsequently subjected to the usual process of alternate wetting and drying. A third lot of two samples was frozen for seven days, sprayed for seven days, and subsequently wetted and dried alternately.

The results of germination tests with these three lots of teliospores are shown in Table 44. The unfrozen spores, which were kept moist constantly, failed to germinate during a period of 42 days. The unfrozen spores, which were alternately wetted and dried, germinated in a rather erratic manner. Their germination was never very abundant. The teliospores subjected to the usual method of freezing, followed by spraying and alternate wetting and drying, germinated consistently and abundantly.

These results show that there is a definite stimulus due to alternate wetting and drying. A still greater stimulus results when this process is preceded by a period of freezing. All the evidence available suggests that the closer the approximation to the conditions to which teliospores are exposed in nature, the more abundant is the germination.

TABLE 45.—Dates of Formation and Germination of Teliospores in Series E.

—	Physiologic form	Host variety	Date first telia formed	Date of full telial formation	Date of first germination
Teliospores formed at 70–75°F.....	30	Garnet	16–1–30	22–1–30	17–3–30
	78	Marquis	.....	.....	.....
	9	Marquis	27–12–29	9–1–30	20–3–30
	80	Garnet	10– 1–30	24–1–30	.....
Teliospores formed at 55–60°F.....	30	Garnet	14– 1–30	11–2–30	13–3–30
	78	Marquis	4– 1–30	20–2–30	13–3–30
	9	Marquis	31–12–29	26–1–30	12–3–30
	80	Garnet	12– 1–30	20–2–30	20–3–30

TABLE 46.—Series E—The Germination of Teliospores of Four Physiologic Forms of *P. graminis tritici*, Frozen for seven Days at −5° C., Followed by Spraying and Alternate Wetting and Drying

Physiologic form	Temperature during telial formation	Period of freezing	Period of spraying	Germination following						
				1st drying (2 days)		2nd drying (3 days)	3rd drying (2 days)	4th drying (2 days)	5th drying (2 days)	6th drying (2 days)
				Mar. 12	Mar. 13	Mar. 17	Mar. 20	Mar. 23	Mar. 26	Mar. 29
30	74·5° F.	7 days	7 days	0	0	1	2	3	3	3
30	59·0° F.	"	"	0	3	5	4	5	5	5
78*	75·4° F.	"	"	0	0	0	0	0	0	0
78	59·2° F.	"	"	0	2	3	3	4	3	3
9*	74·3° F.	"	"	0	0	0	2	4	3	3
9	58·0° F.	"	"	1	3	5	4	4	5	4
80	75·4° F.	"	"	0	0	0	0	0	0	0
80	59·2° F.	"	"	0	0	0	3	2	2	3

*Very few telia on straws.

Explanation of symbols: 0 = no germination; 1 = trace germination; 2 = slight germination; 3 = fair germination; 4 = good germination; 5 = excellent germination

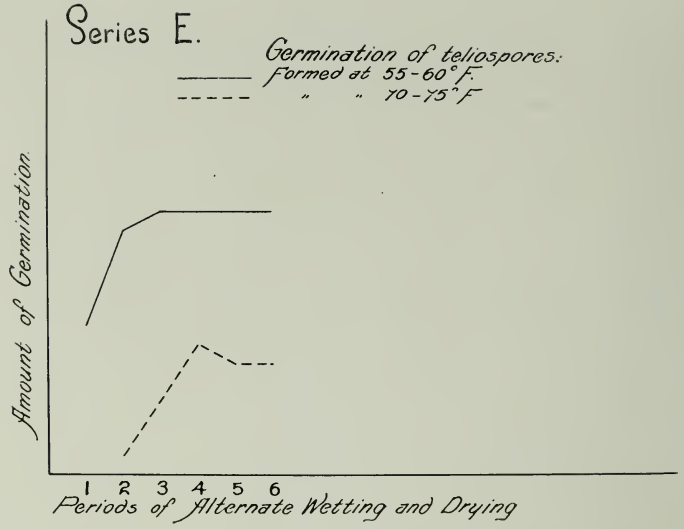

FIG. 17.—A comparison of the germination of teliospores formed at 55-60°F. with that of teliospores formed at 70-75°F. Series E.

Series E.—This series of experiments requires no discussion. The methods employed were the same as described for previous experiments. The results, as is shown in Table 46 and Figure 17, are identical.

ATTEMPTS TO INDUCE TELIOSPORES TO GERMINATE BY CHEMICAL MEANS

Although chemical treatments have been widely used as a means of shortening the dormancy periods of seeds of higher plants, there are but few references to similar treatments of dormant fungous spores. With respect to *Puccinia graminis,* the only successful work of this type known is that of Thiel and Weiss (31). They found that citric acid was effective in stimulating teliospores of this rust to early germination. In the absence of other investigations on dormant fungous spores, a theoretical basis for the possible effect of chemical treatments on the germination of teliospores must be sought in the extensive work which had been done on the seeds of higher plants. Eckerson (7), in a physiological and chemical study of after-ripening in seeds, concludes, that, in the majority of cases, the delayed germination is due to the exclusion of water and oxygen by the seed coats. In certain seeds, however, some change in the embryo is necessary before germination will take place. If the former condition should obtain in teliospores, one might assume that the spore-wall was instrumental in excluding water and oxygen, and thus prevented germination until some modification of the spore-wall had taken place. If such a modification could be brought about by chemical treatments, it is conceivable that the spores might germinate. Among the chemical agents most likely to effect such a change in the spore wall are lipoid solvents and weak or dilute acids.

With these assumptions in mind, some studies were made on the effect of certain chemicals on the germination of teliospores of *Puccinia graminis tritici.* Since the results were negative in all cases, these experiments will be treated as briefly as possible.

The Effect of Citric Acid on Teliospore Germination

The effect of citric acid on the germination of teliospores was tested in six separate experiments, both according to the instructions of Thiel and Weiss (31) and with certain modifications. The teliospores used in the first five of these tests were formed in nature on *Hordeum jubatum* in the fall of 1927 and stored outside in a bag during the follwing winter. These had failed to germinate up to July 11, 1928, when the first test was made. The fact that this telial material germinated later in the summer without any treatment shows that the spores were in a dormant condition and not non-viable as might otherwise have been supposed. Three concentrations of citric acid were used, one per cent, three per cent, and five per cent aqueous solutions. In each test, teliospores on *Hordeum jubatum,* collected in the spring of 1928 and known to be germinable, were subjected to the citric acid in the same manner as the dormant spores to determine if the treatment were in any way harmful to the spores. The results of all five experiments were similar. The dormant spores were not induced to germinate, and the germinability of the viable spores was apparently unaffected.

The sixth experiment was carried out in the winter of 1929-30. The teliospores used were of a known physiologic form and were formed in the greenhouse at a temperature of 55-60° F., a temperature which was known to be a favourable one to the production of viable teliospores. Fifty days after formation, the spores were subjected to citric acid according to the directions of Thiel and Weiss (31). Subsequently, the spores were kept moist in a petri dish lined with wet blotting paper, and examined daily for 28 days, but without any visible sign of germination. Untreated samples of the same material germinated during the above-mentioned period when subjected to alternate wetting and drying.

No explanation of this lack of stimulation is apparent. Since, however, as is elsewhere pointed out in this paper, the conditions of formation have a great influence on the subsequent behaviour of teliospores it is possible that some spores may respond to this treatment more readily than others.

The Effect of Acetic Acid on Teliospore Germination

A number of experiments were made in the summer of 1928 to determine the effect of dilute acetic acid on teliospore germination. The source of teliospores was the same as that for the tests with citric acid. In addition to these, there were tested teliospores formed on wheat in the field during the fall of 1927, which were evidently dormant. Teliospores of physiologic forms 9 and 15 produced in the greenhouse in the spring of 1928 were likewise subjected to these tests. The spores were subjected to N. 0·01 and N. 0·001 solutions of the acid for periods varying from one-half hour to five hours, and were subsequently examined for germination for four successive days. To determine whether any of these treatments were too drastic, a sample of germinable teliospores was included in each test. None of the treatments inhibited the germination of the germinable spores, or stimulated the dormant spores to germinate. The experiments were discontinued when it was found that the untreated teliospores on *Hordeum jubatum* began to show slight germination.

The Effect of Dilute Sulphuric Acid on Teliospore Germination

The experiments on the effect of sulphuric acid on dormant teliospores were carried out during the early fall of 1928. The teliospores receiving the treatments were mainly from two sources: (1) teliospores formed on wheat in the greenhouse in April, 1928; and (2) teliospores formed on *Hordeum jubatum* in nature about two weeks before the experiments were begun. These spores were immersed in N. 0·1, N. 0·01, and N. 0·001 sulphuric acid for periods vary-

ing from a few minutes to one hour for the two higher concentrations, and for periods varying from one to 24 hours for the lowest concentration. There was included in every test a sample of spores known to be germinable, in order to determine if the spores were injuriously affected by treatments. No severe injury was sustained by the germinable spores, as they germinated abundantly with the exception of those immersed in tenth-normal sulphuric acid for one hour. These germinated but slightly. None of the dormant spores germinated within seven days following the treatments.

The Effect of Lipoid Solvents on Teliospore Germination

The source of the teliospores used in this test was the same as that for the tests with citric acid. Three lipoid solvents were used, namely, acetone, ether, and carbon bisulphide, each being used in an undiluted state. The telia were immersed in each solvent for periods of ten and fifteen minutes, after which they were soaked in water for two hours and examined daily for germination for five successive days. Straws of *Hordeum jubatum* bearing teliospores known to be germinable were included in each test to determine whether or not the treatments were too severe. The germinable spores germinated abundantly following the treatments by acetone and ether, but failed to germinate after immersion in carbon bisulphide. On the other hand, no germination was observed in the dormant teliospores.

The resistance of germinable teliospores to acetone and ether is rather remarkable, especially in view of the fact that some of the colouring matter is dissolved out of the spores by these solvents. The loss of colouring matter was apparent when teliospores treated by ether for ten minutes were examined microscopically. These spores were markedly lighter in colour than the untreated ones, the spore-wall appearing grey in contrast with the brown colour of the walls of untreated spores.

The Treatment of Dormant Teliospores by Potassium Thiocyanate

Denny (2, 3, 4) states that several chemicals were effective in hastening the sprouting of dormant potato tubers. Among these were sodium and potassium thiocyanate. As only potassium thiocyanate was available at the time, a few experiments were made to determine its effect on dormant teliospores. Dormant and germinable teliospores were immersed for one-hour periods in 0·5 per cent, 1 per cent, and 2 per cent solutions. The dormant teliospores failed to germinate, while the germinable spores were not adversely affected by the treatment.

Conclusion

The negative results of all these experiments agree with those of Thiel and Weiss with the exception of their work with citric acid which gave positive results. The work was not sufficiently extensive to permit a statement that chemicals are ineffective in breaking or shortening the dormancy of teliospores of *P. graminis tritici*. The chemicals employed in the above experiments are, however, apparently not effective, or at least not in the concentrations used.

It can scarcely be doubted that some of these chemicals effected changes in the spore walls. Unfortunately the chemical nature of the spore wall is not thoroughly understood and consequently the selection of the proper chemical agents to increase its permeability must be based on empirical means. If, however, it is assumed that some of the chemical agents used increased the permeability of the spore wall to water and oxygen, the conclusion seems inevitable that increased permeability is not the factor involved in breaking the dormancy of these spores. There may be a natural process of after-ripening which is dependent on physiological changes in the protoplasm of the spore.

DISCUSSION

The teliospores of *Puccinia graminis* are frequently erratic in germination. This fact is attested, not only by the writer's experience, but also by that of many other investigators. Methods of treatment, which, in certain cases, undoubtedly stimulate germination, are not always successful when employed with teliospores obtained from a different source. Two possibilities can be advanced to account for this behaviour. First, the conditions under which the teliospores are formed may determine to a large extent, their subsequent germinability. Second, the conditions to which they are exposed after formation may be equally important in this respect. These two considerations raise the question of what conditions during formation and subsequent exposure are favourable to the germination of the spores.

Eriksson (9) has suggested that only the teliospores produced in the late autumn succeed in germinating in the following spring. This fact, if true, may be explained in two ways. Conditions in the late autumn may be more favourable to the production of viable spores than those in the summer; or, conditions under which spores produced in the summer remain after formation may destroy their viability. Observations made in nature are somewhat contradictory with respect to this question. Waterhouse (33) states that teliospores which, in Australia, are formed in early summer, remain viable throughout the remainder of the hot, dry summer. Stakman, Kirby, and Thiel (28), report that teliospores formed in early summer in the Southern States fail to infect barberries in the following spring. When such spores, however, are shipped to the Northern States and kept there through the summer and following winter, they germinate normally and cause infection on barberries. This suggests, as pointed out by Lambert (20), that the viability of the spores in the south is destroyed by the high summer temperatures. The inference is that the temperature at which the spores remain after formation, rather than the temperature at which they are produced, determines their viability.

The experiments reported in this paper show, however, that the conditions of teliospores formation determine, in a measure, their subsequent germinability. The striking and consistent differences in the germination of teliospores formed at high and low temperatures cannot be explained unless it is assumed that temperature is the factor responsible. In practically all the experiments, the teliospores were removed from the greenhouse so soon after their formation that it is scarcely possible that conditions subsequent to their formation could have affected their germinability. Apparently, then, it must be concluded that a temperature of 70° to 80° F., is somewhat detrimental to the development of germinable teliospores, and that a temperature of 50° to 60° F., is undoubtedly favourable.

The results from alternate wetting and drying of teliospores are in agreement with those obtained by Klebahn (19) in the winter of 1912-13. This author collected teliospores of *Puccinia graminis* on November 29, 1912. These were subjected to alternate wetting and drying until December 31, after which they were dried in the laboratory until January 10. A germination test made on the latter date resulted in the abundant production of sporidia. It is doubtful if the process of alternate wetting and drying of these spores can be held entirely responsible for their germination, as the spores were exposed to the weather from the time of formation until November 29. Klebahn concluded that alternate soaking with fresh water and drying is the essential requirement for the germination of teliospores. He considers that cold winter weather exerts no favourable influence on their germinability, but that it may, on the contrary, have a retarding effect. The last conclusion is not substantiated by the experiments already reported in this paper. An experiment reported in

Series C. (Table 43), shows that teliospores can germinate after being frozen, even although they are not alternately wetted and dried. There is, on the other hand, no evidence that they will germinate when neither frozen nor kept alternately wet and dry. The most successful germination was invariably obtained when both treatments were combined. The procedure found most satisfactory consisted in freezing the spores for a short period, followed by spraying with cold tap water for about one week, and then alternate wetting and drying. The freezing and the alternate wetting and drying have already been discussed. Spraying is not considered an essential part of the process. It was included with the object of soaking the spores thoroughly with fresh, aerated water before the alternate wetting and drying was commenced. A number of experiments in which fresh running water was substituted for spraying resulted in equally good germination.

The sporidia produced by the teliospores in response to this artificial stimulation appear to be normal in all respects. This is proved both by the consistent germination of the sporidia and by numerous infections of barberries.

The physiological changes which take place in the teliospores, as they pass from dormancy to a germinable condition, are beyond the scope of the present investigation. Whatever they are, it is evident that they can be stimulated, at least in spores formed under favourable conditions, by a proper manipulation of the evironment.

SUMMARY

1. It has been shown that temperature produces striking results on the reactions of some wheat varieties to certain physiologic forms of wheat stem rust. At a relatively low temperature, about 60° F., and a low to moderate light intensity, these varieties are immune. At a higher temperature, about 75° F., and the same light intensity, these varieties are completely susceptible. As the light intensity increases, temperature and other conditions remaining constant, the host reaction is gradually shifted towards susceptibility. Thus temperature and light, when considered as variables of the environment, can, each, result in important changes in the rust reactions of certain wheat varieties. This effect is confined to the reaction of certain wheat varieties to certain physiologic forms. The same varieties when infected by other physiologic forms show no such response to these environmental conditions.

2. Cytological and microchemical studies failed to reveal any morphological or chemical differences in wheat seedlings grown at high and low temperatures.

3. The effect of different concentrations of carbon dioxide on rust development was studied with reference to the so-called "X-reaction." The concentrations used were 0·03, 0·15, 0·75, 1·5, 3·0, and 4·5 per cent. No stimulation of rust development was observed at any of these concentrations. The three highest concentrations, on the other hand, were detrimental to rust development, although not entirely inhibitive.

4. The effect of mineral starvation of seedlings was also studied, with special reference to the development of the X-reaction. The type of infection did not seem to be influenced by mineral deficiency. The infections, however, were less numerous on seedlings grown on solutions deficient in some of the mineral salts.

5. A study was made of the effect of temperature on teliospore formation. Two temperature ranges were chosen for this purpose, namely, 55°-60° F., and 70°-75° F. Teliospore formation was more rapid in the latter temperature range. Considerable differences were observed in the rates of teliospore formation among different physiologic forms. Some began to form teliospores within a few days after the development of uredinia; others, only after a long period in the uredinial stage.

6. The germination of the teliospores formed at the above-mentioned temperatures was compared. Those formed at the lower temperature germinated earlier and more abundantly. It is evident that the conditions of temperature during the formation of the spores have a considerable effect on their germinability.

7. Attempts were made to shorten the dormancy period of teliospores formed in the greenhouse by means of freezing and of alternate wetting and drying. Either of these treatments reduced the period of dormancy. A combination of the two, that is, a short period of freezing followed by alternate wetting and drying, invariably resulted in abundant germination of teliospores formed at low temperatures. Teliospores formed at higher temperatures also responded to this treatment, but their germination was usually more limited. Teliospores formed in nature on *Hordeum jubatum* and *Agropyron repens* in September, 1929, likewise responded to this treatment and germinated abundantly at the beginning of December.

Efforts were made to determine the minimal dormancy period of teliospores produced in the greenhouse. The shortest period between the completion of teliospore formation and the germination of the spores was twenty days. Spores frequently germinated between thirty and forty days after formation.

8. The duration of teliospore germination in certain sori was recorded. The spores were kept alternately wet and dry from the time they began to germinate until germination ceased. Germination began on January 24, 1930, and continued, in some sori, until April 1. This represents a period of sixty-eight days, of which actual germination took place on thirty-one days. There are evidently physiological differences among teliospores in the same sori, a condition which permits a comparatively long period for teliospore germination on each sorus.

9. Attempts to overcome the dormancy of teliospores by chemical means were unsuccessful.

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