REPORT

of

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The Effect of Ecological Factors on the Morphology and Physiology of Puccinia graminis and Puccinia phleipratensis Urediniospores

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A Thesis

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By

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Table of Contents

Page

General introduction 4

Methods and materials 6

Rusts 6

Host plants 6

Inoculation 7

Incubation 9

Observations 10

Experiments 14

Constancy of biologic forms 14

Puccinia phleipratensis (Timothy rust) 14

General statement 14

Inoculations on timothy (*Phleum pratense*) 15

Inoculations on orchard grass (*Dactylis glomerata*). 16

Inoculations on oats (*Avena sativa*) 18

Summary of inoculations 19

Puccinia graminis avenae (oats rust) 22

General statement 22

Inoculation on oats (*Avena sativa*). 22

Inoculation on orchard grass (*Dactylis glomerata*) . 23

Inoculation on brome grass (*Bromus tectorum*) . . . 23

Inoculation on barley, timothy and other grasses . 24

Summary of inoculations 27

Page

Puccinia graminis secalis (rye rust). 27
General statement 27
Inoculations on rye (*Secale cereale*). 27
Inoculations on barley (*Hordeum vulgare*) 28
Inoculations on quack grass (*Agropyron repens*) . . . 29
Inoculations on wild barley (*Hordeum jubatum*) 29
Inoculations on *Elymus virginicus* and *Hystrix patula* 30
Summary of inoculations 30
Puccinia graminis tritici (wheat rust) 33
General statement 33
Inoculations on wheat (*Triticum vulgare*). 33
Inoculations on barley (*Hordeum vulgare*). 34
Inoculations on wild barley (*Hordeum jubatum*) 34
Inoculations on rye (*Secale cereale*) 34
Summary of inoculations 37
General summary of conclusions derived from experiments on the constancy of biologic forms 37
Properties of hosts to unify spore sizes 39
Avena sativa (oats) 40
Hordeum vulgare (barley) 41
Influence of environmental conditions 43
General statement 43
Effect of temperature 43
Effect of humidity 45
Effect of water content 47
Effect of illumination 47

Page

Relations of cultural factors 50

Effect of age on host 50

Effect of age on spore material 50

Summary 53

Bibliography 55

Introduction

The rusts attacking our cereal grasses, and also commonly found on various wild grasses, have been considered to constitute a single collective botanical species, Puccinia graminis Pers., quite uniform structurally, but acting differently parasitically. Ward (6) summarizes the phenomenon of biological specialization in the following words; "While Puccinia graminis infects all of a certain number of hosts, and preserves its morphological characters on all of them, it is so closely adapted to the particular plant it happens to be on at the time, that the uredospores from this host can only attack successfully and directly this particular host or a limited number of its immediate allies." And further, "Each of these specialized forms has received from its host an impress not perceptible in any visible differences of shape, size, color, or other morphological characters, but differing in some capacity of reaction towards another host, " yet, Ward (6, p. 236) suggests, "That the particular specialized race . . . is in course of becoming a species and may during the lapse of time actually become a species of Puccinia, which will eventually show morphological differences in addition to the physiological ones it already shows."

Freeman and Johnson (3, p. 14) also express the opinion that, "What may appear to the eye, and often under the highest power of the microscope, as one and the same species of rust on a number of species, or even varieties, may really not be identical, since they are not interchangeable from one host to the other."

The solution of the question as to whether biologic forms are merely adaptations and differ one from another only in their

behavior, or they are also to a certain degree well established structurally, is of considerable interest not only from a theoretical view point but from a practical one as well. For, as Stakman (4, p. 10) remarks, "If these forms are merely adaptations it ought to be possible to change their parasitic tendencies by restricting or changing their environment."

Most of the extensive studies of the biologic forms of Puccinia graminis both abroad, by Eriksson and Henning, Klebahn, Ward, and others, and in this country, by Hitchcock and Carleton, Carleton, Freeman and Johnson, Stakman, and others, have been devoted to the physiological phase of the problem, while the morphological phase of the question has been but little investigated.

In regard to the effect of physical factors on the virulence of rust attack there seems to exist a harmonious consensus of opinion, especially as far as weather and soil conditions are concerned. For an historical summary of the experimental work along this line the reader is referred to Freeman and Johnson (2) and Stakman(4). But here again little attention has been paid to the effect of the environmental conditions on the morphology of the rust urediniospores. For this reason the object of the present investigation was to determine both the physiological and morphological constancy of biologic races when subjected to various ecological influences, physical as well as biological.

The timothy rust Puccinia phleipratensis Eriks. u. Henn., was included in the investigation for the purpose of comparison with Puccinia graminis avenae to which it is very similar parasitically and from which it may possibly have developed.

Methods and Materials

The methods employed for the principal series of comparative infections were as follows:

Urediniospores of the rusts used in making inoculations were obtained originally from the field and were transferred to different host plants, as further indicated, by artificial inoculations in the greenhouse. The rusts collected for the principal series and the hosts they were found upon is given in the following table.

Table I.— Origin of urediniospores used in inoculations.

Date of collection	Host-plant	Botanical species or biologic races found
Oct. 1 & 23, 1915	Phleum pratense	Puccinia phleipratensis
Oct. 5 & 28, 1915	Agropyron repens	P. graminis secalis
Oct. 7 , 1915	Dactylis glomerata	P. phleipratensis and P. graminis avenae
Oct. 8 , 1915	Hordeum jubatum	P. graminis secalis and P. graminis tritici

The cereal host-plants used were as follows:

Blue stem wheat, Minnesota No. 169

Improved Ligowa oats, Minnesota No. 381

Swedish rye, ... Minnesota No. 3

Manchuria barley, . . Minnesota No. 105

The grass seeds were obtained from the Minnesota Seed Laboratory.

The carefully selected seeds of the grains and grasses were sown in rich loam soil in four-inch clay pots, generally a score of grain seed and about double the number of grass seed in a pot, and allowed to germinate in the "seedling compartment" of the greenhouse. From eight to a dozen plants of the cereals were generally left in each pot and the first leaf of each was inoculated, usually when six or seven days old. The plants were trimmed whenever necessary, leaving only the inoculated blades of each plant. The grass plants did not receive similar treatment, the inoculations being made on all the plants in the pot.

The urediniospores were placed on the leaves with a flat inoculating needle, sterilized in an alcohol flame, the plants having first been moistened in order that the spores might better adhere to the leaf surface. A liberal amount of inoculum was used, altho experiments conducted for the purpose of determining the effect of the quantity of spore material used for inoculation showed no definite results, except for the extent of infection, which was quite consistent. Thus, the total infection of eight consecutive urediniospore generations on wheat of very heavy, moderate and exceedingly light applications gave 93 percent infection or 132 successful infections out of 142 inoculations for the first (heavy) condition, 91 percent or 130 out of 142 for the moderate application and only 88 percent or 104 out of 118 for the light inoculation. The effect on the size of pustules and size of spores as seen from Table II. is variable. In general, whenever infection resulted from inoculations there was no perceptible or consistent difference in the size of pustules, spores or virulence in general.

Table II.--Results of experiments in inoculating wheat plants with varying quantities of inoculating material of urediniospores of Puccinia graminis tritici.

Source of urediniospores and plant inoculated.	Amount of material applied	Infection			Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.	Appr. % inf.		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	Moderate	20	20	100	Strong	35.28-40.32	16.96-23.04	32.64 x 19.84
" "	Heavy	12	12	100	"	34.32-41.92	17.28-23.36	33.60 x 20.16
" "	Slight	12	10	83	"	34.96-41.28	17.60-22.72	33.28 x 20.16

Immediately after inoculation, the pots were placed in shallow pans filled with water, and covered with bell jars. In bright weather sheets of paper were spread over the jars to prevent tipburn of the plants. The plants were usually kept in this condition for 48 hours before being removed to the greenhouse benches in such locations as to reduce as much as possible the danger of accidental infection. For this purpose the plants were kept either beneath double lined muslin cages (in the early fall and spring) or separated by these cages (late fall and during the winter season). An experiment was conducted to find out the effect of the length of incubation period in the water chambers. Ten pots each containing ten six day's old wheat seedlings were inoculated with an equal and liberal amount of viable stem rust urediniospores, and placed in two water pans under glass bell jars. Equal amounts of water were put in both pans and all other conditions were kept uniform. At the end of 12 hours two pots were removed from under the bell jars and set on the bench, after 24 hours the second pair of pots was placed on the bench, and the rest were taken out from the pans in pairs every 24 hours following the second pair, i.e., 48 hours, 72 hours, and 96 hours after inoculation. The first observation was made 80 hours after inoculation and no signs of infection could be noticed on the plants incubated for 12, 24 and 48 hours, but 6 plants of those which had been under 72 hours and 12 of those that were still under the bell jar showed very indistinct but typical rust flecks. The results are given in Table III, being recorded in the following manner: fl. and pus. represent flecks and pustules respectively, (?) doubtful, (-) very indistinct, (±) fairly noticeable, (x) clearly visible,

(x̲x̲) well advanced, and (xx) fully developed.

Table III.--Results of the effect of length of incubation period on rust infection

Period of incubation	Length of time after inoculation, in hours.						
	80	128	144	168	192	240	288
12 hrs	No fl.	1 fl.(?)	1 fl.(?)	1 fl.(-)	1 fl.(x)	1 pu(x̲x̲)	1 pu(xx)
24 hrs	" "	18 " (x)	18 "(xx)	20 "(xx)	17 pu(x)	19 "(xx)	20 "(xx)
			1 pu (-)	13pu (x̲)			
48 hrs	" "	20 " (x)	2 pu (-)	8 pu (-)	15 pu(x̲)	20 "(xx)	20 "(xx)
72 hrs	6 fl(-)	20 "(x̲x̲)	1 pu (?)	5 pu (-)	11 pu(-)	19 "(x̲x̲)	20 "(xx)
96 hrs	12"(-)	20 "(xx)	3 pu (?)	3 pu (-)	9 pu (-)	17 " (x)	20 "(x̲x̲)

As seen from the above table 100 percent infection resulted in all cases except one, that in which the incubation period was only 12 hours. In this case only one leaf of the 20 inoculated leaves became infected, constituting an infection of only 5 percent. Altho in all the four other cases pustules of almost equal size, color and form were finally developed, yet the virulence was varying. The plants kept under the bell jar for 48 hours produced the greatest number of pustules per leaf, those which had been kept under 24 hours and 72 hours respectively, fewer and those which had been under 96 hours, still fewer, while only one pustule appeared on the single infected leaf of the plants kept under 12 hours.

The apparatus used for meteorological observations is fully described in the discussions of the particular experiments

performed. General notes of the behavior of the various cultures were taken at the close of each urediniospore generation before transfers were made to new plants, on the average every two or three weeks. Spore measurements were made of the original rusts found on the grasses in the field and used for the principal series; the next measurements were made on the first following generation and once or twice more during the period the rust was kept in culture.

As a comparative basis of dimensions in this work the biometric "mode" is used in preference to the arithmetical mean. The mode represents the group containing the largest number of individuals of a certain size, thus indicating that this size is the prevailing one among a given spore population. But comparative calculations made show that as a general rule arithmetical means usually fluctuate around the biometric modes, as seen from Table IV. and consequently there is, on the whole, but little difference between the two bases of recording.

Table IV.--Comparative computations of modes and means.

Rust and Host		Biometric mode	Arithmetical mean
Puccinia graminis tritici on wheat	"Check," 1st gener.	33.28x19.84 μ	33.36x19.69 μ
do	" 7th "	32.00x19.84 μ	31.58x19.84 μ
do	" 10th "	32.64x20.16 μ	32.64x20.02 μ

In most cases the figures are almost identical (in two cases they are the same) and only in one case is there a difference of 0.42 μ which may be considered negligible, since two consecutive measurements of the same group of spores may give a similar variation.

As to the number of spores to be measured from a given group it was found that 100 gave equally as good results as 200 or 400, while when less than 100 were used the results were not always representative. Table V. gives the results of measuring different numbers of spores from the same plant in the same day taken from pustules of the same leaves. Wheat and oats rusts were both tried with similar results. As noted from the tables the modes of a population of 100, 200 or 400 spores are the same, but the limits of variation are less in a population of 100 than in those of 200 or 400. It will be seen from the table that at least 100 spores should be used.

Table V.--Result of measuring different numbers of spores.

Source of urediniospores and plant inoculated	Number of spores measured	Spore dimensions		
		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	25	26.88 - 38.72	17.92 - 22.08	32.00? x 19.52?
" "	50	26.88 - 40.32	17.92 - 23.04	33.60? x 19.84
" "	100	26.56 - 40.32	16.96 - 23.36	32.96 x 19.84
" "	200	25.60 - 40.32	16.32 - 23.36	32.96 x 19.84
" "	400	23.68 - 40.32	16.32 - 23.36	32.96 x 19.84

Table V.--Continued.

Avena sativa	25	23.36 - 35.20	17.28 - 22.08	?	?
" "	50	23.36 - 35.20	17.28 - 22.08	27.52?	x 19.52
" "	100	23.04 - 35.20	16.96 - 22.08	29.12	x 19.52
" "	200	21.12 - 36.48	16.32 - 23.04	29.12	x 19.52

In the present investigation two hundred spore measurements were made for each experimental group until January 1, 1916; beginning with this date one hundred spore measurements for length and one hundred for the width, instead of 200 for each.

Stakman (4, p. 31) found that spores from different pustules varied somewhat in average size and he therefore measured spores from a number of pustules in determining the averages. This was also practiced in the present work. The size of spores may vary not only in different pustules but also on different leaves. Table VI. shows that spores from a single well developed pustule were of exactly the same size, as far as modes were concerned, as those taken from ten pustules on 10 different leaves grown in the same pot, while spores taken from 10 pustules from a single blade were somewhat smaller than the above. As a matter of fact the pustules on that particular leaf, altho in the same pot, were not as large as on some other leaves; the difference, however, was very small.

Table VI.--Results of measurements made to determine the amount of variation in size of spores from different pustules.

Source of urediniospores and plant inoculated.	Number of pustules and leaves from which spores were taken for measuring.	Spore dimensions		
		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	1 pu. from 1 leaf	26.24-40.64	17.28-23.04	33.60 x 20.16
do	10 " " 1 "	26.56-39.04	16.96-22.40	32.96 x 19.84
do	10 " " 10 "	24.32-41.92	17.28-23.36	33.60 x 20.16

It was found that the superficial layer of pustules contains larger spores, and if it is removed the remaining spores are considerably smaller in size. But if the pustules are allowed to produce new spores, those on the surface again have the same dimensions as the original fresh spores. For this reason precaution was taken to measure spores from pustules in the same stage of development.

For color determinations Ridgway's Chromotaxia was used.

Experiments

Constancy of biologic races.

Puccinia phleipratensis

General statement

Johnson (3, p. 8) gives the urediniospore sizes of timothy rust as 18 to 27μ in length and 15 to 19μ in width.

Stakman and Jensen (5, p. 214) found that the spores of timothy rust on timothy ranged from 17 to 31μ in length and from 14.5 to 23μ in width, the modes falling at about 26 and 18μ. After one generation on Dactylis glomerata, the timothy rust spores ranged from 17 to 32μ in length, and from 13.5 to 23.2μ in width, while the modes fell at about 25.5 and 19.5μ. At least 100 spores from different pustules were measured. Measurements were also made of spores produced after the rust had been one generation on other hosts, including oats, rye, barley, Lolium perenne, and Avena fatua; but no distinct and consistent differences were apparent, with the exception of the spores produced on barley. These were smaller than those produced on any other host, ranging from 18.5 to 28.3μ in length and from 13 to 20μ in width. The modes were at about 23 and 17μ. Stakman and Jensen also found that the inoculation of timothy rust on timothy nearly always resulted in 100 percent infection, on oats the rust developed in a fairly normal manner, while the infection on Dactylis glomerata was very severe, nearly as severe as that on timothy.

Inoculations on Phleum pratense

The original uredospores were obtained from timothy (Phleum pratense) and orchard grass (Dactylis glomerata) plants on October 1 and October 7, 1915, respectively and, before transferring to new plants, 200 spores from each host were measured. The orchard grass contained both P. phleipratensis and P. graminis avenae, which were eventually separated. The spore dimensions on the field timothy were as follows: 18.88 to 33.60μ in length,

12.80 to 19.20μ in width, with modes falling at 26.24 and 16.00μ. The measurements on the orchard grass (mixed strains) were 21.76 to 40.00μ in length, 12.80 to 21.76μ in width, and 29.76 x 16.64μ for modes, but the frequency polygon, or curve, showed a tendency for the formation of another mode at 26.56μ., suggesting the presence of urediniospores of P. phleipratensis in the group measured.

The details of measurements are given in Table IX. and it will suffice to state here that no distinct and concrete difference was apparent, the extremes being 16.00 to 29.76μ in length and 13.12 to 20.16μ in width for the timothy strain, and 16.32 to 29.12μ in length and 12.80 to 19.84μ in width for the orchard grass strain. The mode averages were 22.83 x 17.17μ for the timothy strain and 23.40 x 17.07μ for the orchard grass strain. Out of a total of 220 inoculations of urediniospores from timothy on timothy 200 or 91 percent were successful, while out of a total of 157 inoculations of urediniospores from orchard grass on timothy, 137 or 90.7 percent were successful, that is, the results are almost identical. The virulence of infection was equally vigorous in both cases, varying with the season. The size of pustules ranged from 0.1 - 0.5 x 0.5 - 5.0 mm., the color varying all the way from antique brown to amber brown and Brussels brown.

Inoculations on Dactylis glomerata

Measurements were made of urediniospores of P. phleipratensis after one generation on orchard grass. The subsequent inoculations were made from the sixth urediniospore generation on

timothy, originating both from timothy and orchard grass. The results are given in Table VII.

Table VII.--Spore dimensions of Puccinia phleipratensis on Dactylis glomerata.

Source of spores	Extreme limits of ranges	Average of Modes
Field timothy	16.64 - 39.12 x 13.76 - 20.80μ	32.08 x 17.28μ
Timothy strain after 6 generations on timothy	17.76 - 38.48 x 14.24 - 20.00μ	23.20 x 17.12μ
Orchard grass after 6 generations on timothy	17.76 - 39.12 x 13.60 - 20.16μ	23.52 x 16.96μ

The detailed results are given in Table IX.

Out of a total of 66 inoculations on orchard grass with urediniospores from timothy 54, or 82 percent, were successful; whereas, out of a total of 47 inoculations on orchard grass, with urediniospores after six generations on timothy, but originally from orchard grass, 38, or 80 percent, were successful, that is, almost uniform infection.

The virulence was moderate and on the whole somewhat weaker than on timothy; the size of pustules ranged from 0.1 to 0.4 by 0.5 to 4.0 mm. The color of the uredinia resembled that of the uredinia on timothy, varying from antique brown to amber brown or Sudan brown.

Inoculations on *Avena sativa*

The first attempt to infect oats with the urediniospores of *P. phleipratensis*, collected on timothy October 1, 1915, failed completely. In the second trial 14 out of 53 inoculated plants produced a small number of pustules, which varied in size from 0.2 - 0.5 x 0.5 - 1.5 mm. Measurements were made of 200 spores. The attempt to confine this rust for a number of generations on oats was not successful, for the virulence of infection constantly decreased after the second generation and infection did not result after the fifth generation, altho strenuous attempts were made to secure successful infection. Transfers were made to oats with rust which had been on timothy one, five, six, seven and eight generations and only the first and last produced more or less successful infection, 50 percent and 38 percent respectively, of which spore measurements were made. Somewhat better results were obtained with *Puccinia phleipratensis* originally from orchard grass and confined to timothy for six successive generations. Three trials resulted in 16 percent, 15 percent and 13 percent infection, respectively. Measurements were made of the first and last of these infections. The results of all measurements are given in Table IX. In Table VIII. are summarized only the comparative results of spore dimensions obtained from the two original sources.

Table VIII.--Spore dimensions of Puccinia phleipratensis on Avena sativa.

Origin of spores	Extreme limits of ranges	Average of modes
Field timothy	17.60 - 30.08 x 14.72 - 20.48μ	23.72 x 17.60μ
Timothy strain, after 1st and 8th generations on timothy.	16.96 - 32.00 x 13.76 - 20.80μ	23.36 x 17.60μ
Orchard grass after 6th generation on timothy	17.60 - 31.36 x 11.84 - 21.12μ	23.52 x 17.60μ

The size of pustules ranged from 0.2 - 0.5 x 0.2 - 3.0 mm. The color of the uredinia varied from amber brown to Sudan brown and burnt sienna.

Summary of inoculations made with Puccinia phleipratensis

The results of inoculations with Puccinia phleipratensis obtained from two different sources, timothy and orchard grass, and grown on three different hosts, timothy, orchard grass, and oats, seem to show a consistent uniformity, retaining their characteristic size, except for small negligible variations, whether parasitizing very congenial or only fairly congenial hosts. The spore shape was dominantly pyriform and the color dull, dirty yellow to grayish. The prevalence of the typical color of the uredinia was also quite apparent in all cases. All the modes fluctuated about those of a long time (over a year old) Puccinia phleipratensis strain on timothy.

Table IX.--Results of inoculations made with Puccinia phleipratensis, Eriks. u. Henn.

Source of urediniospores	Plant inoculated	Infection		Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Phleum pratense, field	Phleum pratense	25	25	Strong	17.92-29.44	13.12-21.12	23.68 x 17.28
" " Ph.5	" "	24	18	Moderate	16.00-28.80	13.44-20.16	21.76 x 16.96
" " " 8	" "	23	23	Strong	16.32-29.76	14.40-19.84	23.04 x 17.28
Dactylis glomerata, field	" "	35	4	Weak	16.64-28.80	12.80-18.88	22.08 x 16.96
" " Ph.5	" "	13	13	Strong	16.32-29.12	13.76-19.84	22.08 x 17.28
" " " 8	" "	21	20	"	18.24-28.48	14.40-19.20	23.04 x 16.96
Phleum pratense, field	Dactylis glomerata	22	22	Moderate	16.64-29.12	13.76-20.80	22.08 x 17.28
" " Ph.6	" "	8	4	"	16.32-28.48	14.40-20.16	22.40 x 17.28
" " " 6D2	" "	20	18	"	19.20-28.48	14.08-19.84	24.00 x 16.96
Dactylis glomerata, Ph.6	" "	12	9	"	16.96-29.44	13.76-20.48	23.36 x 17.28
" " " 6D3	" "	19	17	"	18.56-28.80	13.44-19.84	23.68 x 16.64
Phleum pratense, field	Avena sativa	53	14	Weak	17.60-30.08	14.72-20.48	22.72 x17.60
" " Ph.1	" "	12	6	"	16.96-32.00	13.76-20.48	23.68 x17.28
" " " 8	" "	21	8	"	17.92-28.16	15.04-20.80	23.04 x17.92
Dactylis glomerata, Ph.6	" "	13	2	"	17.60-31.36	11.84-21.12	23.68 x17.28
" " " 6D3	" "	8	1	"	18.56-28.48	14.72-20.48	23.36 x17.92

Table IX.--Continued.

Phleum pratense, Ph_x	Phleum pratense	18	18	Strong	17.60-29.44	13.12-20.48	24.00 x 16.96

Ph = Phleum pratensis

D = Dactylis glomerata

O = Avena sativa

Figures represent generations of confinement of the fungus to the particular host.

(x) indicates a long time confinement of the parasite to its respective host.

Puccinia graminis avenae

General statement

Johnson (3, p. 10), and Stakman and Jensen (5, p. 215) state that *Puccinia graminis avenae* and *Puccinia phleipratensis* are quite similar. Both rusts, according to Stakman and Jensen, can infect directly *Avena elatior*, *Avena fatua*, *Avena sativa*, *Bromus tectorum*, *Dactylis glomerata*, *Elymus spp.*, *Hordeum vulgare*, *Lolium italicum*, *Lolium perenne*, *Phleum pratense* and *Secale cereale*.

In regard to their morphology, Stakman and Jensen say that, "The two rusts are somewhat different, the spores of *Puccinia graminis avenae* being larger."

The object of the work done with this biologic form was to determine the amount of variation which could be induced by growing the rust on different hosts.

Inoculations on *Avena sativa*

Out of the mixed rusts on the orchard grass collected October 7, 1915, *P. graminis avenae* was separated and transferred to oats nine successive times. Measurements were made the first, sixth and ninth generations, and compared with spores of *P. graminis avenae* originally from *Dactylis glomerata* and then kept on oats 23 generations and with spores of the same rust from *Dactylis glomerata* after only one generation on oats. The results are given in Table X. and show that the spores varied in length from 19.20 to 37.12μ. and in width from 13.76 to 25.60μ., while the mode average fell at 28.16 x 19.46μ. The infection was usually very virulent, the uredinia varying in size from 0.3 - 2.0 x 0.8 - 10 mm.

The color of the uredinia varied from amber brown to burnt sienna and argus brown. The spore shape was very variable, from ellipsoid to pyriform to globose. The color of the spores was bright cadmium yellow.

Inoculations on Dactylis glomerata

The results of inoculations with spores from various sources (see Table X) show a tendency for the spores to shorten when confined to Dactylis glomerata, the width remaining practically the same. Altho the spores become more spherical, their color remains the bright cadmium yellow as on oats. The rust grows vigorously on D. glomerata but the uredinia are somewhat smaller than those on oats. The color is amber brown to burnt sienna. The limits of range of the spore sizes were as follows: 18.56 to 35.30μ for length, 12.36 to 24.00μ for width, and the mode averages falling at 25.92 x 19.60μ .

Inoculations on Bromus tectorum

P. graminis avenae was kept three successive generations on Bromus tectorum. The decrease in size was greater than in the case of the orchard grass, the spores becoming both shorter and narrower. More round spores occurred than on either the oats or orchard grass, but the equatorial germ pores could be readily seen and there can be no doubt that they were Puccinia graminis avenae spores. The limits of variation were as follows: 18.56 to 39.12μ for length, 16.32 to 21.12μ for width, and the mode averages were 33.68 x 18.88μ . The color of the spores was the same as that of those on oats and orchard grass. The virulence was slight and the

pustules were small, varying from pin point to pin head in size. The color varied from burnt sienna to chestnut.

Inoculations on barley, timothy and other grasses.

Barley inoculated with spores of Puccinia graminis avenae, originally from Dactylis glomerata collected August 22, 1914, and then confined to oats for 33 successive generations, produced very small pustules, the spores of which were also small, 20.80 to 32.96μ in length with a mode for length of 25.60μ. Only 70 spores were measured in this case and in the following one, as there were no more available. Out of a total of 5 leaves inoculated only one became infected.

Timothy was inoculated with the rust from the same source after having been confined to oats for 31 successive generations. The spores were of practically the same size as those produced on barley.

Both barley and timothy are very uncongenial hosts for Puccinia graminis avenae and the uredinia and spores are very small. This resembles the behavior of P. graminis tritici on resistant varieties of wheat on which Stakman (4, p. 31) found the spores to be smaller than on the susceptible wheat varieties.

When P. graminis avenae from timothy was transferred back to oats the spores acquired their original size.

Puccinia graminis avenae from Dactylis glomerata, after confinement on oats for 36 generations, when inoculated on Hordeum pusillum, Alopecurus pratensis and Lolium temulentum, produced in every case spores of smaller size than those grown on oats. The

Table X.- Results of inoculations made with *Puccinia graminis avenae* Pers.

Source of urediniospores	Plant inoculated	Infection: Lvs. inoc.	Infection: Lvs. infec.	Virulence of attack	SPORE DIMENSIONS: Range of length (in μ)	SPORE DIMENSIONS: Range of width (in μ)	SPORE DIMENSIONS: Modes (in μ)
Dactylis glomerata, field	*Avena sativa*	21	21	Strong	20.80-37.12	16.00-25.60	27.84x19.84
" " "	" "	35	4	Moderate	19.20-35.20	13.76-23.04	26.88x18.56
" " 05	" "	19	19	Strong	22.08-34.88	16.00-22.40	28.48x19.20
" " 08	" "	25	25	Strong	21.12-36.48	16.32-23.04	29.12x19.52
" " 022	" "	23	23	Strong	19.52-46.38	16.00-23.36	28.48x20.16
" " field	*Dactylis glomerata*	18	18	Strong	19.20-32.96	16.00-24.00	25.92x19.84
" " "	" "	15	11	Strong	18.56-35.20	15.36-23.36	26.24x19.52
" " 06	" "	12	11	Strong	19.20-32.00	16.32-22.40	25.60x19.52
" " 06_{D2}	" "	20	19	Strong	20.16-31.68	16.64-22.08	25.92x19.52
" " 06	*Bromus tectorum*	10	10	Weak	18.56-29.12	16.64-21.12	23.68x18.88
" " 06_{Br2}	" "	12	5	Poor	19.20-28.80	16.32-21.12	23.68x18.88
" " 031	*Phleum pratense*	50	5	Poor	20.16-32.64	---------	25.60x-----
" " 033_{Phl}	*Avena sativa*	5	1	1 pustule	22.72-36.16	16.00-22.72	29.44x19.52
" " 033	*Hordeum vulgare*	15	3	Poor	20.80-32.96	---------	25.60x-----

Table X-continued.

Dactylis glomerata	036	Alopecurus pratensis	60	60	Strong	19.84-33.60	16.96- 22.72	26.56x19.84
" "	"	Lolium temulentum	20.	4	Moderate	19.52-33.60	16.64- 22.40	26.56x19.52
" "	"	Hordeum pusillum	28	4	Poor	19.20-30.40	17.28- 21.76	24.32x19.52
Avena sativa	O_x	Avena sativa	23	22	Strong	23.04-35.20	16.96- 22.08	29.12x19.52

O = Avena sativa; D = Dactylis glomerata; Ph. = Phleum pratense; Br. = Bromus tectorum .

difference appeared in length, the width remaining in all cases practically the same. The spore color remained constant, while the spore shape on the grasses tended to become round.

Summary of inoculations made with *Puccinia graminis avenae*

The spores of *Puccinia graminis avenae* seem to be quite easily affected, as far as their size and shape are concerned, by the host on which they grow; the color of the spores, however, remains the same under all conditions, i.e., bright cadmium yellow. Size of uredinia is directly proportional to the size of the spores and vice versa. *P. graminis avenae* is therefore quite variable, a fact which may prove to be of considerable importance in solving problems of biologic forms.

Puccinia graminis secalis

General statement

Puccinia graminis secalis has a number of hosts in common with *P. graminis tritici* and the work with this form was directed partly toward an attempt to determine whether the spore morphology of the two rusts might be made identical by the use of common hosts, as well as toward an attempt to determine the effect of different hosts on this rust alone.

Inoculations on *Secale cereale*

P. graminis secalis was obtained from two sources, viz.,

quack grass (Agropyron repens) and wild barley (Hordeum jubatum). Spores were measured the first, fifth and eighth generations. The size of the spores of the quack grass strain was as follows: extreme lengths 17.92 - 38.72μ, extreme widths 13.44 - 31.44μ, average modes 26.88 x 17.28μ, while those from the wild barley were as follows: extreme lengths 19.20 - 36.48μ, extreme widths 13.44 - 31.12, average modes 26.99 x 17.17μ, or almost identically the same, as far as the average modes are concerned. The shape of the spores was elongate elliptic and the color was a dull, dirty-yellow to grayish. Infection was moderate to strong in virulence, the pustules varying in size from 0.2 - 0.7 x 0.5 - 6.0 mm., color of pustules ranging from Sudan brown to Brussels brown and argus brown.

Inoculations on Hordeum vulgare

One transfer was made to barley directly from quack grass from the field, two sets were made with spores originally obtained from quack grass and wild barley and then kept on rye five generations. Measurements were made from the first and third generation on barley in either set. In addition to this four other sets of measurements of spores from barley having a long and complex history, as indicated in Table XI., were made. The length ranged from 19.20 to 37.44μ, the width varied from 13.76 to 30.48μ, and the average modes fell at 27.64 x 17.21μ, practically the same as on rye. The color and shape of the spores were the same as on rye. The virulence of infection was only moderate, the pustules varying from 0.1 - 0.9 x 0.3 - 3.5 mm. The color of the pustules

was the same as on rye.

Inoculations on Agropyron repens

The rust originally obtained from quack grass in the field was kept on this host eight generations. Measurements were made from the first, fifth and eighth generations with the following results: 17.92 to 35.52μ for extreme lengths, 14.08 to 20.48μ for extreme widths, and 26.35 x 17.39μ for modes. The spore dimensions on the original quack grass host were 18.88 to 37.44μ in length, 13.12 to 21.44μ in width, and mode falling at 28.80 x 17.28μ. Here as in the case of Puccinia phleipratensis the spores produced under field conditions were larger than those developed under greenhouse conditions. In shape and color the spores resembled those on rye. The pustules were 0.1 - 0.5 x 0.2 - 3.5 mm., in size, and Sudan brown or amber brown to chestnut or argus brown in color.

Inoculations on Hordeum jubatum

Wild barley was infected with Puccinia graminis secalis from wild barley, and 200 spore measurements were made from the first generation and 100 from the fifth. As on barley two parallel sets from the fifth generation of rye were made, and spores measured from both series of the first and third generation on the wild barley. The summarized results were: 18.88 - 35.52μ for length extremes, 13.76 - 20.48μ for width extremes, and 26.80 x 17.04μ for average modes. The size and color of the pustules were practically the same as on quack grass.

Inoculations on Elymus virginicus and Hystrix patula

Both of these hosts were inoculated with Puccinia graminis secalis having a long life history, which is given in Table XI. The spore sizes on E. virginicus were 21.76 - 33.28μ for length, 15.04 - 20.16μ for width, and 27.52 x 17.60μ for modes; those on Hystrix patula were 21.12 - 32.96μ for length, 15.04 - 20.48μ for width, and 27.20 x 17.60μ for modes.

Summary of inoculations made with Puccinia graminis secalis

A distinct and consistent uniformity of spore size, shape and color was exhibited by the Puccinia graminis secalis biological race when grown on a number of different hosts. Neither the source of the culture nor the length of time confined to a certain host affected the characteristic morphological features of the race to any marked degree. This is noteworthy because, as will be seen by referring to Table XI., the history of the spores measured varied greatly.

Long time association of P. graminis secalis spores with rye from rye measured 20.48 - 33.92μ long, 14.08 - 19.52 μ wide and 27.52 x 16.64 μ as modes.

Table XI.--Results of inoculations with Puccinia graminis secalis Pers. (Erikss.)

Source of urediniospores	Plant inoculated	Infection		Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Agropyron repens, field	Secale cereale	23	21	Strong	20.16-38.72	13.44-21.44	28.16x17.28
" " R4	" "	42	26	Moderate	17.92-32.00	13.76-20.16	25.60x17.28
" " "7	" "	29	27	Strong	21.12-33.28	14.72-20.16	26.88x17.28
Hordeum jubatum, field	" "	27	24	"	19.84-36.48	14.08-20.80	27.20x16.96
" " R4	" "	28	22	Weak	21.12-34.24	13.44-21.12	26.56x17.28
" " "7	" "	29	27	Strong	19.20-34.88	14.40-20.16	27.20x17.28
Agropyron repens, field	Hordeum vulgare	33	13	Weak	19.20-34.88	15.04-19.84	26.24x17.28
" " R5	" "	20	18	Moderate	19.52-35.20	14.72-19.84	27.52x17.28
" " "5B2	" "	29	26	"	23.04-33.28	13.76-20.48	28.16x16.96
Hordeum jubatum, R5	" "	18	16	Weak	19.20-34.88	14.40-20.16	27.20x17.28
" " "5B2	" "	25	25	Moderate	21.76-33.92	14.72-19.52	27.52x16.96
Agropyron repens, field	Agropyron repens	13	3	Weak	17.92-32.96	14.08-20.48	25.92x17.28
" " A4	" "	29	23	Moderate	19.52-33.92	15.04-20.16	25.60x17.60
" " "7	" "	23	23	Strong	19.52-35.52	14.40-20.16	27.52x17.28
Hordeum jubatum, field	Hordeum jubatum	11	9	Moderate	20.48-35.52	13.76-20.16	26.56x16.96
" " R5	" "	18	9	"	18.88-35.20	14.08-20.48	27.20x17.28
" " "5H2	" "	22	22	"	20.48-33.60	14.08-20.16	27.52x16.96
" " H4	" "	18	14	"	19.84-32.00	13.44-21.12	25.92x16.96

Table XI.--Continued.

Agropyron repens, B13	*Hordeum vulgare*	12	12	Moderate	20.48-37.44	15.36-19.20	28.80x17.28
Agropyron occidentale, R3B4R2B3	" "	16	15	"	21.44-35.52	14.40-19.84	28.16x16.96
Hordeum jubatum, R1W1R6B6	" "	21	16	"	22.72-32.96	14.40-19.84	27.84x17.28
Hystrix patula, R2B4R2B6	" "	17	13	"	21.76-32.32	15.04-20.16	27.20x17.60
Agropyron repens, B1R1B7Er12Ec5Ev5	*Elymus virginicus*	67	67	Strong	21.76-33.28	15.04-20.16	27.52x17.60
Hystrix patula, R2B4R2B3A1B1Ev1	*Hystrix patula*	22	22	"	21.12-32.96	15.04-20.48	27.20x17.60
Secale cereale, R_x	*Secale cereale*	25	24	Moderate	20.48-33.92	14.08-19.52	27.52x16.64

A - *Agropyron repens*
R - *Secale cereale*
E - *Hordeum vulgare*
W - *Triticum vulgare*

H - *Hordeum jubatum*
Er- *Elymus robustus*
Ec- *Elymus canadensis*
Ev- *Elymus virginicus*

Figures represent generations of confinement of the fungus to the particular host. (x) indicates a long time confinement of the parasite to its respective host.

Puccinia graminis tritici

General statement

Freeman and Johnson (2, p. 38) state in connection with an experiment they conducted on the change in morphology of the uredospores by cultivation of stem rust from wheat on barley and from barley on wheat, that "The host plants exercise a strong influence, not only on the physiological and biological relationships, but in some cases even on the morphology of the uredospores" The results obtained in the present investigation are not in accordance with those of Freeman and Johnson. A probable explanation can be found in assuming that the original barley used in their experiments, which is a congenial host to Puccinia graminis secalis, might have contained in addition to the wheat rust spores, which are large in size, also some rye rust spores, which are smaller in size, thus reducing the average size; another reason for the variations they found might be the small number of spores (50 and in some cases only 10 spores) they used for measurements.

Inoculations on Triticum vulgare

The measurements of P. graminis tritici were of spores with varying and complex life histories as shown in Table XII. The summarized results of the measurements were 23.04 - 40.96 μ for extreme lengths, 15.04 - 24.96 μ for extreme widths and 32.32 x 19.58 μ for average modes. The infection was vigorous, the pustules varied in size from 0.3 to 2.0 by 1.5 to 9 mm. The color varied from amber brown to burnt sienna and argus brown.

Inoculations on Hordeum vulgare

As in the case of Puccinia graminis secalis, here also a variety of different strains of Puccinia graminis tritici, as shown in Table XII, were inoculated on barley. The results as summarized are: 23.68 - 41.92μ for extreme lengths, 15.04 - 24.96μ for extreme widths, and 32.25 x 19.77μ for average modes. The infection virulence was on the whole quite vigorous; the pustules varied in size from 0.2 to 1.0 by 0.4 to 3.0 mm.; in color they were amber brown to Brussels or argus brown.

Inoculation on Hordeum jubatum

Wild barley was infected with rusts from the fifth generation on wheat, originated on wild barley at University Farm. The infection was continued for three successive generations and spore measurements made from the first and third, producing the following results: 24.64 - 40.64μ for extreme lengths, 17.28 - 23.36μ for extreme widths and 32.64 x 20.16μ for average modes. The infection virulence was on the average fairly strong, pustules varied in size as follows: 0.2 - 0.7 x 0.5 - 2.5 mm., their color being amber brown or burnt sienna to Brussels and argus brown.

Inoculations on Secale cereale

Spores originated on Agropyron occidentale with a long and complex history were inoculated on rye producing weak infection, the pustules and spores being both considerably reduced in length. The spore measurements were 23.04 - 36.48μ in length, 16.96 -23.04μ in width, and modes falling at 30.08 x 19.84μ.

Table XII.--Results of inoculations with *Puccinia graminis tritici* Pers. (Erikss.)

Source of urediniospores	Plant inoculated	Infection		Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Hordeum jubatum, field	*Triticum vulgare*	16	16	Strong	23.04-40.32	15.68-24.96	31.68x19.84
" " "	" "	31	2	"	23.04-40.32	15.04-23.04	32.32x19.52
" " W4	" "	33	33	"	23.04-38.08	16.00-22.40	32.00x19.52
" " "7	" "	27	26	"	25.60-40.96	16.96-23.36	32.96x20.16
" " W2	*Hordeum vulgare*	22	22	Strong	24.92-40.00	16.00-24.96	32.00x20.48
" " "2B5	" "	23	23	Moderate	26.24-38.72	15.36-23.04	31.36x19.20
" " "2"8	" "	31	31	Strong	25.60-38.72	16.32-22.72	32.00x19.52
" " "5	" "	18	16	"	24.00-40.64	16.64-23.68	32.32x20.16
" " "5B2	" "	25	25	"	24.32-40.96	16.32-23.36	32.64x19.84
" " W5	*Hordeum jubatum*	26	23	Strong	24.96-40.32	17.28-23.36	32.64x20.16
" " "5H2	" "	19	19	Moderate	24.64-40.64	17.60-23.04	32.64x20.16
Agropyron tenerum, B14	*Hordeum vulgare*	26	26	Strong	25.60-38.40	15.04-23.36	32.00x19.20
Agropyron occidentale, W2B14	" "	30	30	"	23.68-41.92	17.28-22.72	32.64x19.84
Hordeum jubatum, B35	" "	27	27	"	25.60-38.72	16.64-23.04	32.32x19.84
" " R2B4R1W2B5	" "	28	28	"	25.28-40.64	16.64-23.04	32.96x19.84

Table XII.--Continued.

Agropyron occidentale, W2B14H1	*Triticum vulgare*	19	19	Strong	25.60-39.68	16.96-22.72	32.64x19.84
" " "2"14"1	*Secale cereale*	14	2	Poor	23.04-36.48	16.96-23.04	30.08x19.84
Triticum vulgare, W_x	*Triticum vulgare*	14	13	Strong	25.60-40.32	16.32-23.36	32.96x19.84

Symbols are the same as in the previous table.

Summary of inoculations made with *Puccinia graminis tritici*

The morphological character of *Puccinia graminis tritici* showed considerable constancy in size, color and shape of spores when grown on congenial hosts. The length was reduced on rye but the cadmium yellow color and predominantly elliptic and ovate spore shape were retained. Long association with different hosts did not cause any noticeable differentiations in size.

The spores of *Puccinia graminis tritici* from wheat associated for a long time with wheat were 25.60 - 40.32μ in length, 16.32 - 23.36μ in width, with modes falling at 30.08 x 18.84μ.

General summary of conclusions derived from experiments on the constancy of biologic forms.

1. *Puccinia phleipratensis*: The size, shape and color of the urediniospores appear to be very little influenced by the host upon which they are produced. The shape is pyriform and color dull, dirty yellowish to grayish; the summarized sizes on the different hosts are given in a comparative table, Number XIII. In general the timothy rust spores are the smallest in size.

2. *Puccinia graminis avenae*: The size and shape are very variable and readily influenced by the host plants. Color constant, bright cadmium yellow, the shape ranges all the way from ellipsoid to pyriform to globose, even when grown on oats.

3. *Puccinia graminis secalis*: Constant in size, color, and shape. Color resembles that of timothy rust spores, length approaches that of oats rust spores on oats and in width practically

the same as that of timothy rust spores. Shape, elongate elliptic.

4. Puccinia graminis tritici: In constancy of size, color and shape it agrees with Puccinia phleipratensis and Puccinia graminis secalis. The spores are larger in size than those of any of the other forms; in width, however, being similar to that of oats rust spores. The spore color is nearly identical with that of P. graminis avenae, and the spore shape varies from elliptic to ovate.

5. Table XIII.--Comparative mode averages of spores of various biologic forms.

Rust form:	Host-plant:	Average modes on different hosts.	Average modes on all hosts used.	Modes on type hosts
P. phleipratensis	Phleum pratense	22.61x17.12 μ	23.14x17.25μ	24.00x16.96μ
	Dactylis glomerata	23.36x17.02 "		
	Avena sativa	23.44x17.60 "		
P. graminis avena	Avena sativa	38.16x19.46 μ	36.08x19.19μ	39.12x19.52μ
	Dactylis glomerata	35.92x19.60 "		
	Alopecurus pratensis	36.56x19.84 "		
	Bromus tectorum	33.68x18.88 "		
P. graminis secalis	Secale cereale	26.93x17.22 μ	27.54x17.19μ	27.52x16.64μ
	Hordeum vulgare	27.64x17.21 "		
	Agropyron repens	28.80x17.28 "		
	Hordeum jubatum	26.80x17.04 "		

Table XIII.--Continued.

P. graminis tritici	Triticum vulgare	32.32x19.58 μ	32.40x19.84 μ	32.96x19.84 μ
	Hordeum vulgare	32.35x19.77 "		
	Hordeum jubatum	32.64x20.16 "		

Type hosts refer to the host plants upon which the rusts of a certain biologic form are most commonly found.

6. When a biologic form succeeds in infecting an uncongenial host, the pustules are as a general rule small in size, and comparatively few abnormally small spores are produced.

Properties of hosts to unify spore sizes.

Stakman (4, p. 27) found that when wheat and einkorn were inoculated with spores from the same plant, the uredospores after growing for a year on wheat averaged 37.84 x 22.76 μ, while those grown on einkorn for a year measured 33.58 x 21.79 μ. The resulted difference appears to suggest the possibility of some hosts to exert a definite influence on the size of spores, but the accompanying two tables, XIV and XV, do not seem to support this theory. As seen from Table XIV, oats has no special properties of unifying the varying sizes of Puccinia phleipratensis and Puccinia graminis avenae altho it is a fairly congenial host for both rusts. The same inability is exhibited by barley to unify the sizes of Puccinia graminis secalis and Puccinia graminis tritici for which it is an equally congenial host.

Table XIV.- Results of experiments to determine capability of *Avena sativa* to unify spore sizes of *Puccinia graminis avenae* and *Puccinia phleipratensis* .

Source of urediniospores	Plant inoculated	Infection		Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.		Range of length (inμ)	Range of width (inμ)	Modes (inμ)
Phleum pratense, field	*Avena sativa*	53	14	Weak	17.60-30.08	14.72-20.48	22.72x17.60
" " Phl	" "	12	6	Weak	16.96-32.00	13.76-20.48	23.68x17.28
" " " 8	" "	21	8	Weak	17.92-28.16	15.04-20.80	23.04x17.92
Dactylis glomerata, " 6	" "	13	2	Weak	17.60-31.36	11.84-21.12	23.68x17.28
" " " 02	" "	8	1	Weak	17.60-28.48	14.72-20.48	23.36x17.92
Dactylis glomerata, field	*Avena sativa*	21	21	Strong	20.80-37.12	16.00-25.60	27.84x19.84
" " "	" "	35	4	Moderate	19.20-38.20	13.76-23.04	26.88x18.56
" " 05	" "	19	19	Strong	22.08-34.88	16.00-22.40	28.48x19.20
" " 08	" "	25	25	Strong	21.12-36.48	16.32-23.04	29.12x19.52
" " 021	" "	32	32	Strong	19.52-36.48	16.00-23.36	28.48x20.16

Table XV.-Results of experiments to determine ability of *Hordeum vulgare* to unify spore sizes of *Puccinia graminis secalis* and *Puccinia graminis tritici*.

Source of urediniospores	Plant inoculated	Infection		Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Agropyron repens, field	*Hordeum vulgare*	33	13	Weak	19.20-34.88	15.04-19.84	26.24x17.28
" " R5	" "	20	18	Moderate	19.52-35.20	14.72-19.84	27.52x17.28
" " "B2	" "	29	26	Moderate	23.04-33.28	13.76-20.48	28.16x16.96
Hordeum jubatum, R5	" "	18	16	Weak	19.20-34.88	14.40-20.16	27.20x17.28
" " "B2	" "	25	25	Moderate	21.76-33.92	14.72-19.52	27.52x16.96
Agropyron repens, B13	" "	12	12	Moderate	20.48-37.44	15.36-19.20	28.80x17.28
Agropyron occidentale R3B4R2B3	" "	16	15	Moderate	21.44-35.52	14.40-19.84	28.16x16.96
Hordeum jubatum, R1W1R6B6	" "	21	16	Moderate	22.72-32.96	14.40-19.84	27.84x17.28
Hystrix patula R2B4R2B6	" "	17	13	Moderate	21.76-32.32	15.04-20.16	27.20-17.60
Hordeum jubatum, W2(Jam)	*Hordeum vulgare*	22	22	Strong	25.92-40.00	16.00-24.96	32.00x20.48
" " "B5	" "	23	23	Moderate	26.24-38.72	15.36-23.04	31.36x19.20
" " "B8	" "	31	31	Moderate	25.60-38.72	16.32-22.72	32.00x19.52
" " H5	" "	18	16	Moderate	24.00-40.64	16.64-23.68	32.32x20.16
" " "B2	" "	25	25	Moderate	24.32-40.96	16.32-23.36	32.64x19.84
Agropyron tenerum, B14	" "	26	26	Strong	25.60-38.40	15.04-23.36	32.00x19.20

Table XV.- continued.

Agropyron occidentale, W2B14	Hordeum vulgare	30	30	Strong	23.68-41.92	17.28-22.72	32.64x19.84
Hordeum jubatum, B35	" "	27	27	Strong	25.60-38.72	16.64-23.04	32.32x19.84
" " R2B4R1W2B5	" "	28	28	Strong	25.28-40.64	16.64-23.04	32.96x19.84

Influence of environmental conditions

General statement

Ward (6, p. 374) notes that differences of temperature, illumination, drought, itc., affecting the transpiration, assimilation and other metabolic processes of the seedlings, also affect the period of germination, incubation and maturation of the rusts.

Effect of temperature

The most favorable temperature for shortening the incubation period and securing vigorous infection appears to be the range between 66.5° and 71° F. This range of temperature is also the optimum one for the production of the largest sized spores. At this temperature infection flecks appear in from six to eight days and pustules rupture the epidermis within another day or two. At temperatures higher than this the development of the pustules is retarded at the rate of one day for every 10° of advance of temperature, but rust will develop in as high temperatures as the host can endure, altho the sizes of the spores are considerably decreased. In low temperatures the development of the pustules is retarded at the rate of one day for every 5° of fall in temperature. Infection results in as low temperatures as the host can endure; the spores are smaller in low temperatures but the difference in size is not as great as in the case of high temperatures, using moderate temperature as a basis for comparison. The uredinia produced under high temperatures are darker than those produced under moderate temperatures while those produced at low temperatures are lighter than those produced at moderate temperatures.

Table XVI.--Effect of temperature on the development and structure of rust spores.

Source of uredinio-spores and plant inoculated	Temperature			Virulence of attack	Infection		Spore dimensions		
	Aver. temp.	Daily tem Max.	Daily tem Min.		Lvs. inoc	Lvs. infec	Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	66.4	96	49	Strong	18	16	25.60-40.00	16.00-23.04	33.28x19.84
" "	79.5	119	66	Moderate	21	21	23.04-40.00	16.32-24.96	30.72x20.16
Triticum vulgare	69.3	84	52	Strong	18	18	22.40-40.32	16.32-23.68	32.00x19.84
" "	81.8	99	70	Moderate	13	13	23.04-36.48	16.32-24.00	29.44x19.84
" "	55.8	70	26	Strong	13	12	26.24-40.00	16.96-22.72	31.36x19.84
Triticum vulgare	69.7	84	57	Strong	24	24	25.92-40.00	16.96-23.36	32.64x20.16
" "	55.8	100	22	"	16	16	25.28-40.00	18.24-22.72	32.64x20.48

The color of the uredinia developed in high temperatures varies from Brussels brown to argus brown; in moderate temperature, it varies from Sudan brown or antique brown to Brussels brown, while in low temperature it varries from amber brown to Sanford's brown.

The high temperatures were secured by means or an electric heater put under a glass bell jar where the plants were kept continuously. For low temperature the plants under a bell jar as in the above case were kept either in an unheated greenhouse or outside, according to conditions. The temperatures were recorded by thermographs from which the records were then computed. Control plants were kept under normal greenhouse conditions. For details see Table XVI.

Effect of humidity.

Plants for this experiment were placed under two glass bell jars, under one of which there were exposed three beakers filled with water to secure a humid atmosphere; under the other bell jar three plates with calcium chloride were placed to absorb the moisture in the air and that of transpiration by the plants. To prevent either evaporation of soil moisture or absorption of moisture by the soil, the surface of the pots were covered with a paraffin layer before they were placed under the jars. A third set of plants were kept as a check under normal greenhouse conditions. For the purpose of determining the relative humidity, hygrometers were employed for each set and readings were taken daily, taking at the same time barometric readings in another end of the same building. From the results shown in Table XVII. it would seem as tho either excessive high or excessive low humidity cause a decrease in the size of spores, but the difference is not

Table XVII.--Effect of humidity on the development and structure of rust spores.

Source of uredinio-spores and plant inoculated	Humidity			Virulence of attack	Infection		Spore dimensions		
	Aver R.H.	Daily hum. Max.	Daily hum. Min.		Lvs. inoc	Lvs. infec	Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	60.4	67.5	47.0	Strong	18	16	25.60-40.00	16.00-23.04	33.28x19.84
" "	85.3	92.5	76.5	Moderate	23	22	23.36-40.32	16.64-23.04	32.96x19.84
" "	52.3	54.0	50.5	Weak	25	23	24.96-40.32	15.36-24.32	32.00x20.16
Triticum vulgare	78.5	83.5	73.0	Strong	18	18	22.40-40.32	16.32-23.68	32.00x19.84
" "	94.6	97.0	91.0	Moderate	6	5	24.96-40.32	15.68-24.96	32.32x20.80
" "	62.2	66.5	59.5	"	7	7	22.72-39.36	15.68-24.64	30.40x19.84
Triticum vulgare	74.3	80.0	68.5	Strong	20	20	25.28-40.32	16.96-23.04	32.64x19.84
" "	93.4	95.0	90.5	"	12	12	24.00-40.00	16.64-24.00	32.00x20.16
" "	71.0	76.0	67.5	"	12	12	23.68-39.04	15.36-24.00	31.36x19.52

very pronounced and not very consistent, and the conclusion is therefore, that the variation caused by humidity is indefinite. The pustules were generally larger on the check plants and smaller on those grown in the dry atmosphere. The color of the spores grown in the humid air was commonly antique brown or amber brown, those produced in the arid air were amber to argus brown, while the checks were Sudan to Brussels brown. In other words, they were darker in dry air and lighter in wet air.

Effect of water content

Three sets of plants were employed, to one of which water was being applied in excessive quantities, to the second set in normal amounts, and to the third set only enough to prevent the plants from wilting. The water content of the soil was determined on the basis of oven dried soil. The plants in the wet soil were slightly more severely attacked and the spores grown on them were larger in size than either the check or the dry set. The set that suffered from drought produced the smallest sized spores. There was no apparent difference in the color of the pustules. The detailed results are given in Table XVIII. The results of this experiment are in accord with those Stakman (4, p. 34) obtained.

Effect of illumination.

Two sets of cultures were employed, one of which was kept beneath a double layer muslin cage, while the other one was exposed to the direct light. Light intensity readings were taken with a simple photometer, charged with "solio" photographic paper.

Table XVIII.--Effect of water content on the development and structure of rust spores.

Source of uredinio-spores and plant inoculated	Water content		Virulence of attack	Infection		Spore dimensions		
	Water applied in cc.	Water present in %		Lvs. inoc	Lvs. infec	Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	250	16.28	Strong	24	24	25.92-40.00	16.96-23.36	32.64x20.16
" "	850	31.65	"	16	16	26.24-40.96	17.60-22.72	33.28x20.16
" "	150	7.24	"	18	18	24.96-38.72	16.32-23.36	32.16x19.84
Triticum vulgare	400	17.12	Strong	20	20	25.28-40.32	16.96-23.04	32.64x19.84
" "	900	32.14	"	20	20	22.72-43.84	16.96-22.40	33.28x19.84
" "	100	5.38	"	20	20	22.40-38.08	16.64-21.76	30.40x19.20

Table XIX.--Effect of illumination on the development and structure of rust spores.

Source of uredinio-spores and plant inoculated	Illumination			Virulence of attack	Infection		Spore dimensions		
	Aver. inten	Daily int Max.	Daily int Min.		Lvs. inoc	Lvs. infec	Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	15.4	28.7	3.5	Strong	18	16	25.60-40.00	16.00-23.04	33.28x19.84
" "	3.3	5.0	0.9	Moderate	23	20	23.04-40.64	14.72-22.72	32.32x18.88
Triticum vulgare	14.0	46.7	6.6	Strong	18	18	22.40-40.32	16.32-23.68	32.00x19.84
" "	2.6	3.5	0.7	"	7	6	22.72-35.84	16.96-22.08	27.84x19.52
Triticum vulgare	17.5	25.0	7.5	Strong	20	20	25.28-40.32	16.96-23.08	32.64x19.84
" "	3.7	10.0	2.0	Moderate	20	20	21.76-36.48	17.28-22.72	29.12x19.84

The percent of intensity was determined with the aid of a "standard" print made in the fall on a bright sunny day. The rust consistently and definitely developed better in fairly high light intensities than in lower ones. The color of the pustules in the shade varied from antique brown to Sudan brown while those in the light ranged from Sudan to argus brown, that is, lighter in shade than in the light.

Relations of cultural factors

Effect of age of host

Two lines of work were carried out. In the first series three sets of oats plants 7 days old, 14 days old, and 21 days old respectively, were inoculated with Puccinia graminis avenae. A uniform amount of inoculating material was applied and similar conditions were maintained for all sets. The plants one week old were slightly more vigorously affected at first but at the end of 10 days the infection seemed stronger on the older plants. The second series consisted of plants 7, 21, and 35 days old respectively. In this series the plants 21 days old appeared to be most strongly infected. Spore measurements were made of all the sets of both series and no differences in the spore sizes or shapes were perceptible.

Effect of age on spore material

The object was to determine the viability of uredinio-spores after different periods of association with various hosts and the effect of the length of association on the rust. For the

Table XX.--Effect of age of host on the development and structure of rust spores.

Source of urediniospores and plant inoculated.	Date of planting	Date of inoculation.	Plant age at inoc. time	Virulence of attack	Infection		Spore dimensions		
					Lvs. inoc	Lvs. infec	Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Avena sativa	12-15-15	12-22-15	7 da	Strong	34	34	24.00-35.52	16.00-22.08	29.44x19.2
" "	12- 8-15	12-22-15	14 "	"	39	39	24.96-34.88	14.72-22.72	29.44x19.2
" "	12- 1-15	12-22-15	21 "	"	24	24	24.96-35.84	16.00-22.40	29.44x19.2
Avena sativa	3-10-16	3-17-16	7 da	Strong	12	12	23.04-35.52	16.96-22.08	29.44x19.5
" "	2-25-16	3-17-16	21 "	"	12	12	21.76-37.12	16.64-21.76	29.44x19.2
" "	2-11-16	3-17-16	35 "	"	12	12	22.72-35.84	16.00-22.40	29.12x19.2

Table XXI.- Effect of age on viability and structure of urediniospores.

Source of urediniospores and plant inoculated.	Length of ass'n. of fungus with its host.	Infection			Virulence of attack	Spore dimensions		
		Lvs. inoc.	Lvs. infec.	App.% of infec.		Range of length (in μ)	Range of width (in μ)	Modes (in μ)
Triticum vulgare	8 days	16	16	100	Strong	25.60-39.68	16.96-22.72	32.64x19.84
" "	14 "	14	13	93	Strong	25.60-40.32	16.32-23.36	32.96x19.84
" "	22 "	14	13	93	Strong	23.36-39.04	15.68-22.72	30.72x19.20
" "	63 "	26	26	100	Strong	24.32-40.96	16.64-23.04	32.64x19.52
Avena sativa	7 days	18	18	100	Strong	24.96-35.52	16.96-23.36	29.44x20.16
" "	20 "	23	22	96	Strong	23.04-35.20	16.96-22.08	29.12x19.52
" "	40 "	12	12	100	Strong	23.04-38.52	16.96-22.08	29.44x19.82
Secale cereale	8 days	16	13	81	Moderate	22.40-34.24	13.76-19.84	28.48x16.96
" "	14 "	25	24	96	Moderate	20.48-33.92	14.08-19.52	27.52x16.64

determination of the first phase of the experiment inoculations were made with rust material at different stages of the development of uredinia. Transfers were made when pustules were merely beginning to break thru the epidermis and two and four weeks afterwards. There was no apparent difference in the infection produced by these methods of inoculations. As for the determination of the effect of age on the structure of the spores, measurements were made at different stages of the development of uredinia, beginning with 7 days after inoculation and ending with 83 days. The three biologic forms of Puccinia graminis were used in this experiment and, as shown in Table XXI, there seemed to be no appreciable effect on the size of the spores, altho the size of the pustules gradually and consistently become larger, which is due to the additional shedding of spores and coalescence of adjacent pustules. The color of the pustules becomes darker with age and the spores lose their coherent floccose consistency and by the least disturbance are separated from the pustules.

Summary

1. The amount of inoculum placed on a leaf has no perceptible effect on the result of infection or size of spores, except in as much as a greater certainty for successful infection is secured.

2. The optimum length of time of incubation in the water pan is 48 hours, thus securing all certainty of infection without causing a tendency to supersensibility.

3. In spore measurements 100 spores obtained from a number

of pustules, are representative of their group. "Modes" are a practical basis for comparisons.

4. Biologic forms are constant not only parasitically but also structurally, the latter recognized by size, shape and color, typical to the given race.

5. Puccinia graminis avenae is an exception to the above rule in as far as shape and size are concerned. The shape is very variable on any host to which the rust may be confined, while the sizes appear to be peculiar to the host it parasitizes.

6. Uncongenial hosts invariably tend to decrease size of pustules and spores.

7. Host plants which are congenial to different biologic races or botanical species, have no power of influencing the size of spores and consequently lack the ability to unify them.

8. Adverse conditions unfavorable for the host are also unfavorable for the parasite, affecting its virulence and size.

9. Optimum temperature for the development of rusts appears to range between 66.5° and 71° F.

10. Sufficiency of water and plentiful light are indispensable for the best growth of the rust.

11. The age of the host seedlings at time of inoculation has no determining effect on the vigor of infection or size of spores, provided they are in a healthy, thrifty condition.

12. The length of association of the rust with its host, after the first pustules burst the epidermis until teliospores are formed, does not impair the viability of the spores, even if the host should die, nor does it exhibit any marked and consistent effect on the size of the urediniospores.

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