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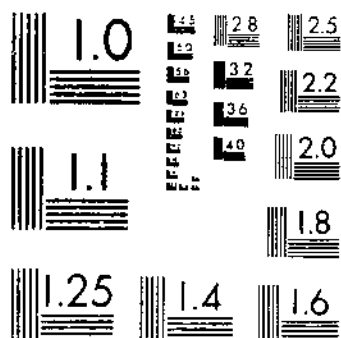
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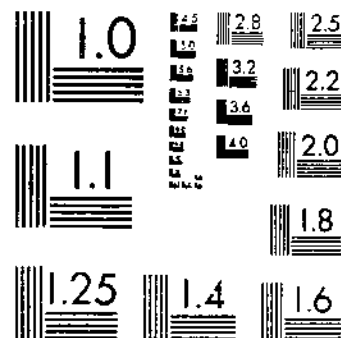
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FACTORS AFFECTING THE DEVELOPMENT OF THE AECIAL STAGE OF PUCCINIA
COTTER, RALPH U. 1 OF 1

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UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

FACTORS AFFECTING THE DEVELOPMENT OF THE AECIAL STAGE OF PUCCINIA GRAMINIS¹

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In Cooperation with the Minnesota Agricultural Experiment Station

CONTENTS

	Page		Page
Introduction.....	1	Experimental results—Continued.....	
Historical review.....	2	Susceptibility of barberry leaves of vari-	21
Methods and materials.....	3	ous ages and species.....	
Experimental results.....	4	Effect of length of incubation period on	24
Susceptible and resistant varieties of Ber-	4	infection of barberries.....	
beris.....		Effect of temperature and moisture on	24
Germination of teliospores.....	10	development of aecia.....	
Relation of age and freezing of teliospores	13	Effect of freezing on rusted barberry.....	25
to infection of barberries.....		Effect of light on development of aecia.....	28
Relation of temperature to infection of	13	Conditions for liberation of aeciospores.....	28
barberries.....		Viability of aeciospores.....	30
Influence of light on infection of barber-	15	Physiologic forms within a single aecium.....	31
ries.....		Discussion.....	34
Time required for infection of <i>Berberis</i>	16	Summary.....	36
<i>vulgaris</i> with teliospores of <i>Puccinia</i>		Literature cited.....	37
<i>graminis secalis</i>			

INTRODUCTION

The aecial stage of *Puccinia graminis* Pers. on barberry furnishes a tremendous amount of primary inoculum for infecting the grain-fields of the upper Mississippi Valley. It has been shown clearly that very destructive local epiphytotics and widespread regional epiphytotics result from aeciospores disseminated from barberries. For this reason a barberry-eradication campaign was begun in 1918 by the United States Department of Agriculture in cooperation with the following States: Colorado, Illinois, Indiana, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin, and Wyoming.

¹ Presented to the faculty of the graduate school of the University of Minnesota as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota.

² Since July 1, 1930, with the Division of Barberry Eradication, Bureau of Plant Industry. Acknowledgment is made to E. C. Stakman and J. J. Christensen for helpful suggestions during the progress of this work, and to M. N. Levine for valuable suggestions in the preparation of the tables and in verifying the identification of many of the physiologic forms of *Puccinia graminis tritici* obtained from aeciospores. The writer is indebted to A. H. Blakeslee, of the Carnegie Institute for Experimental Evolution, for the *Berberis vulgaris atropurpurea* parent and the hybrids between *B. thunbergii* and *B. vulgaris atropurpurea*.

From 1918 to 1927, inclusive, approximately 16,000,000 bushes, sprouts, and seedlings were found and destroyed in these States.³ In 1927, 10 years after the campaign started, 1,638,554 bushes, sprouts, and seedlings were found. During May and June of each year a large proportion of the remaining bushes were rusted. One heavily rusted barberry bush may bear about 70,000,000,000 aeciospores at one time (15).⁴

In determining the potential destructiveness of the rust produced on these bushes it is necessary to take into consideration the susceptible and resistant varieties of *Berberis* and *Mahonia*, the factors affecting infection of the bushes, subsequent development of the rust, the amount of inoculum produced under certain conditions, the liberation and dissemination of the aeciospores, the vitality and viability of the spores, and the infection of grains and grasses by the aeciospores. A study of the aecial stage, therefore, is extremely important from the standpoint of ascertaining the factors affecting its rôle in initiating rust epiphytotics.

Barberries, however, probably serve not only as a source of inoculum but also as a breeding ground for new parasitic strains or physiologic forms. It has been known for some time that the sexual process of the rusts initiates the aecial stage of the life cycle. It seems entirely probable, therefore, that different varieties and physiologic forms may hybridize on the barberry and produce new parasitic entities. Color is lent to this supposition by Craigie's (4) recent demonstration that *Puccinia graminis* is heterothallic. The fusion of two strains of opposite sex is prerequisite to the formation of aecia; hence it seems a foregone conclusion that dikaryophytes with new parasitic capabilities probably are produced on barberries. The work done by Allen (1) and Hanna (8) along these lines had not been published when the writer was preparing manuscript for this publication. Waterhouse (19), Newton, Johnson, and Brown (14), and Stakman, Levine, and Cotter (17) have adduced recent evidence to show that new physiologic forms of *P. graminis tritici* have been artificially produced on the barberry by crossing varieties of stem rust and physiologic forms within the variety. It is essential, therefore, to a complete understanding of the present habits and the possible future development of forms of stem rust to investigate this phase of the problem also. For these reasons the writer undertook a comprehensive investigation of the factors affecting the development of the aecial stage of *P. graminis*.

HISTORICAL REVIEW

The relationship between the common barberry and stem rust was suspected as early as the seventeenth century, or possibly even earlier. It is said that a law requiring the eradication of the bush was passed in Rouen, France, in 1660. It was known definitely that laws requiring its eradication in some of the colonies were passed long before the Revolutionary War. It was not, however, until late in the eighteenth century or the beginning of the nineteenth that definite experiments were made to determine the exact relationship between barberries

³ HUTTON, L. D., and BARINGER, J. W. ANNUAL REPORT OF THE BARBERRY ERADICATION CAMPAIGN, 1927, WITH SUMMARIZED RESULTS FOR 1918-1927, INCLUSIVE. U. S. Dept. Agr., Bur. Plant Indus., Off. Cereal Crops and Diseases Mimeographed Pamphlet. 31 p., illus. February, 1928.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 37.

and rust. According to Cobb (3), Joseph Banks suggested in 1805 the possibility that the rust on barberry and the rust on wheat belonged to the same species. In the period from 1807 to 1816, Schoeler, according to Freeman and Johnson (7), planted small grains around barberry plants and found that oats and rye were in danger of destruction by rust when growing near them. In 1816 he made rather crude but effective inoculations by rubbing rusted barberry leaves on rye plants moist with dew. Schoeler marked the plants and about five days later observed that they were heavily rusted, whereas those elsewhere in the field were free from rust. According to Freeman and Johnson (7), in 1818 a German farmer gathered the dust that fell from the cups on barberry leaves and took it to a far distant rye field where there were no barberries and placed it on some of the plants. Within five or six days rust appeared on these plants but was not evident elsewhere in the field.

It was not until 1852, however, that the genetic relationship between the so-called summer rust, *Uredo*, and the autumn rust, *Puccinia*, was demonstrated by Tulasne (7). He showed also that the teliospores would not germinate until the spring following the fall in which they were formed. It remained for de Bary to show in 1865 (7) that barberries would become infected when inoculated with teliospores and that grains would become infected when inoculated with aeciospores. De Bary's results have been repeated and confirmed so often that there is no question as to the genetic relationship of the rust on the barberry and that on grains and grasses. De Bary's discovery immediately stimulated more detailed observations regarding the responsibility of barberries in initiating epiphytotics of stem rust.

In 1918 an extensive barberry-eradication campaign was undertaken in the 13 grain-growing States in the upper Mississippi Valley in the United States. This activity immediately created a demand for information regarding the susceptibility of different species and varieties of *Berberis* and for detailed information on the factors affecting the development of the aecial stage in general. Stakman and Levine compiled in 1923⁶ a list of susceptible and resistant barberries, and this was followed by supplementary lists by Melander and by Melander and Lambert in 1923 and 1924.⁶

The writer began a detailed study of the susceptibility of various species and varieties of barberry in 1925. Further facts regarding the historical development and knowledge concerning the aecial stage of the rust will be found in the body of this bulletin in connection with the particular experiments to which they apply.

METHODS AND MATERIALS

In the barberry-eradication campaign it was found necessary to specify definitely the species and varieties of barberry that were susceptible to rust and that therefore should be placed in the list of quarantined species. Inquiries have come in from time to time concerning the susceptibility of certain species, but unfortunately no information was available regarding many species grown in botanical gardens, arboreta, private estates, and parks. The publication by Stakman

⁶ STAKMAN, E. C., and LEVINE, M. N. A PARTIAL REPORT ON THE SUSCEPTIBILITY AND RESISTANCE OF BERBERIS AND RELATED GENERA TO STEM RUST. Cereal Courier [Office of Cereal Crops and Diseases] 15(24): 278-287. Sept. 30, 1923. [Alimnographed.]

⁶ MELANDER, L. W. STUDIES OF THE RELATION OF BERBERIS SPECIES TO PUCCINIA GRAMINIS PERS. [Unpublished thesis, University of Minnesota. 1924.]

and Levine in 1923⁷ of a list of susceptible and resistant species helped to clear up the doubt in many cases, but there were still many species of barberry whose reaction to rust was unknown. Some barberries could not be condemned for destruction by the workers in the barberry-eradication campaign because the question of their susceptibility to rust had not been settled. These plants were in some cases a potential menace to grainfields, for it was possible that the barberries might rust shortly after the visit of the barberry field men and might broadcast inoculum over the grainfields before it was found that the plants of that particular species rusted and should be removed. To procure information on this subject, the writer undertook a greenhouse inoculation study of as many species, varieties, and hybrids of *Berberis* as were obtainable.

The following methods were used in inoculating barberries: (1) A few drops of a suspension of teliospores in water were placed on the young leaves or shoots of the barberry with a loop needle. The plants were then kept 48 hours in a moist chamber. (2) When it was desired to secure heavy infection on all parts of the plant, heavily rusted straw was placed on wire screens supported above the plants to be inoculated. The incubation period with this method was usually 72 hours, although it was reduced to 24 hours with good results when the telia had been presoaked in water 12 to 24 hours. After incubation the plants were placed on the greenhouse bench in the evening in order to avoid solar injury to the tender tissues. Pycnia usually appeared about the fifth or sixth day.

The rust varieties used in the experimental work were *Puccinia graminis tritici* (Pers.) Eriks. and Henn. and *P. graminis secalis* (Pers.) Eriks. and Henn. Five different collections of *P. graminis secalis* were used in the various phases of the work.

Most of the barberries tested for susceptibility were sent to University Farm, St. Paul, Minn., from Bell, Md., by B. Y. Morrison, of the Bureau of Plant Industry, United States Department of Agriculture. A smaller number was obtained from Highland Park, Rochester, N. Y., and others came from unknown sources. The common barberries used as checks in most of the experiments were supplied by L. W. Melander, in charge of barberry eradication in Minnesota.

The degree of infection was necessarily based on the individual barberry leaf, as there were not always large numbers of young leaves on the plants when inoculations were made. When only a single pycnium occurred on a leaf the infection was designated as very light; when there were 2 to 4 on a single leaf it was designated as light; 5 to 10 pycnia on an individual leaf indicated moderate infection; if the pycnia exceeded 10 in number the infection was designated as heavy; and when pycnia on a leaf were numerous and nearly confluent the infection was considered very heavy. Although not perfect, this scale has been found to satisfy the requirements of the work.

EXPERIMENTAL RESULTS

SUSCEPTIBLE AND RESISTANT VARIETIES OF BERBERIS

In Table 1, which is a compilation of the lists of Stakman and Levine, Melander, Melander and Lambert, and Cotter, are listed those species and varieties of *Berberis* whose susceptibility to *Puccinia graminis* is known.

⁷ STAKMAN, E. C., and LEVINE, M. N. Op. cit. (See footnote 5.)

TABLE 1.—*Species and varieties of Berberis known to be susceptible to Puccinia graminis, as compiled by Stakman and Levine, Melander, Melander and Lambert, and Colter*¹

STAKMAN AND LEVINE, 1923

No.	Host	Source of information			Degree of infection
		Original observations		Literature references ²	
		Artificial inoculation	Natural infection in field		
1	<i>Berberis actinensis</i> Presl			Saccardo; Sydow	Normal.
2	<i>B. alscuthensis</i> Hort.		+		
3	<i>B. altana</i> Pall.			Saccardo; Klebahn	
4	<i>B. amurensis</i> Rupr.	+	+	Saccardo; Sydow; Jaczewski; Klebahn.	
5	<i>B. angulosa</i> Wall.		+		
6	<i>B. aristata</i> DC.	+	+	Bolley; Klebahn; Saccardo; Sydow.	Moderate.
	(<i>B. coriaria</i> Royle)			Butler and Hayman.	
7	<i>B. asiatica</i> Roxb.			Sydow	
8	<i>B. atropurpurea</i> Regel.		+	Klebahn	
9	<i>B. brachybotrys</i> C. Gay. (<i>B. brachybotrydis</i> C. Gay)			Saccardo Sydow	
10	<i>B. bretschnelderi</i> Rehd.		+		Heavy.
11	<i>B. brevipaniculata</i> C. Schneid.	+			
12	<i>B. buxifolia</i> Lam.			Sydow	
13	<i>B. canadensis</i> Mill.	+	+	Bolley; Klebahn; Saccardo; Sydow.	
	(<i>B. caroliniana</i> Loud.) (<i>B. fischeri</i> Hort.)		+	Saccardo	
14	<i>B. coryi</i> Veitch (<i>B. wilsonae</i> subcaululata Schn.)	+			Moderate. Do.
15	<i>B. crataegina</i> DC.		+		
16	<i>B. cretica</i> L.		+		
17	<i>B. declinata</i> Schrad.			Arthur	
18	<i>B. declinata oxyphylla</i> C. Schneid.		+		
19	<i>B. diaphana</i> Maxim.	+			Moderately resistant.
20	<i>B. dioxyphylla</i> Franch.	+			
21	<i>B. durobrivensis</i> C. Schneid.	+			
22	<i>B. emarginata</i> Willd. (<i>B. vulgaris emarginata</i> Gord.)		+	Bolley	
23	<i>B. emarginata britzensis</i> C. Schneid.		+		
24	<i>B. fendleri</i> A. Gray		+	Arthur	Heavy.
25	<i>B. fremontii</i> Torr.		+		
26	<i>B. fuchsoides</i> Hort.		+		
27	<i>B. haenatocarpa</i> Wooton.		+		
28	<i>B. heteropoda</i> Schrenk.		+	Jaczewski; Sydow	
29	<i>B. ilicifolia</i> Forst.	+	+	Klebahn; Sydow	Heavy.
30	<i>B. integerrima</i> Bunge.			Jaczewski	
31	<i>B. laxiflora</i> Schrad.		+		
32	<i>B. leichtlinii</i> Hort.	+	+		
33	<i>B. lucida</i> Schrad.	+			
34	<i>B. lycium</i> Royle			Klebahn	Moderately resistant. Do.
35	<i>B. macrophylla</i> Hort.			Saccardo	
36	<i>B. mechanii</i> C. Schneid.		+		
37	<i>B. nepalensis</i> (DC) Spreng.			Butler and Hayman; Klebahn; Sydow.	
38	<i>B. neubertii</i> Lein.		+	Bolley; Klebahn; Saccardo; Sydow.	
39	<i>B. oblonga</i> C. Schneid.		+		Heavy.
40	<i>B. provincialis serrata</i> C. Schneid.	+			
41	<i>B. pruinosa</i> Franch.	+			
42	<i>B. regeliana</i> Koehne. (<i>B. amurensis japonica</i> Rehd.) (<i>B. vulgaris japonica</i> Regel)		+		
43	<i>B. sibirica</i> Pall.		+	Arthur; Jaczewski; Saccardo; Sydow.	
44	<i>B. sieboldii</i> Mig.	+	+	Klebahn	Heavy.
45	<i>B. sinensis</i> Desf. (<i>B. chinensis</i> Polr.)		+	Arthur; Sydow.	
46	<i>B. stapfiana</i> C. Schneid.	+	+		
47	<i>B. subcaululata</i> C. Schneid.		+		

¹ A plus mark (+) in the column entitled "Artificial inoculation" indicates that when greenhouse inoculations on that particular species were made, infection occurred, the variety of inoculum used being unknown unless otherwise stated. A plus mark in the column entitled "Natural infection in field" indicates that plants found growing in the field, in arboreta, or in gardens were found naturally infected. A blank indicates that no inoculations were made on that particular species.

² Citations in list of Stakman and Levine. (See footnote 5.)

TABLE 1.—Species and varieties of *Berberis* known to be susceptible to *Puccinia graminis*, as compiled by Stakman and Levine, Melander, Melander and Lambert, and Cotter—Continued

STAKMAN AND LEVINE, 1923—Continued

No.	Host	Source of information			Degree of infection
		Original observations		Literature references	
		Artificial inoculation	Natural infection in field		
48	<i>B. swaseyi</i> Duckl.	+		Arthur	Moderately resistant.
49	<i>B. thibetica</i> C. Schneid.	+			
50	<i>B. trifoliolata</i> Morle	+		Arthur	
51	<i>B. umbellata</i> Wall.			Butler and Hayman	Heavy.
52	<i>B. vulgaris</i> L.	+	+	Arthur; Bolley; Butler and Hayman; Jaczewski; Klebahn; Saccardo; Sydow.	
53	<i>B. vulgaris alba</i> Weston		+		
54	<i>B. vulgaris asperma</i> Don		+		Moderate.
55	<i>B. vulgaris atropurpurea</i> Regel		+		
56	<i>B. vulgaris fructoviolacea</i> Hort.		+		
57	<i>B. vulgaris lutea</i> Don		+		Somewhat resistant.
58	<i>B. vulgaris macrocarpa</i> Jaeger		+		
59	<i>B. vulgaris mitis</i> Hort.			Sydow	
60	<i>B. vulgaris nigra</i> Don		+	Sydow et al.	Heavy.
61	<i>B. vulgaris purpurea</i> Hort.		+		
62	<i>B. vulgaris sanguinolenta</i> Hort.		+		
63	<i>B. vulgaris spathulata</i> Gord. (<i>B. chinensis</i> Poir.)		+		Moderate.
64	<i>B. vulgaris sulcata</i> Hort.	+	+		
65	<i>B. vulgaris violacea</i> Willd.		+		
66	<i>B. aquifolium</i> Pursh	+		Jaczewski; Sydow	Somewhat resistant.
67	<i>B. diversifolia</i> (Sweet) Steud.			Arthur	
68	<i>B. glauca</i>			Arthur; Sydow	

MELANDER AND LAMBERT, 1923

1	<i>B. coryl</i> Veitch	+	—	—	—
2	<i>B. haematocarpa</i> Wooton	+	—	—	—
3	<i>B. lucida</i> Schrad.	+	—	—	—
4	<i>B. swaseyi</i> Duckl.	+	—	—	—
5	<i>B. thibetica</i> C. Schneid.	+	—	—	—

MELANDER, 1924

1	<i>B. chinensis</i> Poir.	+	—	—	—
2	<i>B. koronae</i> Palib.	+	—	—	—
3	<i>B. vanfleeteri</i> C. Schneid.	+	—	—	—

COTTER, 1925-1931

1	<i>B. acuminata</i> Franch.	+	—	—	Heavy.
2	<i>B. nemulans</i> C. Schneid.	+	—	—	Pycnia.
3	<i>B. oetnensis</i> Presl	+	—	Saccardo; Sydow	Heavy.
4	<i>B. aggregata</i> C. Schneid.	+	—	—	Moderate.
5	<i>B. aggregata prattii</i> C. Schneid.	+	—	—	Pycnia only.
6	<i>B. nulesuthensis</i> Hort.	+	+	—	Heavy.
7	<i>B. amurensis japonica</i> Rehd. (<i>B. regelliana</i> Koehne).	+	+	—	Light.
8	<i>B. arvensis</i> Hort.	+	—	—	Heavy.
9	<i>B. asiatica</i> Roxb.	+	—	Sydow	Heavy; pycnia only.
10	<i>B. atropurpurea</i> C. Schneid.	+	—	—	Heavy.
11	<i>B. bergmanniae</i> C. Schneid.	+	—	—	Moderate; pycnia only.
12	<i>B. brachypoda</i> Maxim.	+	—	—	Moderate.

* From this point on, *secalis* was used as inoculum unless otherwise shown.

* First recorded artificial infection, although natural infection was noted previously.

* First observed by Stakman and Levine.

* The variety of inoculum used was tritici.

TABLE 1.—Species and varieties of *Berberis* known to be susceptible to *Puccinia graminis*, as compiled by Stakman and Levine, Melander, Melander and Lambert, and Collier—Continued

COTTER, 1925-1931—Continued

No.	Host	Source of information			Degree of infection
		Original observations		Literature references	
		Artificial inoculation	Natural infection in field		
13	<i>B. californica</i> Jepson.....	+			Light; pycnia only.
14	<i>B. crataegina</i> DC.....	++	++		Moderate.
15	<i>B. declinata oxyphylla</i> C. Schneid.....	++	++		Heavy.
16	<i>B. dieckmannii</i> Fedde.....	++			Light.
17	<i>B. dulcis nana</i> Hort.....	++			Pycnia; light.
18	<i>B. emarginata</i> Willd.....	++	++	Bolley and Pritchard.....	Heavy.
19	<i>B. emarginata britzensis</i> C. Schneid.....	++	++		Do.
20	<i>B. fendleri</i> A. Gray.....	++	++	Arthur; Durrell and Lungren.....	Moderate.
21	<i>B. fremontii</i> Torr.....	++	++		Light.
22	<i>B. francisci-ferdinandi</i> C. Schneid.....	++	++		Heavy.
23	<i>B. fuchsioides</i> Hort.....	++	++		Do.
24	<i>B. gagnepainii</i> C. Schneid.....	++			Pycnia; light.
25	<i>B. gilgiana</i> Fedde.....	++			Very light.
26	<i>B. heteropoda</i> Schrenk.....	++	++	Jaczewski; Sydow.....	Moderate.
27	<i>B. henryana</i> C. Schneid.....	++			Light.
28	<i>B. hookeri</i> Leun.....	++			Do.
29	<i>B. hybrida serrata</i> Hort.....	++			Heavy.
30	<i>B. japonica</i> Spreng. (<i>B. boabel</i> Fort.).....	++			Light.
31	<i>B. knightii</i> Hort. (<i>Wallchiana</i> var. <i>latifolia</i>) (<i>B. xanthoxylon</i> Hassk.).....	+			Do.
32	<i>B. koehneana</i> Schneid.....	++			Moderate.
33	<i>B. levis</i> C. Schneid. (Bell 149) *.....	++	(8, 10)		Light.
34	<i>B. lychnum</i> Royle.....	++	++		Moderate.
35	<i>B. masonii</i> C. Schneid.....	++	++		Do.
36	<i>B. morrissonensis</i> Hayata.....	++			Light.
37	<i>B. notabilis</i> C. Schneid.....	++	++		Heavy.
38	<i>B. neubertii</i> Hort.....	++	++	Bolley and Pritchard.....	Very heavy.
39	<i>B. nevadensis</i> A. Gray (<i>Mahonia nevadensis</i> Fedde).....	+	++		Heavy.
40	<i>B. oblonga</i> C. Schneid.....	++	++		Do.
41	<i>B. parvifolia</i> Sprague.....	++	++		Light.
42	<i>B. polareti</i> C. Schneid.....	++	++		Do.
43	<i>B. polareti latifolia</i> C. Schneid.....	++	++		Do.
44	<i>B. polyantha</i> Hemsl. (<i>B. aggregata prattii</i> C. Schneid.).....	++	++		Moderate.
45	<i>B. prattii</i> C. Schneid. (<i>B. aggregata prattii</i> C. Schneid.).....	+	++		Do.
46	<i>B. serotina</i> Lange (<i>B. chinensis</i> Poir.).....	++	++		Do.
47	<i>B. sinensis</i> Desf. (<i>B. chinensis</i> Poir.).....	++	++	Arthur; Sydow.....	Light.
48	<i>B. soulieana</i> C. Schneid.....	++	++		Do.
49	<i>B. subcaulata</i> C. Schneid.....	++	++		Do.
50	<i>B. umbellata</i> Wall.....	++	++		Heavy.
51	<i>B. vernae</i> C. Schneid.....	++	++		Light.
52	<i>B. virens</i> Hook f.....	++	+		Do.
53	<i>B. viridis</i> Hort.....	++	++		Moderate.
54	<i>B. vulgaris atropurpurea</i> Regel.....	++	++		Do.
	(<i>B. vulgaris purpurea</i> Bertin).....	++	++	Sydow et al.....	Heavy.
55	<i>B. vulgaris shoyalle</i> Hort.....	++	++		Do.
56	<i>B. wilsonae</i> Hemsl. and Wils.....	++	++		Moderate.
57	<i>B. wilsonae</i> Hemsl. var. <i>Autumn Cheer</i>	++	++		Heavy.
58	<i>B. wilsonae</i> Hemsl. var. <i>Fredy</i>	++	++		Light.
59	<i>B. wilsonae</i> Hemsl. var. <i>Sparkler</i>	++	++		Do.
					Very light.

* First recorded artificial infection, although natural infection was noted previously.

† First observed by Stakman and Levine.

‡ Both tritici and scutellari used as inoculum.

§ Number given at U. S. Plant Field Station, Glenn Dale, Md.

|| Observed by Butler and Hayman.

||| Observed by Klebahn.

||| Pycnia only on previous trials.

||| The variety of inoculum used was poae.

It will be seen from Table 1 that the number of susceptible species and varieties of *Berberis* far exceeds that of the immune varieties.

The question naturally arises as to whether reaction to rust is correlated with gross morphological characters. In general, those species which are susceptible to *Puccinia graminis* resemble *Berberis vulgaris* somewhat in the size, shape, and texture of the leaves, while those resembling *B. thunbergii* are likely to be resistant. Of course, there are exceptions to this generalization. For example, *B. wilsonae* Hemsl. and Wils. is characterized by small, lanceolate leaves, while *B. vulgaris* has relatively large and oval leaves, but both are quite susceptible to *P. graminis*. *B. hookeri* Lem. (susceptible) differs widely from *B. vulgaris* in leaf texture, the former possessing leaves that are hard and leathery in texture when mature, while those of *B. vulgaris* are soft and more or less tender. The leaves of *B. neubertii* Hort. (very susceptible) are about 2 inches wide and 3 inches long, while those of *B. vulgaris* are less than 1 inch wide and about 1½ inches long. As a general rule, however, there seems to be a close correlation between the *B. vulgaris* type of leaf and susceptibility to *P. graminis*.

Certain indications have pointed out that while the plants of a botanical species of *Berberis* may be phenotypically similar, they may be genotypically dissimilar with respect to susceptibility to *P. graminis*, or there may be a very narrow range of conditions within which infection may take place on certain species. In one shipment of *B. hookeri* there were 12 plants. Within a period of two years 18 inoculations were made on these plants and only one aecium resulted, but this lone aecium was perfectly normal in all respects. Other species that may not be homozygous for rust resistance are *B. aristata* DC., *B. brachypoda* Maxim., *B. gilgiana* Fedde, and *B. soulieana* C. Schneid.

In Table 2 are given those genera and species of the Berberidaceae that did not rust when artificially inoculated in the greenhouse. The variety of rust used in the inoculations is given, the term "variety" here being used to designate one of the group forms of *Puccinia graminis*. The number of trials for each species is the total of the inoculations made by Stakman and Levine plus those of the present author. All the species here listed are not to be considered definitely immune, since further inoculations may prove that some of the species, except *B. thunbergii* DC. or its varieties, are susceptible. The first list of immune barberries, made three years ago, contained more than double the number presented in Table 2, so conclusions as to resistance or immunity of any species, based on a small number of negative results should not be regarded as of great value.

TABLE 2.—Genera and species of Berberidaceae that did not rust as a result of artificial inoculation with teliospores of five varieties of *Puccinia graminis*

Genera and species	Number of inoculations with varieties of <i>P. graminis</i>				
	Agrostidis	Avenae	Poa	Secalis	Tritic
<i>Berberis beaniana</i> C. Schneid.	0	0	0	11	0
<i>B. coccinea</i> Hook.	0	0	0	7	0
<i>B. edgeworthiana</i> C. Schneid.	0	0	0	12	0
<i>B. ottawensis</i> C. Schneid.	0	0	1	5	0
<i>B. potaninii</i> Maxim.	0	0	1	7	1
<i>B. repens</i> Lindl.	0	0	0	18	34
<i>B. stenophylla</i> Mast.	0	0	0	11	1
<i>B. thunbergii</i> DC.	0	4	0	21	7
<i>B. thunbergii atropurpurea</i> Hort.	12	0	14	248	38
<i>B. thunbergii maximowiczii</i> (Regel) C. Schneid.	0	13	0	24	10
<i>B. thunbergii minor</i> Rabd.	0	0	0	3	0
<i>Caulophyllum thalictroides</i> (L.) Michx.	0	11	0	5	19
<i>Diphylleia cymosa</i> Michx.	0	1	0	2	0
<i>Jaffersonia diphylla</i> (L.) Pers.	0	0	0	0	2
<i>Podophyllum peltatum</i> L.	0	0	0	0	2

A few of the barberries, previously listed as susceptible, exhibit what may be a physiologic rather than a morphologic resistance, as probably is the case with the majority of those barberries now considered or proved immune. For example, mature leaves of *Berberis asiatica* are rather leathery, but the young leaves are soft and smooth. When this species was inoculated with teliospores of the *tritici* variety of *Puccinia graminis*, a heavy pycnial infection resulted, but no aecia formed. (See pl. 5.) When examined with a hand lens, a single outgrowth, possibly an abortive aecium, was sometimes seen on the dorsal surface of the leaf. On this species the fungus was able to penetrate the leaf and produce the initial infection, but for some reason was prevented from completing the normal development of the rust.

The same phenomenon—heavy pycnial infection with subsequent production of a few or no aecia—is frequently seen in varying degrees on the compound-leaf barberries, the Mahonias of some authors. *Berberis fremontii* Torr., *B. haematocarpa* Wooton, *B. nevinnii* A. Gray, *B. swaseyi* Buckl., and *B. trifoliolata* Moric. are infected without great difficulty. Aecia, however, fail to develop in about half the number of infections. With *B. aquifolium* Pursh and *B. californica* Jepson, infection has been difficult to secure, and only a few pycnia usually are present, with no aecia. In Europe, however, infection has been reported on the fruits of *B. aquifolium*, and in the United States and Canada on the leaves of *B. aquifolium* in a few cases.

Although those species and varieties of *Berberis* that resemble *B. vulgaris* seem in general to be susceptible to rust and those of the *B. thunbergii* type resistant, there is some observational evidence that hybrids between the two sometimes may have the susceptibility of *B. vulgaris* and many of the morphologic characters of *B. thunbergii*. It seemed worth while, therefore, to study the rust reaction of hybrids between these two species in order to ascertain whether there is a correlation between morphologic characters and susceptibility to rust. The results are summarized in Table 3.

TABLE 3.—Reaction of *Berberis vulgaris* × *B. thunbergii* hybrids to *Puccinia graminis tritici* and *P. graminis secalis*

Crosses	Reaction of hybrids ¹ to—			
	<i>P. graminis tritici</i>		<i>P. graminis secalis</i>	
	Resistant	Susceptible	Resistant	Susceptible
<i>B. vulgaris</i> × <i>B. thunbergii</i> F ₁ (F ₁ individuals).....	¹ C. B. 1	² B. C. 1 B. C. 2 B. C. 3	C. B. 1
	³ 207 (3, 9, 14)	⁴ 207 (x)	² 201 (1, 2, 5, 9, 10, 11)	201 (0, 8)
			² 204 (3, 9, 10, 11)	202 (1, 2, 3) 204 (2)
<i>B. vulgaris</i> × <i>B. thunbergii</i> F ₂ (F ₂ individuals).....			² 205 (3, 8, 9)	⁴ 207 (x, 2, 3, 5, 10, 14, 18, 19, 22, 27)
			² 208 (2)	
			⁴ 207 (x, 9, 11, 15, 23, 28, 29, 30)	
<i>B. C. 1</i> × <i>B. vulgaris</i> (back cross).....		203 (2)	203 (2)	203 (1, 2)

¹ Individuals are grouped according to F₂ families and back crosses. For example, 201 (1, 2, 5, 9, 10, 11) means individuals 1, 2, 5, 9, 10, and 11 of family 201.

² According to Blakeslee, B. C. 1, 2, and 3 and C. B. 1 are F₁'s from crosses made in 1914 between *Berberis thunbergii* and the purple-leaved variety of barberry.

³ Numbers given by A. H. Blakeslee, Carnegie Institute for Experimental Evolution, Cold Spring Harbor, N. Y.

⁴ x=plants plowed up in the nursery and replaced without numbers.

The plants used in this study were furnished by A. H. Blakeslee, of the Carnegie Institute for Experimental Evolution at Cold Spring Harbor, N. Y. They were crosses between *Berberis vulgaris atropurpurea* and *B. thunbergii*. The plants were removed from the field at that place and shipped to St. Paul, Minn., where they were potted and subsequently inoculated on the appearance of new leaves. They were inoculated with *Puccinia graminis tritici* form 18 from Laramie, Wyo.; *P. graminis secalis* form 9 from Wanamingo, Minn.; and *P. graminis secalis* form 11 from Litchfield, Minn.

The purple-leaf common barberry parent was determined as susceptible. The other parent, *Berberis thunbergii*, is known to be immune. Of the four F_1 individuals referred to in footnote 2 of Table 3, one, B. C. 1, was susceptible, while three, B. C. 1, B. C. 2, and B. C. 3, did not rust as a result of the five inoculations made. Of the three individuals obtained from a back cross of B. C. 1 \times Purple, two were susceptible and the third was rust free in three inoculations. Of these inoculations, only the series in which the checks were infected are included.

Of the four individuals obtained from a cross of B. C. 1 \times B. C. 3, three of the four F_2 individuals are susceptible, the status of the other being unknown.

Data are available on the reaction of 38 F_2 individuals belonging to six F_2 families of a population of 45. Of this number, 22 were immune from and 16 susceptible to *Puccinia graminis secalis* in repeated inoculations. These small numbers constitute a ratio of three to two and are not definitely indicative of the number of genetic factors involved. Nor is it possible to ascertain from the reaction of either the F_1 or F_2 generation whether resistance or susceptibility is dominant.

In this particular cross, susceptible plants were in general like the susceptible parent in morphologic characters. The leaf color of most of the plants was green, very few purple-leaf plants being present. All the purple-leaf plants were susceptible. The immune individuals resembled the *Berberis thunbergii* more than the *B. vulgaris* parent; therefore, it would seem that there is a correlation between morphologic characters and resistance to *Puccinia graminis*. This makes the eradication of barberries easier, as apparently there is but little ground for the fear that there may be numerous hybrids that look like *B. thunbergii* but rust like *B. vulgaris*.

GERMINATION OF TELIOSPORES

The species and varieties of *Berberis* susceptible and those resistant to rust having been determined, experiments were made to ascertain the conditions governing infection of the susceptible varieties. *B. vulgaris* was the principal species used in the experiments. Obviously, the first factor to be considered in studying the sequence of infection in the asexual stage is the germination of the teliospores.

It has been known for many years that the germination of teliospores is capricious. DeBary (2, p. 24) observed that they rarely germinated immediately after their formation. It is definitely known that a resting period is required before they can germinate. Just what is the nature of the change that occurs between the time of formation of the teliospores and that of their physiologic maturity is not known. Numerous attempts have been made to determine the factors affecting maturation and germination, but the problem is still very imperfectly understood.

McAlpine (11), Klebahn (9), Thiel and Weiss (18), and Lambert (10) made experiments to determine whether temperature affected the maturation of the spores, but all concluded that this factor alone exerted only a minor influence. Likewise, Klebahn (9) and Lambert (10) concluded that moisture alone had very little influence on the maturation of teliospores. Lambert (10) also found that light had very little effect. Waters (20) has shown that the formation of telia may be hastened by placing plants infected with *Uromyces appendiculatus*, *Puccinia sorghi*, *P. suaveolens*, *U. trifolii*, or *U. polygoni* in the dark. In general, it may be said that the teliospores formed in the fall usually do not germinate until the following spring or the season corresponding to it.

Numerous attempts have been made to break the period of dormancy of the spores, but most of them have failed. Thiel and Weiss (18) found in the winter of 1920-21 that teliospores treated with citric acid germinated readily, while nontreated spores from the same lot did not germinate. Similar attempts since that time, however, have been unsuccessful. Lambert (10) tried to stimulate germination by using several chemicals, but found none effective.

After the maturation of the teliospores, germination may be influenced by moisture and temperature and probably less by light and hydrogen-ion concentration. Melhus et al. (13) found that "at a humidity of 95.6 per cent, only a slight production of sporidia was obtained," and "the most prolific sporidia formation occurred in a saturated atmosphere."

Dietel (5) thought the minimum temperature for the germination of *Puccinia graminis* teliospores to be 9.5° C., the maximum 23°, and the optimum 22°. Melhus et al. (13, p. 290) state: "* * * sporidia formation may occur only between 5° and 25° C. and seems most profuse at 20° C. * * *" Lambert (10, p. 11) states: "* * * the spores germinated well at 12° to 18° C. but they germinated most consistently at 18° C."

Lambert (10, p. 11-12) found "no consistent differences" between germination of spores in diffuse sunlight and in darkness. He further states that "some collections of teliospores will germinate in a fairly wide range of H-ion concentration," and that "different collections of teliospores differ considerably with regard to the time required for germination."

When teliospores of the *tritici* and *secalis* varieties were used to inoculate barberries for 2 days, then dried for periods of 1 to 10 days before being used to inoculate other barberries, the infection produced by both varieties was practically the same in both trials.

With regard to the longevity of mature teliospores, Lambert (10, p. 13) says: "It has been observed repeatedly that under natural conditions they usually are comparatively short-lived." Regarding the best method of storing telia to retain their viability, he (10, p. 13, 15) states: "It will be noted that material kept dry and at the lowest temperatures remained viable for the longest time." He mentions that telial material of *Agropyron repens* was placed in storage at approximately 2° C. and that "Cotter continued his inoculations with the spores * * * and was able to obtain infection until spring." This material was left in cold storage during the summer.

On August 31, 1926, *Berberis lycium*, *B. pruinosa*, *B. vulgaris*, and *B. (Mahonia) repens* were inoculated with the material mentioned.

A very light infection occurred on *B. vulgaris*. Another series, inoculated the same day with the same material, consisted of *B. aggregata prattii*, *B. brachypoda*, *B. leichlinii*, and *B. vulgaris* used as a check. On *B. leichlinii* a moderate infection of pycnia appeared September 13, and aecia were noted on September 27. A very light infection, apparently monosporidial, appeared on *B. vulgaris* September 14. No aecia appeared. From these data it seems that telia may retain their viability for a year and a half when kept in a dry place at a temperature slightly above the freezing point. These telia were formed in the fall of 1924 and produced some infection the latter part of August, 1926.

In the spring of 1926 teliospores on *Agropyron repens* (L.) Beauv. were collected near Red Wing, Goodhue County, Minn. These telia were used in the inoculations made in 1926 and 1927. The last inoculations with this material were made in May, 1927. On May 12 *Berberis arvensis*, *B. heteropoda*, *B. lycium*, and *B. notabilis* were inoculated with teliospores from this source. A moderate pycnial infection appeared on *B. arvensis* May 21, and aecia appeared on June 6. A light pycnial infection appeared on *B. lycium* May 20, no aecia being observed. A moderate infection of pycnia appeared on *B. notabilis* May 20 and aecia appeared on June 1. On May 16 *B. alessuthensis*, *B. aristata*, *B. haematocarpa*, *B. swaseyi*, and *B. vulgaris* were inoculated with this same material. Light infection occurred on *B. alessuthensis* and *B. haematocarpa* and moderate infection on *B. swaseyi* and *B. vulgaris*. Aecia developed on *B. vulgaris* 12 days later. Inasmuch as these teliospores were formed in the fall of 1925 and caused infection in the spring of 1927, it appears that spores of this collection were still germinating well a year and a half after the formation of the spores and a year after having been placed in the cold-storage room. In this connection, Eriksson (6) showed in 1898 that teliospores from wheat stored for two years in a dry laboratory germinated after having been kept in the open during the winter.

In the upper Mississippi Valley teliospores under outdoor conditions usually begin to germinate in April or May, and the question arises how long they will remain viable under such conditions. Under outdoor conditions, Melhus et al. (13) obtained plentiful germination in April and May, but spores left out of doors would not germinate in June or July. Lambert (10) found similar conditions at St. Paul in 1923 and 1924. The teliospores from a collection of telia on wheat collected at Black Earth, Wis., and placed out of doors at St. Paul by the writer germinated well on February 23, 1926. The teliospores from this collection did not germinate March 9, 1926, or later. Teliospores on *Agropyron repens*, left out of doors at St. Paul in the winter of 1926, germinated fairly well in March but very indifferently two weeks later. The teliospores from *A. repens* left out of doors at St. Paul in the fall of 1928 germinated weakly on March 26, 1929, but not at all on April 10. As snow or rain had fallen after the first inoculation in each of the cases cited, the teliospores probably had germinated before the second inoculation was made. In these cases the teliospores had been germinating before and after the first inoculation; so when the second inoculation was made, all of the teliospores had germinated. Teliospores left out of doors during the winter at St. Paul germinated before the middle of April in 1926, 1927, and 1928.

Infection may not occur even though the teliospores have germinated and the sporidia have lodged upon the barberry leaf. In nature the conditions at the time the barberry leaves are unfolding are extremely variable. Rains may be intermittent, so that the film of moisture necessary to the life of the sporidium may disappear before the germ tubes can penetrate the leaf; the temperature may drop so low that growth of the germ tubes is very slow, while the cuticle and epidermis of the leaf is thickening to such an extent that the germ tube can not penetrate it; or the teliospores may germinate before the barberry leaves appear. A study of the conditions under which infection of barberry leaves may occur is desirable to clear up the present state of uncertainty as to the influence of moisture, temperature, light, age of leaves, and the necessary incubation period on the infection of barberries. To this end several experiments were made.

RELATION OF AGE AND FREEZING OF TELIOSPORES TO INFECTION OF BARBERRIES

The writer observed that from teliospores collected in the spring of 1925, 1926, 1927, and 1928 a much heavier infection of barberries resulted than was obtained from spores of the same collection a year later. On three occasions telia were tested a year and a half after collection, and the germination and resultant infection were very weak, even when the spores had been kept in a cold, dry place. If teliospores are not kept at a low temperature (about 0° C.) and dry, they lose their viability rapidly. Occasionally they lost all power to produce sporidia within two weeks from the time germination was first noticed in the collection.

Freezing viable telia of the *tritici* and *secalis* varieties at a temperature of -5° C. for two days did not appreciably lower the germination of the teliospores below that of the check, as determined by the amount of infection produced on barberries.

RELATION OF TEMPERATURE TO INFECTION OF BARBERRIES

As temperature fluctuations at the time of the appearance of the first barberry leaves usually are very great, it is of the highest importance to determine the effect of temperature on the infection of barberries. Experiments therefore were made to determine the importance of this factor.

Lambert (10, p. 19) found that "the greatest amount of infection occurred at 17° to 18° C." He states:

This experiment indicates that the combined process of teliospore germination, sporidia formation, liberation, and germination, the infection of barberry leaves, and the development of aecial cups are retarded or prevented by temperatures higher than 22° C.

To test this point, the writer repeated the experiment, using slightly different temperatures. The teliospores used as inoculum were scraped from the straw and placed upon the surface of distilled water in Syracuse dishes, which were then placed at temperatures of 10°, 14°, 21°, and 26° C., one dish of teliospores from wheat and one from *Agropyron repens* being placed at each temperature. At the end of two days many spores presumably had germinated and produced sporidia, for infection was readily secured with spores so treated in previous experiments. Only susceptible varieties of Berberis, principally *B. vulgaris*, were used, except as otherwise noted. Before inoculation the barberry leaf was wetted and the bloom removed,

after which a drop of the telial suspension was placed on the young leaf by means of a loop needle inserted below the surface of the liquid and then lifted so as to carry with it the teliospores and their sporidia almost undisturbed. After the plants had been thoroughly wetted with water, they were placed in containers in a temperature tank for two days. The plants were sprayed with water at intervals of about three hours during the day for two days in order to maintain the film of moisture around the spores. At the lower temperatures it was not difficult to maintain this film of moisture, but at 24° to 26° C. and 31° to 35° it was more difficult. Notes were taken on the first appearance of infection, as shown by the appearance of pycnia, on the first appearance of aecia, and on the degree of infection. The results are given in Table 4.

TABLE 4.—Effect of temperature on rate and degree of development of rust infection on *Berberis vulgaris* inoculated with teliospores of *Puccinia graminis tritici* and *P. graminis secalis* and incubated in a continuously saturated atmosphere

Telial host	Temperature for spore germination	Number of days required for appearance of pycnia and aecia and degree of infection ¹ produced at temperatures of—											
		8°–10° C.		12°–14° C.		16°–18° C.		19°–21° C.		24°–26° C.		31°–35° C.	
		Pycnia	Aecia	Pycnia	Aecia	Pycnia	Aecia	Pycnia	Aecia	Pycnia	Aecia	Pycnia	Aecia
	° C.												
Agropyron repens...	9–10	9+	23+			8+	21+	6++	21++	9+	23+		
	12–14			8+	25+			8++	7++	14+	0	8+	0
	19–21	21+	0			9+	21+	9+	25+	21+	27+		
	21–26	21++	27++	12+	28+	21+	0	9+	20+	8+	25+		
Wheat.....	12–14							8++	7++				
	21–26			8++	24++			8+	0	8+	28+		

¹ The degree of infection is indicated by the following symbols: ++, Heavy; +, moderate; +—, light; +—, very light; 0, none; —, no trial.

It will be seen from Table 4 that only one very light infection resulted at 31° to 35° C.; hence it may be concluded that the combined process of teliospore germination, formation, liberation, and germination of sporidia, and barberry infection was greatly inhibited, if not prevented, by temperatures higher than 26° C. The optimum temperature seems to be 19° to 21°. Lambert (10) thinks the optimum for barberry infection is 17° to 18° and that infection does not take place readily above 22°, while Melhus et al. (13) state that the maximum for formation of sporidia is 25° and that a saturated atmosphere is necessary. This, however, is difficult to maintain at temperatures of 24° to 26° and 31° to 35°.

After inoculation the barberries listed in Table 4 were held two days at the same temperature at which the teliospores germinated. Then four of the five plants in each series were changed to the incubating temperatures, where they remained until notes were taken on all the plants. However, in this experiment one factor has not been eliminated, namely, the possibility that the teliospores incubated at the germinating temperature might not have germinated at that temperature, but might have germinated when the inoculated plant was transferred to the incubating temperature. To remedy this defect further inoculations of *Berberis vulgaris* were made.

Teliospores from *Agropyron repens* from Rochester, Minn., were placed in distilled water and incubated two days at each of five temperatures. Five plants of *Berberis vulgaris* were inoculated with the spores incubated at each temperature, and these plants were held at the same temperature for two days, during which they were sprayed with water from an atomizer every three hours during the day. At the expiration of this period four of the five pots held at each temperature were shifted, one to each of the other four temperatures, where they were left until the notes had all been taken. When shifting the four pots the plants were thoroughly dried before being placed at the different temperatures. This procedure obviates the possibility of infection occurring at a temperature other than the original spore-germination temperature. The results of the inoculations are given in Table 5.

TABLE 5.—Influence of temperature on the rate and degree of development of pycnial infection on *Berberis vulgaris* inoculated with teliospores of *Puccinia graminis secalis* and incubated two days in a saturated atmosphere, dried, and replaced in the chambers

Temperature (° C.) for spore germination	Number of days required for appearance of pycnia and degree of infection ¹ produced at temperatures (° C.) of—				
	7°-9° C.	15°-18° C.	19°-21° C.	24°-26° C.	31°-35° C.
7-8	15+-	11+-	8++	8+	8+
8-9	10+-	7++	5+	6++	7+-
15-16	0	0	7+	7+-	6+
16-18	8+++	6+	4++	0	0
19-20	8+-	4++	4++	4++	0
20-21	13+	7+	7+	8++	0
24-26	11+	0	0	11+-	0
31-33	0	0	0	0	0
33-35	0	0	0	0	0

¹ +++, Very heavy; ++, heavy; +, moderate; +-, light; +--, very light; 0, none; -----, no trial.

The results given in Table 5 are a little more clear-cut than those in Table 4. The data indicate that the processes of germination of teliospores, formation, liberation, and germination of sporidia, and penetration of barberries do not take place freely at temperatures of 24° to 26° C. and 31° to 35°, as no infection was secured from the teliospores incubated at these two temperatures. The smallest amount of infection resulted from the teliospores incubated at 7° to 9°, while moderate infection developed at the three lower temperatures, from the telia germinated at 15° to 18° and 19° to 21°. It is noteworthy that infection appears first at the higher temperatures and last at 7° to 9°, the optimum temperature being 19° to 21°.

The evidence presented seems to show that teliospores of *Puccinia graminis* rarely if ever germinate and produce and liberate sporidia at temperatures higher than 26° C. This is in accordance with the results of Melhus et al. (13), who found 25° the maximum for production of sporidia, and Lambert (10), who found that there was light infection at 22° to 23° and little or none at 26°.

INFLUENCE OF LIGHT ON INFECTION OF BARBERIES

Next to temperature and moisture, light is perhaps one of the most important factors in the development of rusts. Therefore, the effect

of light and darkness on infection of barberries was also investigated.

Lambert (10, p. 22) found that—

Different amounts of light during the period of incubation apparently had no effect on the percentage of leaves infected, but the number of infected petioles was markedly greater on the plants kept in total darkness than on those exposed to constant intense light.

Heavily rusted straw was supported on wire screens above *Berberis vulgaris* and wetted twice daily. By this method heavy infection usually is secured. After removal from the incubators, one of the plants was kept in constant light under a 500-watt light suspended 4 feet above the table, one was placed on the greenhouse bench where it was exposed to alternate light and darkness, and the third was kept in a dark room at a temperature of about 10° C. until sufficient time for the appearance of pycnia had elapsed. The results are given in Table 6.

TABLE 6.—Influence of light on production of pycnia on *Berberis vulgaris* inoculated with teliospores of *Puccinia graminis secalis* in the greenhouse

Date of inoculation	Number of days required for appearance of pycnia and aecia under indicated light conditions after removal from moist chambers, and degree of infection ¹ produced					
	Alternate light and darkness (diurnal)		Constant light		Constant darkness	
	Pycnia	Aecia	Pycnia	Aecia	Pycnia	Aecia
Jan. 12.....	8 +++	18 ++	10 +--	18 ++	0	0
	11 +++	++	8 ++	18 ++	11 +++	+++
Jan. 19.....	9 +	+	0	0	0	0
	8 +	23 +	9 +	+	14 +	+

¹ +++, Very heavy; ++, heavy; +, moderate; +--, light; +--, very light; 0, none.

No conclusions can be drawn from the data in Table 6, because the plants kept in total darkness were held at a temperature of 10° C., while the plants in constant light and alternate light and darkness were kept at a temperature of 20°, so the results are not comparable. While the cold temperature would tend to retard the appearance of infection whether in total darkness or in constant light, the fact that infection did appear as soon on the plants inoculated January 12 and kept in total darkness as on the plants subjected to alternate light and darkness would tend to discredit the idea that light alone has any decided effect on the occurrence of pycnial infection. Later experiments carried out at a temperature of 10° indicated that the infection becomes apparent slightly earlier on plants kept in constant light during the processes of inoculation and incubation than on those inoculated and incubated in chambers kept constantly dark.

TIME REQUIRED FOR INFECTION OF *BERBERIS VULGARIS* WITH TELIOSPORES OF *PUCCINIA GRAMINIS SECALIS*

As far as the writer knows, no one actually has determined the time necessary for infection of barberries from the time the telia are first wetted until infection occurs.

The question is important because of the fact that in nature the teliospores germinate on straw, where they produce sporidia which

must be blown to barberry plants, where they germinate and produce germ tubes that penetrate the host. Environmental factors therefore must be favorable for a longer time to insure infection of barberries than in the case of infection of gramineous hosts with aeciospores and urediniospores. Just how long conditions must be favorable is not known.

To solve this problem, a suspension of teliospores in water was prepared and used immediately to inoculate *Berberis vulgaris* plants, which were at once placed in moist chambers where they were kept moist for the duration of the experiment.

No infection resulted when *Berberis vulgaris* plants inoculated with teliospore suspension from *Agropyron repens* were incubated for 17, 18, 24, 30, and 42 hours, respectively, in various series at greenhouse temperature. Other plants in these series became infected when incubated for 41, 48, 50, 66, 90, 113, and 119 hours, respectively, at greenhouse temperature. Development of pycnia was very light on the plants incubated 41 hours; the infection on the plants incubated 50 hours was only slightly heavier; and the infection resulting from longer incubation periods was moderate to heavy. The results indicate that a period of at least 66 hours was necessary for the germination of the teliospores, formation of the promycelia, production, liberation, and germination of the sporidia, and subsequent infection. The severity of infection was but little greater when the incubation period was 72 hours or longer. This, then, means that barberries probably become heavily infected in nature only when favorable weather conditions prevail more or less continuously for two or three days.

To determine the minimum length of time necessary for susceptible barberries to become infected under the most favorable conditions, teliospores from *Agropyron repens* collected near Rochester, Minn., were soaked two days in water and used as inoculum. The incubation chamber was a tall metal can covered by a pane of glass and with about an inch of water in the bottom to supply the necessary humidity. The potted barberry plants were placed in the chamber, wooden sticks were inserted into the soil in the pots, and a coarse $\frac{1}{2}$ -inch mesh wire screen was placed on top of the wooden sticks. The telial material was placed upon this wire screen so that the sporidia fell in a shower over the plants below. Twice a day this material was wetted with water. Four barberry plants were designated as a series in each chamber, and certain plants of each series were removed at definite times. Plants exposed to the sporidial shower $1\frac{1}{2}$, 2, 3, 8, and 24 hours did not become infected; but plants in the same series exposed 21, 29, 33, 45, 46, 47, 48, 55, 69, and 71 hours became infected. The infections were light on the plants exposed 21 and 29 hours, moderate on those exposed 33 hours, and heavy on those exposed 45 hours. It was to be expected that plants exposed for longer periods to the sporidial shower would be heavily infected, and such was the case.

In these experiments the minimum time required for liberation and germination of sporidia and barberry infection was 21 hours. However, the limited available data and the wide gaps in time of exposure in the various groups made further information desirable. This experiment also raised the question as to how long the telial material would have continued active in supplying inoculum if the

experiment had been continued. Another point deemed worthy of investigation was the length of time that must elapse before the teliospores kept in a moist atmosphere discharge the maximum number of sporidia.

Telia on quack grass from Rochester, Minn., were soaked in tap water two days prior to being used as a source of inoculum. Five pots of *Berberis vulgaris* were exposed to the sporidial shower from this material. One pot was removed after exposure for 28 hours, and developed a light infection; the other four were exposed 45 hours and developed light to moderate pycnial infections.

Four plants of *Berberis vulgaris* were exposed to the telia used in the foregoing series. One plant, subjected 6 hours, did not become infected, while a second plant, exposed 24½ hours, became heavily infected. The other two plants, exposed 27 hours, also became heavily infected.

The same rusted straw was used again as a source of inoculum for a third series of *Berberis vulgaris*. All four of the plants were exposed to the sporidial shower 45 hours and eventually developed a moderate to heavy pycnial infection. The results show that sporidia were being liberated 116 hours from the time straw was first wetted and 50 hours from the time the first infected barberry was removed from the incubation chamber.

As a check on the foregoing series, and in order to determine whether collections of telia on *Agropyron repens* would cause similar reaction, telia from Hennepin County, Minn., were similarly soaked for two days before they were employed as a source of inoculum.

As in the first series of the previous experiment, one of the plants was exposed to the supposed sporidial shower 28 hours and the other three were exposed 45 hours, but no infection resulted. The results show that each teliospore collection is a law unto itself. These two collections were gathered about the same time from localities similar in climate and not very widely separated. Barberry infections were obtained from the Rochester collection 96 hours after the telia had been soaked in water; from the Hennepin collection barberry infection developed after the rusted straw had been soaked 117 hours. The known time of spore discharge of the Hennepin teliospores is 46 hours and of the Rochester material 88 hours. The Rochester material germinated first and over the longer period of time. The telial material was used again for a supply of inoculum, and some infection resulted. A plant exposed 6 hours did not show any signs of infection, but plants exposed 24 hours became lightly infected, as did two plants exposed 27 hours. In the third series with this telial material, four *Berberis vulgaris* plants, exposed 44 hours to the sporidial shower, became lightly or moderately infected.

It is evident that for some reason germination of the teliospores in the Hennepin collection did not occur so readily as in the Rochester collection, although both collections were gathered within a few days of each other from localities 80 miles apart. The first infection recorded from the use of the Hennepin material was on a plant exposed 24 hours in the second series and 117 hours after the material was first wetted. The first recorded infection from the use of the Rochester material was on a plant exposed to the shower of sporidia for 28 hours after the telia had been soaked in water 48 hours. The known time of spore discharge by the Hennepin teliospores covered

a period of at least 46 hours, as shown by the infections that occurred on the barberries exposed to the sporidial shower during that time. The known time within which the Rochester teliospores produced sporidia is 88 hours, as judged by the infections produced on barberries removed from the incubation chamber during that time.

In a third experiment rusted quack grass from an unknown source was similarly soaked for two days before being used to inoculate barberries. The material was divided into two parts, the second series of barberry plants being inoculated six hours later than the first, with telia soaked six hours longer.

In the first series, plants exposed 5½ and 8½ hours did not become infected, while plants exposed 26 and 33 hours became heavily infected. In the second series, infection occurred on all the plants, even though they had been exposed to the sporidial shower only 7, 23, 26, and 30 hours, respectively, the infection ranging from light on the plant exposed 7 hours to very heavy on the plant exposed 30 hours.

In the second half of the experiment three plants exposed 1, 2, and 3 hours, respectively, did not become infected. The fourth plant, exposed 20 hours, became heavily infected. Plants of the second series exposed to this telial material were all heavily infected after 15, 18, 24, and 39 hours.

The teliospores from the two series were combined to furnish inoculum for a series of plants of *Berberis vulgaris*. Infection occurred on all members of the series after exposures of 5, 12, 24, and 49 hours.

A third series exposed to this combined telial material became infected after an exposure of 48 hours. Two plants of *Berberis vulgaris* were subsequently inoculated with this material. One light infection was noted on one of the two plants after an exposure of 23 hours. When two plants of *B. vulgaris* and one of *B. canadensis* were inoculated with the same material after the removal of the previously inoculated plants, light infection occurred on the *B. canadensis* plant and on one of the *B. vulgaris* plants after an exposure of 25 hours. This telial material had first been immersed in tap water at noon February 9. The first infection obtained was on a plant exposed to a shower of sporidia for 26 hours and removed 72 hours from the time the teliospores were first wetted. The last infection obtained was on two plants removed from the incubation chamber after exposure to a sporidial shower for 25 hours produced by telial material wetted in tap water for 264 hours, or 11 days.

From these results it appears that barberry plants may be infected by telial material that has been continuously wet for 11 days. Such a continuous discharge of sporidia means that under natural conditions, during long periods of rain, the barberries in the vicinity of heavily rusted grains or grasses are exposed to a constant shower of sporidia, so that infection is almost certain to occur on the barberry plants. If there are periods of alternate rainfall and dry, warm weather successive infections on barberry may result. If barberry leaves are not unfolded when the first teliospores germinate, little or no infection occurs; but as the successive periods of germination of teliospores occur, some and possibly all of the barberry leaves are very likely to become infected.

To complete the data, another double series of plants was exposed to teliospores from quack grass from an unknown source. To gain some idea of the time when the first sporidia were formed, half of the telial material was used as inoculum two hours after it had been placed in tap water, the other half being used three days later, because in one of the preceding experiments it seemed that the teliospores did not germinate in the tap water in which they were soaked but did germinate shortly after being placed on the screens above the plants, where they had access to plenty of oxygen and moisture.

In the first series, in which the telia had been presoaked two hours before being used as inoculum, *Berberis vulgaris* plants exposed to possible infection by sporidia for periods of 21 and 29 hours, respectively, became lightly infected, while those exposed for 45 and 70 hours, respectively, became heavily infected. In a series inoculated with the other half of this material, three *B. vulgaris* plants exposed for 1, 2, and 3 hours, respectively, did not become infected, whereas the fourth plant, exposed 47 hours, became heavily infected. In a third series, exposed to telia from the first series, plants exposed 9 and 24 hours did not become infected. The other plants, exposed 33 and 48 hours, respectively, became moderately infected.

From the foregoing it is evident that the 2-day presoaking was not necessary for the germination of the teliospores. The first plant removed from the incubation series, 21 hours after inoculation and 23 hours after the water was put on the telia, was infected. While the degree of infection was light, further experimentation might have revealed that infection may take place in a still shorter time. From the facts here given, however, one can say that the processes of germination of teliospores, and formation, liberation, and germination of sporidia, with subsequent penetration of the barberry leaf, can, under favorable conditions, take place in a minimum time of 23 hours.

The other half of this telial material, presoaked three days before using, failed to produce infection on a *Berberis vulgaris* plant exposed to it 3 hours, but did produce infection on plants exposed 46, 55, and 71 hours, respectively. The degree of infection was, however, very light. In a second series inoculated with this telial material, no infection was secured after exposures of 3, 5, and 23 hours. Thus, it appears that presoaking the telia for two days promotes germination, and but little infection usually results if the material is kept moist longer than two days. The writer believes that soaking the telial material for two to six hours before using it for inoculation will provide for the least loss of sporidia with the maximum number of germinated teliospores. Furthermore, short periods of rain, followed by conditions unfavorable for dissemination of sporidia and infection of barberry plants, would result in considerable loss of potential infective power, as far as the pathogene is concerned. It will be recalled that in the previous experiment the teliospores germinated over a period of 11 days when suspended on a wire screen in a moist chamber. In this experiment it appears that nearly all the teliospores germinated in three days when taken from the same collection, kept under the same conditions, and soaked three days in tap water.

Under natural conditions most of the teliospores from low places, such as swamps, may germinate at about the same time, while those on grains or grasses in higher, well-aerated places may germinate at different times.

SUSCEPTIBILITY OF BARBERRY LEAVES OF VARIOUS AGES AND SPECIES

It is known that the age of barberry leaves at the time of inoculation is a factor in infection with *Puccinia graminis*. Melhus et al. (13) state:

Repeated attempts in the greenhouse and field under varied moisture and temperature conditions failed to produce infection on the more mature leaves * * *. The marked difference in the susceptibility of young and old leaves of the barberry may be due to the thickness of cuticle and epidermis, considering that infection is accomplished by penetration of the tissues and not through invasion of stomata.

Melander and Craigie (12) think that the leaves of susceptible species become virtually immune with age.

The leaves of the barberry plant can unfold and grow under conditions that do not permit infection. If, then, only the very young leaves are susceptible, many of them would escape infection if weather conditions were not favorable during the period of their immaturity. If, on the other hand, the leaves remain susceptible for a considerable period, the chances of infection would be much greater.

To obtain evidence on this point, rapidly growing plants of *Berberis vulgaris* were examined daily for 11 days, and to each new leaf a tag was attached bearing the date the leaf unfolded. On the eleventh day, eight of these barberry plants were inoculated with sporidia of *Puccinia graminis secalis*. Pycnia appeared five days later. Notes were taken on the number of leaves infected on the several dates, the age of the leaves, and the degree of infection produced on each leaf each day. Some of the results are given in Table 7.

TABLE 7.—Influence of age of barberry leaves on development of rust in the greenhouse

Age of leaves (days)	Berberis vulgaris										Berberis aetnensis							
	Number of leaves inoculated	Number of leaves infected at indicated number of days after inoculation					Number of leaves inoculated	Number of leaves infected at indicated number of days after inoculation				Number of leaves inoculated	Number of leaves infected at indicated number of days after inoculation					
		7	8	9	10	15		5	8	10	12		7	8	9	10	15	
1.....	3		2	3	3	3	7	1	6	6	7	2	2	2	2	2	2	2
2.....	4	4	4	4	4	4	3	1	3	3	3	5	5	5	5	5	5	5
3.....	17	8	0	13	14	17												
4.....	6	2	2	3	6	6	1	1	1	1	1	11	5	6	8	10	11	
5.....	2		1	1	1	1	1		1	1	1							
6.....	0	1	3	4	4	6	5		2	6	5							
7.....	13	5	6	13	13	13	1		1	1	1	2	1	1	2	2	2	2
8.....	3	1	1	2	2	3	4			3	4	8	1	6	6	7	8	8
9.....	0		1	2	2	3						3	2	2	3	3	3	3
10.....	14	1	3	8	10	12						9	1	2	5	8	9	9
11.....	0		2	5	6	9	2				2	1		1	1	1	1	1
12.....	14			8	10	14	4		2	4	4	9		4	5	7		
13.....							4			2	2							
14.....							7			2	2							
15.....							7			2	3							
16.....							5				1							
Total.....	100	22	34	66	75	91	51	3	16	30	36	60	17	28	37	46	60	

The data given in Table 7 show that infection appears first on the younger leaves and two or three days later on the older leaves. This probably is due to the difficulty the fungus experiences in puncturing

the cuticle of the older leaves and perhaps in penetrating the tissues of the older leaves before ramifying inside the tissues. Since heavy infection was noted on leaves 14 and 15 days old, apparently the age of the leaf is not a factor in determining the resistance or susceptibility of any given species, provided the fungus is able to penetrate the leaf, the infection being just as heavy on leaves inoculated when 15 days old as on those inoculated when 1 day old.

It is evident from Table 7 that leaves of *Berberis vulgaris* may be infected by *Puccinia graminis* as late as 16 days after they first appear. As many of the tags showing the age of the leaves were dislodged during the inoculations, the ages given in Table 7 are not so great as they otherwise might have been; but since leaves that apparently were at least 2 days older than the tagged 16-day leaves were moderately or heavily infected, it is thought that if none of the tags had been dislodged leaves still older than 16 days might have proved susceptible.

Undoubtedly, insolation exerts some indirect influence on the ease with which the fungus can penetrate barberry leaves. In the experiments, the results of which are given in Table 7, 9 of the 17 days during which barberry leaves were being tagged were cloudy, while the sun shone brightly on 8 days. The forenoons of 2 days were cloudy and the afternoons were sunny. These days were included in the totals as 1 cloudy and 1 sunny day. Had all the days covered in this experiment been sunny, it is quite possible that infection would not have occurred on common barberry leaves 16 days old, as Melander and Craigie (12) found that leaves of *Berberis vulgaris* 10 days old were somewhat resistant. There was no correlation between the degree of infection and the presence or absence of sunshine on the day the leaves appeared, as leaves of the same date showed the extremes of infection, from very light to very heavy.

From Table 7 it is clear that the total numbers of leaves of *Berberis vulgaris* inoculated and infected are the same, with one exception, up to and including the eighth day, whereas in *B. aetnensis* the total number of leaves inoculated and infected correspond up to and including the twelfth day. With *B. vulgaris* it was possible for every tagged leaf to become infected up to the twelfth day. All the 12-day leaves tagged on that day became infected, but not all the 9-day and 10-day leaves were infected. Infection became more uncertain, however, on leaves 13 to 16 days old, and on the sixteenth day only one of the five inoculated leaves became infected, and then but very lightly. In the case of *B. vulgaris* there seems to be a gradual hardening off of the leaves after the eighth day, although there is no evidence that this occurs in leaves of *B. aetnensis*. The results support the conclusions of Melander and Craigie (12, p. 99), who state: "Leaves of *B. vulgaris* 10 days old are fairly resistant to *P. graminis*. This would indicate a close correlation between resistance and the thickness of the outer epidermal wall." But the results also show that leaves remain at least moderately susceptible for 10 days to two weeks, especially if weather conditions are such as to prevent them from hardening. This fact, of course, is favorable for the pathogene during the spring.

Observations were made to determine those parts of the common barberry that are most susceptible to infection by *Puccinia graminis*. Data were gathered on a number of infected plants. The degree of



Leaves of *Herbaris Hietolia* infected with *Puccinia graminis aenolia*. Note the profuse growth of aecia on the petioles, resulting in the death of the leaves



Barberry vulgaris infected by *Puccinia graminis scutis*

infection was noted on the leaves, stems, petioles, spines, leaf spines, and growing points of the stems. Field observations were made on infection of the flowers of *Berberis vulgaris*. The greenhouse data are given in Table 8.

TABLE 8.—Susceptibility, in the greenhouse, of various plant parts of *Berberis* to black stem rust¹

Infection courts	Number of replications according to degree of infection indicated					
	B. vulgaris normals			B. vulgaris atropurpurea		
	Moderate	Heavy	Very heavy	Moderate	Heavy	Very heavy
Foliage:						
Petioles.....	3	6	7			2
Leaves.....	3	5	13		1	2
Serrates.....	10	1		3		
Stem:						
Sprigs.....	1	1	7			
Spines.....	2		4			
Apices.....	1	1	1			

¹ Extremely heavy infection was once noted also on the nodes of *B. vulgaris normals*.

The data in Table 8 show that any young or tender tissue on any part of the plant aboveground may be infected. Infection is more readily noticeable on the leaves than on any other part of the plant because of the much greater surface exposed to infection in comparison with that on the other parts of the plant. If infection occurs, however, other parts of the plant are just as susceptible as the leaves. Even the flowers may be infected. Common barberry collected at Rochester and Albert Lea, Minn., in 1928, was heavily rusted on leaves and sepals, with light aecial infection on peduncles and petals. Apparently there was also an infection on one of the stamens in one of the flowers. When *Berberis gilgiana* was artificially inoculated when blooming, infection developed on one of the petals as well as on the leaves.

In the early spring, barberry plants are subjected to a wide range of weather conditions. The frequent spring rains are often accompanied by lower temperatures, and this combination is especially favorable for teliospore germination and barberry infection.

Berberis vulgaris is generally supposed to be the species of barberry most susceptible to the attack of *Puccinia graminis*. In the writer's experiments, however, other species have become more heavily rusted and have been injured to a greater degree than *B. vulgaris*. Two such are *B. ilicifolia* Forst. and a strain of *B. canadensis* Mill. found in one section of Illinois. Frequently the entire leaf area of this *B. canadensis* strain became infected, with subsequent chlorosis and death of the leaves. Leaves of *B. ilicifolia* were frequently killed outright when inoculated in the same series with *B. vulgaris*, while on the leaves of *B. vulgaris* the infected areas were not continuous nor did any leaves die as a result of the attack of the fungus. There was little or no infection on the petioles of *B. vulgaris*, but there were very numerous aecia on *B. ilicifolia* three weeks after inoculation. The type of infection on *B. ilicifolia* is shown in Plate 1, and an un-

usually heavy infection on stems of *B. vulgaris* is shown in Plate 2. In Plate 3 is shown an infected whorl of leaves of *B. vulgaris*. It is evident from Plates 2 and 3 that the leaves of *B. vulgaris* are not killed as are those of *B. ilicifolia*. Even leaves having the exceptionally heavy infection shown in Plate 4 may live for weeks.

Of those species tested, *Berberis asiatica* Roxb. seems to be one of the most resistant. Leaves of *B. asiatica* are illustrated in Plate 5. In this case very small pycnial infections appeared at the same time as those on *B. vulgaris* and within 10 days became dry and brown. No aecia formed, whereas on *B. vulgaris* abundant aecia appeared. (Pl. 2.) Apparently the leaves of *B. asiatica* toughen rapidly and prevent the rust from spreading in the tissues.

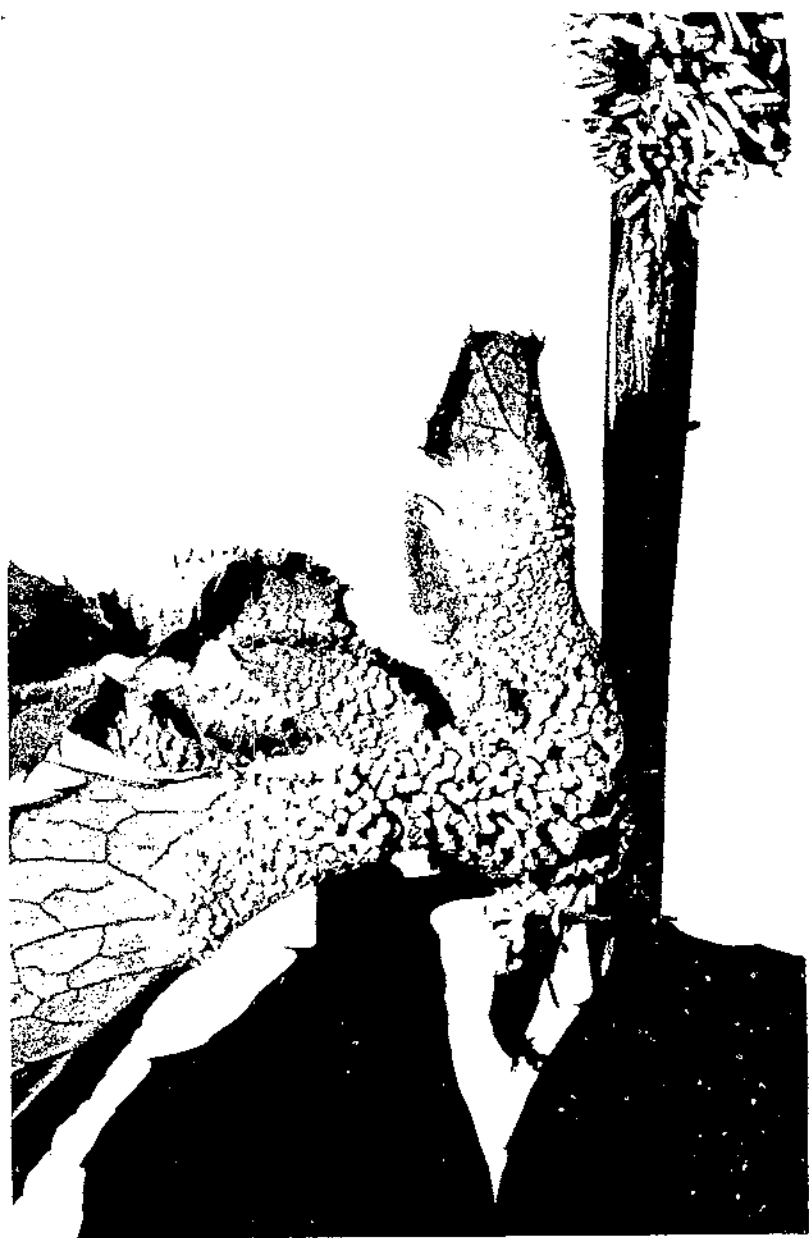
EFFECT OF LENGTH OF INCUBATION PERIOD ON INFECTION OF BARBERRIES

If warm days follow the penetration of the sporidial germ tubes, the appearance of pycnia is accelerated, according to the data resulting from artificial inoculations. In the greenhouse the incubation period of the rust in the barberry is much shorter at temperatures of 20° to 30° C. than at temperatures below 10°. At the higher temperatures, 24° to 26°, pycnia appeared 3 to 10 days after inoculation, depending on the amount of consequent infection. Pycnia appeared on the third day upon the plant with the most infections, and on the tenth day upon one of the plants with the least infection. It was found that the first infection appeared on the most heavily infected plants and the last infection appeared on the very lightly infected ones. This would indicate that the amount of inoculum determines to some extent the rapidity with which infection becomes evident, as a leaf infected by a large number of sporidia produced pycnia before other leaves on the same plant that were inoculated with a few sporidia. During the last five years the writer has found that the pycnia first appear on the fifth to the tenth day following inoculations when the plants are kept under ordinary conditions in the greenhouse. Light alone seems to have no influence on the length of the incubation period of the fungus.

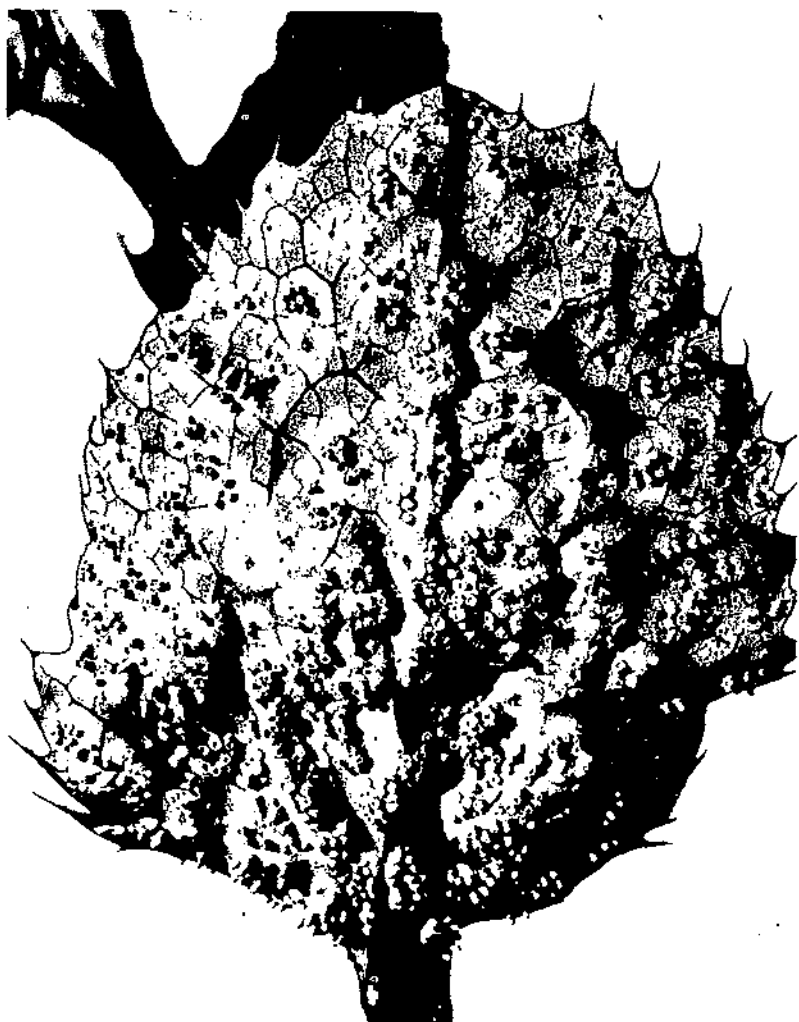
Wetting the barberry leaves and removing the "bloom" from them do not favor their infection. In a few of the writer's experiments those plants that did not have the leaves wetted nor the "bloom" removed were those that became most heavily infected. If more series had been inoculated the results might have been exactly the opposite of those already obtained.

EFFECT OF TEMPERATURE AND MOISTURE ON DEVELOPMENT OF AECIA

Plants of *Berberis vulgaris* with a moderate to heavy development of pycnia on the leaves were placed in the temperature chambers and observations were made to determine the time required for aecia to appear and mature. The results are given in Table 9.



A whorl of leaves of *Herberis vulgaris* infected by *Puccinia graminis steudii*, showing the manner in which all the leaves in a whorl may be heavily infected. Part of an aecial cluster on the stem above. Note length of aecial cups. $\times 5$



Individual leaf of *Berberis vulgaris* infected by *Puccinia scutis*. This leaf had practically no green areas between the individual infections. $\times 5$

TABLE 9.—Effect of temperature on development of aecia and opening of aecial cups on *Berberis vulgaris* on which pycnia had been produced after inoculation with *Puccinia graminis tritici* and *P. graminis secalis* in the greenhouse

Experiment No.	Tallal host	Number of days required for appearance of aecia and for opening of aecial cups at temperatures of—									
		7°-8° C.		15°-16° C.		20°-21° C.		25°-26° C.		31°-32° C.	
		Ap- pear- ance	Open- ing	Ap- pear- ance	Open- ing	Ap- pear- ance	Open- ing	Ap- pear- ance	Open- ing	Ap- pear- ance	Open- ing
1.....	<i>Agropyron repens</i>										
2.....	(L.) Beauv.....	14	15	12	14	10	11	10	10	10	10
3.....	do.....	14	17	9	10	9	10	9	9	9	9
4.....	do.....	11	(1)	12	14	10	(1)	9	10	9	9
5.....	do.....	16	16	15	15	16	17	14	16	7	(1)
6.....	<i>Triticum vulgare</i>	13	15			10	11	8	(1)	9	(1)
	Vill. 1.....			14	14	10	11	10	(1)	10	10

¹ Cups not open at expiration of experiment.

² According to the rules of botanical nomenclature the name of this species is *T. aestivum* L., but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

The data show that temperature has a marked effect on the appearance of aecia after pycnia have developed. At 25° to 26° C. aecia developed at the same time as at 31° to 32° except in those series where the amount of infection on the plants was not the same. The lowest temperature (7° to 8°) had the most retarding effect on the development of aecia. Temperature alone had no influence on the number of aecia, for as many developed at 7° to 8° as at 31° to 32°. There was, however, a very noticeable difference in the length of the aecial cups. At the lowest temperature the aecial cups were two to three times as long as at the highest temperature. At the temperature of 31° to 32° the aecial cups opened very soon after their formation and before they had become much elongated. They were rarely 1 mm long. The aecial cups formed at 7° to 8° were 2 to 3 mm long before they opened.

From an empirical experiment it appears that the influence of moisture on the formation of aecia is irregular, because in two trials aecia formed on the plants in the wet chamber after the formation of aecia in the dry chambers, whereas in a third trial aecia formed on the plant in the wet chamber before they did on either the plant held under dry conditions or on the check.

EFFECT OF FREEZING ON RUSTED BARBERRY

Under natural conditions infected barberries frequently are subjected to wide extremes of temperature. The leaves often unfold and become infected during warm weather in the early spring and may be exposed later to freezing temperatures. Experiments therefore were made to determine the effect of low temperature on the rust in infected plants.

Plants of *Berberis vulgaris*, some bearing aecia of the *tritici* variety and some bearing those of the *secalis* variety, were exposed to freezing temperatures in low-temperature rooms. One plant was left in the greenhouse as a check, one was held at 0° C. for 11 days, one was held at 0° for 10 days and at -5° for 24 hours, and the fourth was held at

0° for 10 days, -5° for 24 hours, and -10° for 24 hours. The plants held at -5°, -10°, and -26° were placed at 0° for 24 hours after being frozen, in order to allow them to thaw out before being removed to the greenhouse.

After the removal of the plants from the 0° C. room, the aeciospores from each plant were used to inoculate a susceptible host. Little Club wheat was used for the *tritici* variety and Petkus rye for the *secalis* variety.

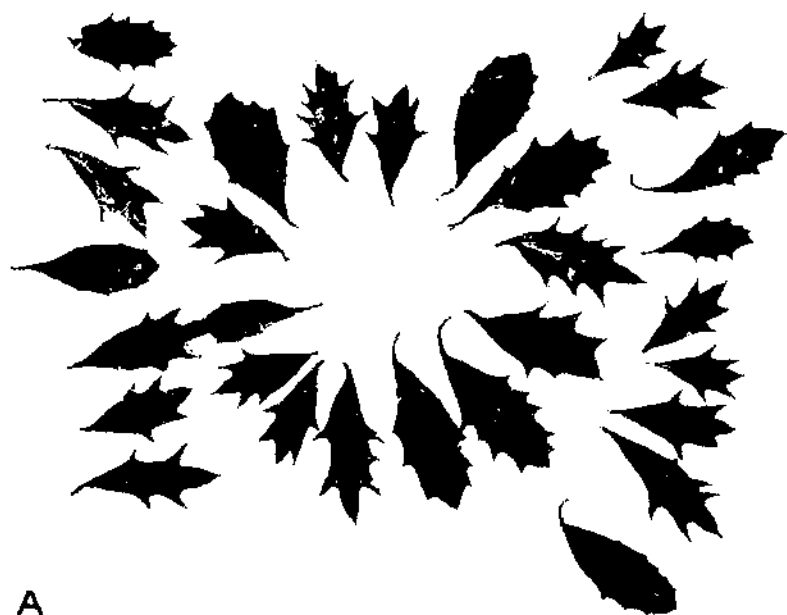
Only one uredinium resulted from the inoculations. This was on Petkus rye inoculated with aeciospores from the plant kept at 0° C. and only 1 plant out of 17 became infected. It is evident, however, that a temperature of 0° did not destroy the ability of the aeciospores to germinate. Craigie³ found that some aeciospores germinated after having been exposed 70 minutes to a temperature of -6°. As the aecia used in these experiments were from 51 to 67 days old when the plants were removed from the cold rooms, it is more than likely that many of the aeciospores had lost their viability even before they were frozen. As no other aecial material was available at the time, the experiment was not repeated.

A number of *Berberis vulgaris* plants bearing pycnia were likewise subjected to subzero temperatures. One plant was held at 0° C. for 5 days, another for 10 days, a third at -5° for 11 days, and two others were held at 0° for 10 days, -5° for 24 hours, and -26° for 20 hours.

In both the aecial and the pycnial experiments all the plant parts above ground were killed at -5°, -10°, and -26° C., although new leaves subsequently appeared on the plants frozen at -5°. At 0° some of the tender shoots were killed, but new leaves appeared later on the older stems. All the barberry leaves in the aecial series and all the young leaves in the pycnial series were killed at 0° except one of the leaves on the plant bearing pycnia, which subsequently produced a normal aecium. This aecium formed 12 days after the plant had been removed from the 0° room, while aecia developed on the check plant 6 days after the other plants had been removed from the freezing rooms. From this fact it would appear that the rust fungus can live at a temperature as low as the host can withstand, for the rust was able to complete its development when the barberry was placed under favorable conditions, even after having been held at a temperature that killed the younger leaves on the plant.

In a later experiment two series of four plants each of *Berberis vulgaris* were inoculated with sporidia at 16° to 18° C. and then placed in the 0° C. room. At the expiration of a week one of the plants was removed and placed in a greenhouse at a temperature of about 20° C., and four days later a pycnium appeared on one of the young leaves that had not been killed. The second plant in the first series was removed from the 0° C. chamber two weeks after having been placed there. Pycnia appeared three days later. Some time afterwards ammonia escaped from the cooling system and killed the leaves; therefore, three of the six remaining plants were removed from the cold chamber 16 days after being placed therein, and the remaining plants were removed 18 days later. There was no infection on the leaves that later unfolded on the first three plants, but a pycnium appeared on a leaf spine and another on a petiole of one

³ CRAIGIE, J. H. THE LIBERATION, GERMINATION, AND VITALITY OF THE AECIOSPORES OF PUCCINIA GRAMINIS. [Unpublished thesis, Univ. of Minn. 1925.]



A



B

Leaves of *Barbarea asarifolia* showing pycnidial infection produced by *Puccinia graminis tritici*. A, Upper side of leaves; B, lower side of leaves, showing failure of aecia to form from the pycnia. (Photographed 66 days after appearance of pycnia.)

of the three plants last removed from the cold room and subsequently kept at 20° C. In another set, nine barberry plants were exposed to rusted *Agropyron repens* straw from Minneapolis, Minn. After removal from the incubation chambers one plant was placed in the greenhouse as a check and the others were placed in the 0° C. chamber, being removed successively at the end of 9, 14, 21, 28, 35, 42, and 49 days. No infection was observed on the plants held 35, 42, or 49 days in the cold room; the leaves were killed and no infection appeared on the leaves that unfolded later in the greenhouse. Pycnia, however, appeared on the plants that were held 9, 14, 21, and 28 days in 12, 7, 3, and 5 days, respectively, after being removed from the 0° C. chamber.

To test the resistance of the mycelium further, four plants with heavy pycnial infection on the leaves were used. On one plant, kept in the greenhouse as a check, aecia appeared 13 days from the time of removal from the incubation chamber. The other three plants were placed in the 0° C. chamber and were removed successively after the expiration of 8, 28, and 91 days. Aecia appeared on the 8-day and 28-day plants 10 days after their removal. The pycnia on the plant exposed 91 days were dry when the plant was removed from the cold chamber, but nectar reappeared 10 days later, followed by the formation of aecia on 8 of the 16 infected leaves 20 days after their removal from the chamber.

Eight other common barberry plants were inoculated with teliospores of *Puccinia graminis secalis* from Chatfield, Minn., and after pycnia had appeared they were placed in the 0° C. room. They were removed 8, 14, 21, 28, 35, and 61 days later. Aecia appeared on the plants held 8, 14, 21, 28, and 61 days, but not on the 35-day plant. The pycnia on the plant exposed 61 days were dry when removed but subsequently produced more nectar, and aecia formed normally.

Thus it appears that barberries that have been infected may not show any visible sign of the presence of the rust for relatively long periods of time during cold weather, but pycnia may appear when conditions become favorable. In line with this evidence, an observation made in the field may be of interest. On May 3 pycnia were observed on *Berberis alesuthensis* at University Farm, St. Paul, Minn. Three days later pycnia also appeared on *B. vulgaris* near by. The weather then became cool. On May 23 aecia had formed on *B. alesuthensis* and were just appearing on *B. vulgaris*. It therefore required 20 days for the appearance of aecia, about four times as long as usually is required under favorable conditions.

From the data presented it would appear that in nature the rust may infect barberries about the middle of April, produce pycnia early in May, and develop aecia the latter part of the month under the retarding influence of low temperature. This also would indicate that the teliospores are ready to germinate at the time of the first spring rains and that the barberries may be infected even while in the bud, although the infection may not become apparent for weeks if low temperatures prevail. In the field, infections occasionally have been observed on the leaf spines of escaped common barberries, indicating that sporidia were being produced even before the leaves unfolded.

EFFECT OF LIGHT ON DEVELOPMENT OF AECIA

To determine whether light has any influence on the development of aecia, a number of barberries were inoculated by means of a shower of sporidia and placed on the greenhouse bench until pycnia appeared. The infected barberries were placed under the following conditions of light: Constant light, constant darkness, and alternate light and darkness. A dark chamber was improvised, consisting of a metal cylinder sunk in the sand of the greenhouse bench and covered by a pane of glass painted with a black, light-proof paint.

All the plants used were moderately or heavily covered with pycnia at the time they were subjected to the various light conditions, and data were taken on the first appearance of aecia. These data are given in Table 10. It would appear that light alone influences the production of aecia very little, although Lambert (10) states that "the development of aecial infection after penetration (of sporidia) appeared to be favored by intense light." While aecia usually appeared first on plants kept constantly in the light, in some cases they appeared equally early on those exposed to alternate light and darkness, and in two cases they appeared first in total darkness. It is thought, therefore, that light exercises only an indirect influence. Plants kept in the darkness became etiolated, and the infected leaves were much distorted. Infection was far heavier on the petioles of the barberry plants kept in darkness than on those kept in the light, because the petioles did not become so tough in the dark as under normal conditions. These results corroborate those of Melander.⁹

TABLE 10.—Influence of light on development of aecia on *Berberis vulgaris* on which pycnia had been produced after inoculation with *Puccinia graminis secalis*

Series No.	Time required for formation of aecia, and degree of infection ¹			Series No.	Time required for formation of aecia, and degree of infection ¹		
	Constant light ²	Constant darkness	Alternate light and darkness		Constant light ²	Constant darkness	Alternate light and darkness
	Days	Days	Days		Days	Days	Days
1.....	9+	0	11++	4.....	10++	9+	11++
2.....	6+	0	6+	5.....	13++	0	8+
3.....	10+	9++	11++	6.....	12+	0	13+

¹ ++, Heavy; +, moderate; +-, light; 0, none.

² Constant light consisted of sunlight in the daytime and the rays from a 1,000-watt Mazda lamp suspended 3 feet above the plants at night.

CONDITIONS FOR LIBERATION OF AECIOSPORES

The potential rôle of barberries in initiating rust epidemics depends, of course, upon the time they become infected, the number of spores produced on them, and the distance to which the aeciospores are disseminated from them. The liberation of the aeciospores is prerequisite to their dissemination. It is known that they are forcibly ejected from the aecial cups, but the conditions under which they are discharged are not precisely known. Craigie¹⁰ states: "Moisture is necessary to induce rapid spore liberation. The greatest activity takes place in a saturated atmosphere." This being

⁹ Melander, L. W. Op. cit. (See footnote 8.)

¹⁰ CRAIGIE, J. H. Op. cit. (See footnote 8.)

true, the circumstance favors the pathogene, because the spores will be liberated most abundantly when the best condition for infection, that is, a saturated atmosphere, prevails. The writer therefore made observations to determine the conditions under which the aecia open and discharge spores most readily. Fourteen aecial cups of various ages were placed in a Petri dish, on the bottom of which was placed a moist filter paper. Forty minutes later 2 of the aecial cups had opened; 55 minutes later 3 had opened; 65 minutes from the time the experiment was begun 5 of the aecia had opened; 2 hours and 20 minutes later 7 of the 14 had opened and 1 of the cups was discharging spores; and 5½ hours later 10 of the 14 cups were open and 2 were discharging spores. Nineteen hours later 11 of the 14 aecia were discharging spores and the other 3 were open.

It was desirable that the age of the aecial cups be known, for aeciospores are capricious in germination and in viability. Aecial cups of known ages were taken from two series of *Berberis vulgaris* plants inoculated with sporidia of *Puccinia graminis secalis* and placed in moist chambers. Notes were taken on the time required for the aecial cups to open and discharge spores. The results are given in Table 11.

TABLE 11.—Influence of age of aecia on their opening and the subsequent discharge of aeciospores in a moist chamber¹

Series No.	Age of aecia	Aecia	Hours in moist chamber	Aecia		Series No.	Age of aecia	Aecia	Hours in moist chamber	Aecia	
				Opening	Discharging spores					Opening	Discharging spores
	Days	Number	Number	Number	Number		Days	Number	Number	Number	Number
1-----	15	5	3	3	3	1-----	37	5	3	13	2
	18	5	3	14	1		26	8	6	8	1
	22	7	3	7	1		30	14	6	13	1
	24	2	3	12	0		32	13	6	13	2
	24	4	3	14	0	2-----	33	16	6	16	8
	25	1	3	1	0		37	4	6	4	0
	36	6	3	15	1		39	9	6	8	0
	37	7	3	17	0						

¹ Aeciospores of *Puccinia graminis secalis* only.

² Cups crumbled without discharging spores, except as indicated in last column.

Aecia that were 24 and 25 days old failed to eject aeciospores forcibly in the first series, while aecia 15, 18, 22, and 36 days old did violently discharge aeciospores in a moist chamber. Among those that were 37 days old there were some from which the spores were forcibly discharged and others from which they were not.

In the second series, aecia that were 37 and 39 days old failed to discharge aeciospores, while aecia 29, 30, 32, and 33 days old did forcibly discharge aeciospores in a moist chamber.

It is evident that the various aecial cups from the same plant vary in their ability to discharge aeciospores in a moist chamber, but in the foregoing experiments some aecia discharged their spores 37 days from the date of appearance of aecia on the barberries. Under natural conditions this circumstance would give the fungus a greater chance to complete its life cycle by infecting grains or grasses. Under fairly dry conditions the aecia on the barberry leaves would remain dormant, while under the moist conditions sometimes present the

aecia would open and discharge the aeciospores, which then would have a much better chance of infecting grains or grasses than if discharged under dry conditions.

VIABILITY OF AECIOSPORES

In order to test the viability of aeciospores formed under greenhouse conditions, Petkus rye was inoculated with aeciospores of *Puccinia graminis secalis* from the living plants at various times after their formation. Three different telial collections were used to produce the aecia. After the rye was inoculated with the aeciospores it was placed in the incubator for 48 hours, then removed and placed on the greenhouse bench in a booth free from other sources of inoculum. The results are given in Table 12.

TABLE 12.—Effect of age of aeciospores of *Puccinia graminis secalis* on infection of Petkus rye

Age of aeciospores (days)	Plants		Age of aeciospores (days)	Plants	
	Inoculated	Infected		Inoculated	Infected
	Number	Number		Number	Number
8	31	18	29	25	6
20	25	6	29	30	9
22	35	7	33	31	13
28	37	25	36	19	0
28	28	0	46	48	5
29	29	1	74	22	0

No infection was obtained with aeciospores of the Rochester (Minn.) collection, which were 74, 36, and 29 days old, respectively, at the time they were used as inoculum, although other spores 29 and 46 days old caused infection. The numbers of plants infected were 1 out of 29 and 5 out of 48, respectively.

When the plants of Petkus rye and Little Club wheat were removed from the incubators after inoculation with aeciospores, the hard, horny aecial cups had been transformed into a flocculent mass of spores. Microscopic examination failed to disclose any germination at this time, and the spores floating on the surface of distilled water failed to germinate two days later. It seems that these aecia crumbled and did not discharge aeciospores. The aecial chains separated from one another and finally broke up without germination of the spores. This phenomenon has been observed in aecia a month old or older and apparently is a sign of age in the aecia.

Uredinia were produced on 4 of 31 plants inoculated with 33-day-old aeciospores from Hennepin County collections. Flecks appeared on leaves of 9 other plants.

Using aeciospores from an unknown source, infection was obtained 8, 20, 22, 28, and 29 days after the date of formation of the aecia. The numbers of plants infected were as follows: 15 of 31 with 1 flecked, 5 of 25 with 1 flecked, 5 of 35 with 2 flecked, 23 of 37 with 2 flecked, 7 of 30 with 2 flecked, and 6 of 25 with none flecked, respectively.

The aeciospores in the foregoing experiments differed in their ability to infect Petkus rye, but infection occurred on this susceptible host with spores from aecia that were 46 days old. Thus, the chances for infection of grains or grasses are much greater than if the aeciospores

were relatively short-lived, in which case they might be liberated in the spring at periods when no susceptible hosts were present and no infection would occur. When the aeciospores are liberated and remain viable for relatively long periods, their chances of falling on susceptible hosts are much greater.

As stated previously, these experiments were made in the greenhouse. Whether the results would have been the same under field conditions is not known, but it seems logical to assume that in the field the aeciospores would not have lived so long, because, exposed to rain and dew, they probably would have germinated more promptly.

PHYSIOLOGIC FORMS WITHIN A SINGLE AECIUM

Even if aeciospores are successfully liberated from the aecial cups and disseminated, the question of their infective capacity still remains. Barberries usually become infected from the near-by grains or grasses. Obviously, if barberries are infected with a variety or physiologic form of rust that will not attack the gramineous hosts near the bushes, epiphytotics will not develop near them, and it may be assumed that the source of the barberry infection was some distance away from the infected barberries. It is therefore important to know which varieties and physiologic forms predominate on the barberry in order better to understand the occurrence of rust in any year.

There is a possibility, of course, that hybridization may occur on barberries. It seemed desirable, therefore, to analyze the parasitic capabilities of the spores within single aecial cups. For this purpose telia on Red Sask wheat were used as the inoculum for susceptible barberries, and the resulting aeciospores constituted the source of all the cultures identified in this work. This telial material was secured from Olaf S. Aamodt, formerly associate pathologist in the Division of Cereal Crops and Diseases, Bureau of Plant Industry, stationed at University Farm, St. Paul, Minn. He stated that the material was the result of inoculation with nine known physiologic forms of *Puccinia graminis tritici*, namely, forms 1, 18, 19, 21, 23, 29, 33, 36, 39, and with some unidentified material collected in the spring-wheat region. Some physiologic forms, e. g., form 21, produce telia rapidly on Red Sask, even in the greenhouse, so the wheat was heavily rusted with telia. The rusted wheat was kept out of doors exposed to the weather during the winter, but the teliospores were found to be viable in April, when some of the barberry inoculations were made. Two of the species, *Berberis declinata oxyphylla* and *B. canadensis*, were heavily rusted. Two cultures of Little Club wheat were inoculated with aeciospores from *B. declinata oxyphylla*, and heavy infection occurred on the majority of the plants in both cultures. One culture was given to M. N. Levine and J. M. Wallace for identification. The writer identified the rust on plants in the other culture. Levine and Wallace identified the rust given them as a new form, *P. graminis tritici* form 51. The author found that the collection he identified comprised a mixture of *P. graminis tritici* forms 18 and 33. In Wallace's collection there was evidence of form 18 also, although it was not completely isolated and identified. These collections or strains were the result of inoculation with aeciospores from a number of aecia.

Four pot cultures of Little Club wheat, one of Manchurian barley, and one of Petkus rye were inoculated with spores from single aecia

from *B. canadensis*. The cups were picked off with a pair of tweezers and placed on the wetted leaf, one cup to a leaf. The six cultures were incubated 48 hours in the usual manner, then placed on the greenhouse bench. The amount of infection varied. In the case of culture No. 1 of Little Club wheat, 2 out of 21 leaves were infected; culture No. 2, 4 out of 19; culture No. 3, 4 out of 15; and culture No. 4, 5 out of 22. One flecked plant was noted in No. 1, 3 flecked plants in No. 3, and 2 flecked plants in No. 4. Of the barley plants, 2 out of 21 were susceptible, while 1 was flecked. None of the Petkus leaves proved susceptible, although two of the leaves were flecked.

The rust on each of the susceptible leaves was treated as an individual strain, and an effort was made to inoculate a susceptible host from each leaf, then isolate the culture of rusted plants and identify this rust as a separate unit. The results are given in Table 13. Because of the press of work, not all of the strains were kept in culture. Consequently, only 10 of 17 possible strains were identified. From these 10 strains, five physiologic forms of *Puccinia graminis tritici*, with a possible sixth, were isolated, in which the technic of Stakman and Levine was followed (16).

Study of the data obtained reveals the fact that forms 18 and 35 were found associated in the rust strains five times, forms 21 and 33 occurred together four times, forms 18 and 33 twice, forms 21 and 35 three times, and forms 34 and 35 once. Forms 34, 36, 51, and 52 appeared but once during the course of the investigation, form 51 appearing in the multisporous culture identified by Wallace and Levine, and forms 34, 36, and 52 appearing in the single-cup cultures identified by the author. Form 51 was not known before it was identified by Wallace and Levine in the mass-aecial culture. Form 52 was identified twice in 1928 from field collections, one of which was from northern Minnesota, and it was also in culture at St. Paul in another greenhouse at the time it was identified, so the possibility of its having been a contamination can not be dismissed entirely.

TABLE 13.—*Physiologic forms of Puccinia graminis tritici isolated from Berberis spp., inoculated with teliospores produced on Red Sask wheat grown at University Farm, St. Paul, Minn., 1926*

Source of culture	Aecial host	Rust ¹ strain	Number of times physiologic forms indicated were isolated							
			18	21	33	34	35	36	51	52
Many sori.....	<i>B. declinata</i> ory- phylla.....	1	1		1					
		2	1						1	
		LC-1a		1	1					
		LC-2a		1	1					
		LC-2b	1	1			1			
		LC-2c	1				1			
Single cup.....	<i>B. canadensis</i>	LC-3a		1	1					
		LC-3d	1		1		1	1		
		LC-4c	1	2	1		1			
		LC-4g	1				1			
		B-1a				1	3			
		B-1b		1			1			1

¹ LC=Little Club wheat; B=Oderbrucker barley. Numbers refer to primary host; letters following numbers refer to rust strain from each host.

² Identity not definitely established.

³ Identity of all but 1 of the cultures doubtful.

⁴ Identity of 1 of the cultures doubtful.

The experiment indicates that when the telia used for inoculating barberry contain more than one form it is improbable that only one form can be secured by using single aecial cups. To be sure of having only one form present in the culture, it would be necessary to use only single chains of aeciospores, or better still, single aeciospores from a single aecial cup. The results obtained suggest the possibility that forms 51 and 52, and perhaps form 34, originated through hybridization on the barberry. As some of the collections of uredinia used in inoculating the Red Sask wheat had not been identified before Aamodt used them, the possibility can not be excluded that forms 34, 51, and 52 may have been present in the field and passed through the barberry unchanged. However, form 51 has never been identified before or since, so it seems strongly probable that it was the result of hybridization. The presence of two or more forms in the strains from single aecial cups indicates that the aecia are not the product of the fusion of the hyphae of a single form at the base of the aecium. If two or more physiologic forms make up or form a single aecium, then, theoretically, there is almost no limit to the number of physiologic forms of stem rust that may be identified in the progeny of a single aecium.

In the rust forms identified from these various cultures not more than two physiologic forms usually were present. In one culture, LC-4c, forms 18, 21, 33, and 35 were definitely identified. In this culture, supposedly derived from a single aecium, four different physiologic forms were present. When several forms are obtainable from a single aecium it is most probable that hybridization will sometimes occur and possibly evolve new forms, or yield forms already known. This may have been the explanation of the appearance of forms 34, 51, and 52. Form 51, new, differs from form 33 only in its reaction to Vernal emmer of the differential hosts. Vernal is susceptible to form 51 and highly resistant to form 33. Form 52 differs from another of the identified forms, form 18, on but 2 of the 12 differentials. Kubanka shows an X-type reaction to form 52, and Vernal is susceptible; Kubanka is susceptible to form 18 and Vernal is highly resistant. Both these forms may have originated as the result of a slight change in the chromosomal arrangement of the cells, one form acquiring the ability to attack Vernal emmer, the other having two factors altered, enabling the pathogene to attack Vernal and produce an indeterminate reaction on Kubanka. It is possible that this indeterminate reaction on Kubanka or some other host is the result of a heterozygous condition of the rust. Inoculating barberry with telia of such a heterozygous rust might break up the rust into two forms, one of which would be able to attack Kubanka severely, the other unable to produce more than a light infection on the same host.

Of the known physiologic forms of *Puccinia graminis tritici*, used to inoculate Red Sask wheat during the summer and fall, only four were isolated the following spring. These were forms 18, 21, 33, and 36. Those not identified in the summer and found in the spring were forms 34, 35, 51, and 52. However, it should be remembered that some unidentified material also was used in inoculating the Red Sask wheat, so the possibility of their presence in the unknown material can not be excluded.

DISCUSSION

It is evident from the foregoing experiments and observations that most of the species of *Berberis* tested are susceptible to *Puccinia graminis*. Susceptible species in general resemble *Berberis vulgaris* morphologically; those that did not rust resemble *B. thunbergii*. This also was true of the limited number of progeny of *B. vulgaris* × *B. thunbergii* that were tested.

The infection produced on barberries depends upon a number of factors: Age of teliospores, length of incubation period, temperature, age of barberry leaves, and moisture available for germination of teliospores. Teliospores a year or more old produced fewer sporidia than those collected in early spring, as judged by the barberry infections. Teliospores kept cool and dry retained their ability to infect barberry for a year and a half; when exposed to natural conditions the spores lost their viability within a few weeks. Teliospores wetted continuously for 11 days germinated and the resultant sporidia infected the barberry plants. This is significant in nature because teliospores often are exposed to rains before the barberry leaves have unfolded, and if all of them germinated promptly at such times the amount of effective inoculum would be greatly reduced. Barberries may be infected by viable sporidia from teliospores at any time during an 11-day period of rainy weather, provided the temperature is favorable, for there may be a constant discharge of sporidia during this period. The telial material retains its infective power for some time, even when exposed to continuous moisture, and may retain it longer under conditions of intermittent rain.

The minimum length of time necessary for infection of barberries, including germination of the teliospores, formation and liberation of the sporidia, and the entrance of the sporidial germ tubes into the plants, was 41 hours when the teliospores were removed from the straw before inoculation and 21 hours when the plants were inoculated with heavily rusted straw. Plants exposed five hours to a shower of sporidia in an incubator became infected. The length of time required for infection, as well as the severity of infection, depends on a number of factors, the principal ones of which are moisture and temperature. It was found that teliospores do not germinate, produce sporidia, and cause infection readily at temperatures higher than 26° C. The optimum temperature for the entire process required for infection of barberries is fairly low, lying between 12° and 21°. After infection once occurs, temperature again is important in determining the length of the incubation period. Aecia are produced most rapidly at temperatures ranging between 20° and 30°, and the incubation period may be lengthened from 3 days at 31° C. to 15 days at 8°. Moisture has very little effect on the degree of infection, and sunlight in the greenhouse apparently has only an indirect effect. It probably is important principally in the hardening of the leaf tissues. If there is abundant light, the tissues are likely to harden rather rapidly and the period of their susceptibility is shortened. When, on the other hand, there is relatively little light, the tissues remain more delicate and the period of susceptibility is lengthened, as in the case of the petioles of barberry leaves kept in constant darkness.

Leaves, young stems, spines, petioles, peduncles, sepals, and berries may become infected. The impression has been general that leaves

retain their susceptibility only a few days. If this were true, it would be necessary that conditions for infection be favorable when barberry leaves first unfold in the spring. It has been found, however, that leaves of *Berberis vulgaris* may remain susceptible 16 days after the buds unfold. Leaves of *B. aetnensis* may remain susceptible for 12 days; actually this period probably is longer than that for *B. vulgaris*, as there were indications that leaves of *B. aetnensis* were not becoming resistant with increase in age as was the case with *B. vulgaris*. This period of susceptibility during which infection may occur if environmental conditions are favorable is fairly long.

Barberries often become infected quite early in the spring and may therefore encounter freezing temperatures. If such temperatures were more harmful to the rust than to the barberry tissues, the amount of potential inoculum produced on barberries would be greatly reduced in the spring. It was found, however, that freezing kills the tissues of the host plant before it kills the rust fungus. Barberries with a moderate development of pycnia on the leaves were kept 91 days at a temperature of 0° C., but when they were again placed on the greenhouse bench the fungus was able to produce normal aecia. It is therefore apparent that the rust on the barberry can withstand temperatures as low as those endured by its host.

After aecia are once formed in nature there often are periods of dry weather. During these periods the aecia may remain capable of discharging viable spores for 37 days after the appearance of the aecia on the plants. This means, then, that the aecia may remain dormant on fairly dry leaves and resume activity when the air becomes moist enough to enable the aecial cups to open and discharge spores. As the aeciospores are discharged only under moist conditions and germinate only in a moist atmosphere, the rust fungus can withstand unfavorable conditions for some time in the aecial stage. Under greenhouse conditions the aeciospores remained viable as long as 46 days after the date of their appearance on plants of *Berberis vulgaris*.

The results show that the aecial stage of *Puccinia graminis* is fairly well adapted to withstand the variable weather conditions so likely to occur in the barberry-eradication area in the spring. There are a fairly long time and a wide range of environmental conditions under which the aecial stage may develop, discharge spores, and cause infection on grains and grasses.

The recent discovery of heterothallism by Craigie (4) has strengthened the belief that hybridization between rust forms may occur on the barberry. The writer's results with cultures of *Puccinia graminis tritici* from single leaves inoculated with individual aecial cups indicate that in telial collections containing more than one physiologic form there is a possibility of new combinations of the known forms. As many as four physiologic forms were isolated from one culture. If four different physiologic forms can be isolated from a culture it is highly probable that at some time some of the physiologic forms in the collection may fuse to form entirely new combinations or physiologic forms. In this work one such form, form 51, was obtained, and another, form 52, was isolated, although form 52 was found in the field the year following the collection of the telia. Form 51 never has been described, so it is very probable that this form originated on the barberry through hybridization of other forms.

The work of Newton, Johnson, and Brown (14) and Stakman, Levine, and Cotter (17) on hybridization in *Puccinia graminis* involved making many cultures from single aecia. From conversation with Newton it appears that individual unopened aecia were used, and very rarely did more than one physiologic form appear in any one culture. In the writer's results it was unusual to find but a single physiologic form present. This obvious discrepancy in the results may perhaps be explained by the fact that in the writer's experiments at least nine known physiologic forms, and probably more, were present in the telial collection used to inoculate the barberries from which the uredinal cultures were derived. In the experiments of Newton et al. only one or two forms were present in their telial material.

SUMMARY

A study was made of the factors affecting the development of the aecial stage of *Puccinia graminis*.

A list of susceptible and immune species and varieties of *Berberis*, as determined by the writer and others, is given.

In individuals from a *Berberis vulgaris* × *B. thunbergii* cross there seemed to be a correlation between the morphologic characters of *B. vulgaris* and susceptibility to *P. graminis*.

Teliospores of *P. graminis* may remain viable at least a year and a half when kept dry and at a temperature near the freezing point.

Teliospores of *P. graminis* do not germinate and produce sporidia at temperatures higher than 26° C., or if they do, they do so very rarely.

Barberries may become infected when exposed to teliospores that have been wetted for 264 hours, or 11 days.

Temperatures ranging from 12° to 21° C. are the most favorable for the germination of teliospores, infection of barberry bushes, and production of aecia. Freezing infected barberries killed the host plant before it killed the rust.

Light and moisture had no decided effect on the production of pycnia or aecia, although there was some evidence that the absence of light may increase and prolong the susceptibility of barberry leaves because the tissues remain succulent.

The minimum length of time necessary for barberry infection by teliospores was 21 hours, although infection occurred after a 5-hour period of exposure to a shower of sporidia in a moist chamber.

On *Berberis vulgaris*, infection may occur on the leaves, stems, spines, petioles, sepals, or berries.

Leaves of *B. vulgaris* 16 days old were susceptible to the attack of *P. graminis*.

Aecia of *P. graminis* may discharge aeciospores 37 days after the appearance of the aecia, and the aeciospores may infect rye 46 days after the appearance of the aecia on the barberry leaves.

Two physiologic forms of *P. graminis tritici* were isolated from a number of uredinal cultures produced by single aecial cups, although the forms found were not the same in all cultures. In one case four physiologic forms were identified from such a culture. The telia used to inoculate the barberries contained nine or more physiologic forms.

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