

3789450



A U S T R A L I A N R U S T S T U D I E S

By W.L. Waterhouse,
The University of Sydney.

27/1/29

C O N T E N T S

- Introduction.
- Aecidial stage of Puccinia graminis.
- Teleutospore stage of P. graminis.
- Uredospore stages of P. graminis and P. triticina.
- Specialisation.
- Historical.
- Methods of study.
- Purity of differential hosts.
- Occurrence of natural crosses.
- Effects of varying environmental conditions in the plant house upon rust development.
- Specialisation in P. graminis tritici.
- Identity of forms in Australasia.
- Colour differences between forms.
- Distribution of the forms.
- Hosts of P. graminis tritici other than wheat.
- Specialisation in P. graminis avenae.
- Identity and distribution of forms in Australia.
- Hosts of P. graminis avenae other than oats
- Specialisation in P. triticina.
- Biometrical studies of the morphology of spore forms.
- Oversummering of rust.
- Stem rust of wheat.
- Stem rust of oats.
- Leaf rust of wheat.
- Breeding for rust resistance.
- Introduction.
- Crossing technique.
- Germination as affected by age of the grain.
- Wide crosses.
- Wheat x barley.
- Wheat x rye.
- Interspecific wheat crosses.
- Vulgare crosses.
- Canberra x Thew.
- Federation x Canberra.
- Federation x Thew.
- Riverina x Thew.
- Federation x Japanese Bearded.
- Further crossing work.
- Oat crosses.
- Acknowledgments.
- Summary and Conclusions.
- A Bibliography.

INTRODUCTION



Cereals are the most important of the crop plants grown in Australia. Wheat is the most widely grown of the cereals, being one of the staple products of the country. This is indicated by the fact that in the season 1927-1928 there were 12,263,979 acres under wheat, yielding a total of 116,737,082 bushels. Far reaching benefits will therefore accrue from anything which can be done to eliminate losses in the yield. That great losses do occur is well known, and amongst the causal agents, plant diseases reduce yields in a remarkable degree. The disease known as rust is no exception.

A detailed account of the history of cereal rusts in Australia is beyond the scope of this paper. A great deal of information has been compiled, but from it only a few brief extracts will be given.

Rust occurred in wheat in the very early days of colonisation of Australia. It was one of the many obstacles encountered. The difficulties met with by the early settlers in their wheat growing have been reviewed by Potts (118) but some further references to the original accounts may be of interest.

The earliest available record is that written by David Collins, the Secretary to the first Governor of the Colony. Writing in February 1788 of the disembarkation of the first colonists, Collins (31) refers on page 7 to the clearing of a piece of land, -- the site of the present Botanic Gardens, -- where wheat was first sown. In August of the same year, referring to this land he says (p. 41) "The seed wheat that was sown here did not turn out any better than that at Norfolk Island: in some places the ground was twice cropped, and there was reason to apprehend a failure of seed for the next year." The next reference on p.88, written in December 1789 states, "In the course of this month the harvest was got in; the ground in cultivation at Rose Hill produced upwards of 200 bushels of

82108D

wheat, about 35 bushels of barley, and a small quantity of oats and Indian corn; all of which was intended to be reserved for seed. At Sydney, the spot of ground called the Governor's Farm had been sown only with barley, and produced about 25 bushels."

Collins on p.442 writing of December 1795 says, "The harvest was begun this month. The Cape wheat (a bearded grain differing much from the English) was found universally to have failed. An officer who had sown 7 acres with this seed at a farm in the district of Petersham Hill, on cutting it down found it was not worth the reaping. This was owing to a blight; but everywhere the Cape wheat was pronounced not worth the labour of sowing." The "blight" may well have been a plant disease, possibly rust. "Petersham Hill" includes the present site of the University of Sydney.

Early despatches from Governor Hunter (67) give further information regarding these happenings. A communication written in March 1796 (p.554) states, "We have got our harvest in, and it is, upon the whole, in point of quantity as well as quality, very superior to anything which this country has before experienced, although a few blights and other accidents had disappointed the expectations of some very industrious settlers." In a further despatch (p.557) dated April of the same year is another reference to this "destruction of the crops of some of the settlers by blight." The estimated wheat yield in that year was from "35,000 to 40,000 bushels." The acreage under wheat was stated to be 2721½ acres, so that the harvest yielded an average of from 12 to 14½ bushels per acre.

Rumsey (125) refers to the first recorded attack of rust in Australia. It occurred in the Dundas district at Brush Farm owned by Captain Cox. This is close to the present Eastwood station. What is evidently a quotation from the "Sydney Gazette" reads as follows :-

"October 21, 1803. A more beautiful appearance of a successful harvest never flattered the expectations of a farmer within 3 weeks of being ripe; ears were full, plump and well coloured, and in every respect gratifying to look at. In 3 days it was completely destroyed by rust. The produce of 266 acres was not worth £20. A loss of £4,000 was made, -- an enormous amount in those days." Potts (118) quotes this same statement. Maiden (91) states that rust attack was the reason for wheat growing being discontinued at Farm Cove.

Atkinson (7^A) refers to the prevalence of smut in the wheat crops, and says (p.43) that "rust sometimes appears, but is not very common."

Montague Smith (106^A) notes on page 9 that the plains and forest lands of the Hunter River district of N.S.W. suffered from rust in wheat. He states that the only places which escaped were patches of virgin soil, although the weather conditions were favourable for rust development.

Even with the extension westwards into the drier climates, rust damage was not eliminated. Severe losses occurred from time to time in the various States. Grove (56) states on p.253 that in 1889 when there was a wet and muggy spring, the loss due to rust for the whole of Australia was estimated to be between 2 and 3 million pounds sterling. The importance of this "red rust" led to the convening of conferences of delegates from the different States to consider the problem. Several of these conferences were held, commencing in the year 1890. The work of these conferences is summarised in various numbers of the Agricultural Gazette of N.S.W. (126) (128) (129) (130). Only one of the full reports has been available for consultation. It gives interesting sidelights on the problem as it existed then, and impresses one with the enthusiasm and soundness of the workers in those days. It must be remembered that what are now commonplace facts regarding rust were then quite unknown.

At the "Rust in Wheat Intercolonial Conference" held in 1892 (129A) figures were given which showed the importance of the disease. Anderson (p.13) estimated the damage done in 1891-1892 season at over 2 bushels per acre sown. This season was stated to have been a favourable one as regards small loss from rust.

McAlpine (p.18) considered that the loss in Victoria that year varied from 2 bushels to 12 bushels per acre, and in some instances more. Farrer at this conference (p.25) affirmed that he had the greatest faith in the world that the solution to the rust problem could be found.

Gobb (26) (27) (28) (29) (30) broke much new ground in his rust investigations and made many contributions to the subject. It is interesting to find Sir J.B. Lawes (129) expressing the opinion that the solution would be found in a rust-proof variety of wheat.

McAlpine (105) has made the most valuable contribution to our knowledge of Australian rusts. It is the only work of its kind available.

Despite the wonderful wheat breeding work done by Farrer, losses from rust continued. Thus in 1903 the N.S.W. losses through rust attack were estimated by Mr. A.H.E. McDonald, then Chief Inspector of Agriculture, to amount to nearly 3,000,000 bushels, worth more than £400,000. The same authority computed the rust loss in 1916 at more than 5,000,000 bushels, the damage totalling over £2,000,000.

Following the lead given by Farrer, attention has been paid by wheat-breeders to selection for rust-resistance. Considerable success has been achieved in this work. An even greater saving has been effected through the development of rust-escaping varieties. These mature early enough to greatly lessen and to frequently render negligible the attack by rust.

In 1915 the late Captain H. Stephens was appointed the first Walter and Eliza Hall Agriculture Research Fellow of the

University of Sydney. He commenced an investigation of the rust problem, but after holding the Fellowship for only three months, enlisted for active service overseas. Science in general and Australia in particular suffered a great loss when he was killed in action. Results of the greatest value would have certainly been forthcoming had he been spared to carry forward the work which he so ably began.

In 1918 the writer was appointed to the Fellowship left vacant by the death of Captain Stephens, and upon returning from abroad in 1921, the studies herein recorded were commenced.

The nature of the problem demanded extensive studies as well as intensive research into many aspects of the subject. Whilst definite conclusions are now possible from some of the lines of work, in others it will be many years before definiteness can be reached. But some of the general results obtained seem worth recounting at this stage. These largely have to do with investigations carried out in the plant house, the only practicable method to follow if full control of the work is to be maintained. But as far as possible attention has also been given to field results. In this work particularly the co-operation with the N.S.W. Department of Agriculture has been invaluable.

The work deals with rusts present on cereals and certain of the grasses. On wheat, the only rusts recorded in Australia are Puccinia graminis tritici E. & H. which causes stem or black or summer rust, and P. triticina Erikss. which causes orange leaf rust or spring rust. The yellow stripe rust caused by P. glumarum (Schm.) E. & H., has not been recorded. On barley two rusts occur, P. graminis tritici E. & H. which causes stem rust and P. simplex (Körn) E. & H. which causes leaf rust. On oats there are two rusts, P. graminis avenae E. & H. which produces the stem or black or summer rust, and P. coronata Cda. which causes the crown or leaf rust. There are also two rusts on rye, P. graminis tritici E. & H. the cause of stem or black

or summer rust, and P. dispersa E. & H. which causes leaf rust. The genetic connection between these cereal rusts and certain of the rusts on grasses is discussed later in this paper.

THE AECIDIAL STAGE OF PUCCINIA GRAMINIS

This stage in the life history of Puccinia graminis is of extreme importance in the Northern Hemisphere. The eradication of barberries in Denmark has led to a most marked reduction of stem rust in that country (88). The barberry eradication campaign in U.S.A. has repeatedly shown that the destruction of barberries results in a diminution of rust attack. More recently (165), it has been shown that physiologic forms may originate in this stage on the barberry.

Results in 1921

Prior to 1921 it was believed that the Australian stem rust was incapable of infecting the barberry. In October of that year it was shown (162) that teleutospores from wheat straw grown at Glen Innes produced normal infections of a susceptible barberry growing in the open at the University of Sydney. Inoculations of seedling wheat plants with aecidiospores from the aecidia on this barberry gave rise to the uredospore stage. This culture was studied in regard to its specialisation, as was also the English culture referred to in the same paper. The latter was found to be Form 27. Both were kept in culture for several years and frequently tested on the differential hosts to be certain of their identity. The Australian culture proved to be Form 46. It was kept in culture in the plant house for four years and used in various tests. This same form was isolated from uredospore material on wheat sent from exactly the same locality in December 1921 and again in December 1922.

Results in 1922

In March rusted wheat straw was received from Pusa, India. It was exposed to winter conditions at Hawkesbury Agricultural College until October, when the teleutospores gave

abundant germinations. They were then used for barberry inoculations. Sixteen days after the date of inoculation, spermagonia and aecidia were produced. Aecidiospores were used to inoculate "Federation" wheat seedlings, but without result. No reason could be given for the failure. A recent visitor from India, Mr. Robertson Brown, has stated that "Federation" grown in India is resistant to stem rust, although other varieties are very susceptible. This may perhaps explain the negative result obtained. Nevertheless a later test with rust from this locality gave Form 16. "Federation" is quite susceptible to this form.

Results in 1923

In April, when the positive results from the teleutospore germinations of Hawkesbury Agricultural College material were unexpectedly found, barberries which had been cut back and were putting out new growth were inoculated with the viable teleutospores. No infections were obtained. The form of rust used in this test was Form 43.

In the same month, a package of wheaten straw showing heavy teleutospore infections of stem rust was received from A.D. Cotton Esq., of Kew, England. The straw had been collected by Miss K. Sampson at Milton, Wales, and then exposed to winter conditions until March, when it was forwarded to Sydney. Upon arrival the teleutospores were viable, giving abundant germinations. Barberry plants (some of them the identical plants used for the above-mentioned H.A. College work) were successfully inoculated, spermagonia beginning to show in eight days and aecidia in twelve days. From inoculations of Federation wheat with aecidiospores, the uredospore stage was produced. This proved to be Form 27. It has since been kept in culture and is still alive. It was interesting to find that the form (27) was the same as that which had been obtained in the 1921 work from straw collected at the same place.

It is clear, then, that the conditions for barberry infections were quite suitable, and that the failure to secure infections from the H.A.College teleutospore material was due to something in the material itself.

In October when the H.A.College material was giving abundant germinations, further extensive barberry inoculations were made. Again the results were negative in every instance. This seemed to indicate that Form 43 cannot infect the barberry.

Results in 1924

At the end of September barberries were inoculated with viable teleutospores from rusted straw collected from H.A.College and known to comprise Forms 43 and 46. Spermagonia were produced but no aecidia.

Further barberry inoculations were made with material from Rutherglen, Victoria, which had been exposed at Epping, N.S.W. Uredospore tests with this same material had shown that Form 46 was present. As a result of the inoculations spermagonia were produced. The unusual incidence of a cold snap at this time checked the growth of the barberries and may have prevented the formation of aecidia.

Another batch of rusted wheat straw was received from Devonport, Tasmania, in March 1924. Uredospore tests showed that it was Form 46. The straw, which showed heavy teleutospore as well as uredospore infections, was exposed to winter conditions at Epping, N.S.W., and tested at the end of September. It proved viable. Barberry inoculations gave abundant infections. Spermagonia and aecidia were produced in quantity. Uredospore cultures on Federation were obtained and proved to be Form 46. This culture has been frequently compared with the uredospore culture from the original straw. No differences in parasitism have been detected.

Results in 1925

It has been pointed out that some evidence seemed to be forthcoming that Form 43 cannot attack the barberry. In this year's tests, wheat varieties were selected at H.A. College on the basis of their known reaction to Form 43 and Form 45, and teleutospore material of three varieties susceptible to each saved. As described in the account dealing with the "Teleutospore Stage" abundant germinations were obtained in September.

During September, six sets of barberry inoculations were made. The barberry plants in two of the sets were well-established bushes of Berberis vulgaris growing in the open, and in the other four sets, were sturdy but rather small plants growing in pots. From one set of inoculations, no infections were obtained. This set was on the outdoor plants. During the 48 hours incubation of the young twigs in lamp-chimneys, hot dry westerly winds were experienced. Attempts were made to keep the cotton wool plugs of the chimneys damp throughout the incubation, but it is believed that these unusual weather conditions were responsible for the failure of this set of inoculations. All the other sets in the plant house gave infections with production of spermagonia and aecidia (Plate I.) In every instance the infections came from the rust on Alaska Branched or American Club, which were known to be infected with Form 45. The barberries infected in this test were Berberis vulgaris, B.vulgaris var sanguinellenda, B.Georgiana, and B.Lucidii. The three last-named were grown from seed kindly supplied by Dr.M.N.Levine in 1920. No case of infection from teleutospores of Form 43 was found. A series of fixations of leaves inoculated with sporidia of Form 43 was made for sectioning and examination. The happenings in the infection of the young leaf by the sporidia had already been traced (161) and these preparations were on hand. But a further series of fixations of leaves inoculated with sporidia from

Form 45 was made at the same time and under identical conditions as those made with Form 43.

The sections of the young leaves show that the sporidia of Form 43 bring about a penetration of the cuticle in the ordinary way. In material fixed 48 hours after inoculation, the uninucleate hyphae are present in the epidermal cells and appear to be normal in every way. Further studies of later stages in inoculations with Form 43 are in progress.

From the aecidiospores, uredospore cultures were obtained on Federation and have been studied for specialisation. The form agrees closely with Form 45. On the ordinary basis for determination, it is classed as the same. That is to say, both must be listed as showing a resistant reaction on Kanred. But there is a consistent difference between them. The stock culture of Form 45 gives no infection whatever on Kanred. The aecidial culture, - tentatively listed as 45A - has always given a "2" reaction on this host under the same conditions. The same consistent differences are shown on the Kansas wheats forwarded by Dr.L.E.Melchers under the names P762, P1066, and P1068, as well as on several other varieties. A further interesting difference between the stock culture of Form 45 and this aecidial culture called 45A is shown later in connection with the biometrical study of the uredospores. Here it is found that the spores of Form 45A are very significantly narrower than those of the stock Form 45.

Further Results

In 1926 teleutospores of Forms 34, 43, and 45 were viable in September. In that month and in October inoculations of barberries were made, with negative results in each case. On both occasions hot dry conditions prevailed during the incubation period out of doors and are believed to have been responsible for the failure.

In 1927 viable teleutospores on wheat straw from India and of Forms 34 and 45 together present on straw from Glen Innes were used. Barberries were inoculated at the end of September. The Indian material produced spermagonia only. The Glen Innes material produced spermagonia and aecidia. These were fixed and used for class preparations, not being tested in the uredospore stage.

The results obtained in 1928 were of unusual interest. They have been reported in a separate paper (165).

In April 1929 a parcel of oaten straw with heavy teleutospore infections was received from Miss K.Sampson of the University College of Wales. The material had been collected in County Kildare, Ireland, by Dr.Murphy. The spores proved to be viable and were used to inoculate a young barberry which, although it was autumn, was putting forth some young growth. At the same time two other barberries in pots were cut back and brought into a warm spot in the plant house where later they were used for a further series of inoculations. Very heavy aecidial infections were procured and uredospore cultures obtained on Algerian oats. One of the barberry infections is illustrated in Plate I. Specialisation studies, (referred to later in this paper) have shown the presence of Forms 1 and 7 of *P. graminis avenae*, and that in respect of colour and spore size this rust differs from the Australian rust.

Summary

Further evidence has been obtained to show that Australian stem rust of wheat can attack the barberry. An exception seems to occur in Form 43. Plants of *Berberis vulgaris* known to be susceptible to other forms of *P.graminis tritici* have repeatedly failed to give infections when inoculated under ideal conditions with viable teleutospores of this Form 43. As early as 1925, from infections of a barberry with teleutospore of Form 45, a form was isolated which resembles Form 45 but consistently differs from it in certain respects. This aberrant form has not been met with in any other isolation. Overseas teleutospores have given rise to forms unlike those present in Australia.

THE TELEUTOSPORE STAGE OF P. GRAMINIS

In order to link up with studies of the aecidial stage of P. graminis, germination studies were carried out.

Numerous attempts were made to repeat the results reported by Thiel and Weiss (151) for hastening spore germination. Using teleutospores which had not been exposed to weather conditions throughout the winter, these workers found that as a result of treating the spores with a weak solution of citric acid, immediate germinations were obtained. Despite many attempts made in different seasons, in no instance was the result repeated. Various samples of citric acid, both "pure" and "commercial" were tried. It therefore became necessary to fall back upon the method of exposing the rusted straw to weather conditions in the open, and then testing the germination capacity.

Results in 1922

Following upon the successful infection of the barberry which had been obtained with teleutospores from wheat straw collected at Glen Innes, (162) it was decided to test rusted material collected at other places. Wheat straw showing heavy teleutospore infection was collected from the Government Experiment Farms at Bathurst, Cowra, Glen Innes and Grafton in December of 1921. Each collection was divided into equivalent parts, of which one was sent to each of these farms to be there exposed to winter conditions. Samples of this exposed straw were forwarded to Sydney for examination at intervals.

McAlpine (105) states that teleutospores which have been exposed to winter conditions commence to germinate in warm days about the end of September. Arrangements were made for the first batch of material to be forwarded about the end of July. Thereafter the fortnightly submissions were not evenly

maintained from all the farms, and hence all the results from the various places are not strictly comparable.

The tests were made by floating a number of teleutosori in covered watch-glasses of tap water and examining under the microscope after a period of about 18 hours. The results are set out in the accompanying table. The sign xxxx indicates that germinations were very abundant. The sign xxx indicates that germinations were plentiful, xx means that they were scarce, x that they were very rare, and - that they were absent altogether.

It will be seen that material collected from all the centres gave germinations after having been exposed at all the centres. The results obtained from material kept at Bathurst and Cowra were not so good as those from Glen Innes and Grafton. The similarity between the climatic conditions at the two former stations would lead one to look for the similarity which is shown between their results. But the conditions at Glen Innes and Grafton are very different. Glen Innes on the Northern Tablelands experiences severe winters, whilst Grafton on the Coast has very mild conditions. Yet the two sets of material gave closely approximating results. These show an extended period over which germinations occur, when compared with those from Bathurst and Cowra. The drier conditions at the latter stations probably explain this happening.

Taking next into consideration the source of the material, it is seen that little difference is observable between the rusted straw which had been collected at Glen Innes, Bathurst and Cowra. But the Grafton rust showed a more restricted germination when kept at both Glen Innes and Grafton.

T A B L E 1.

Results of tests for viability of teleutospores of *P. graminis tritici* collected in different localities and kept at different places.

Straw kept at Glen Innes

Source of Material	Dates of examination for spore germination.									
	24.7.22	8.8.22	11.9.22	2.10.22	11.10.22	25.10.22	6.11.22	23.11.22	4.12.22	18.12.22
Bathurst	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	-	-	-
Cowra	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	-	X	-	-
Glen Innes	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX	-	-	-
Grafton	-	-	-	X	XXX	-	-	-	-	-
	Straw kept at Grafton									
Bathurst	17.8.22	25.9.22	XXX		XXXX	XXXX	XXXX	23.11.22	4.12.22	18.12.22
Cowra			XXX		XXXX	XXXX	XXXX	XXX	XXX	-
Glen Innes			XXX		XXX	-	-	XX	-	-
Grafton			XXX		-	XXX	XX	XXX	-	-
	Straw kept at Bathurst									
Bathurst	4.8.22	12.9.22	XXXX	XXXX	XXXX	XXXX	XXXX	14.11.22	30.11.22	13.12.22
Cowra			XXXX	XXXX	XXXX	XXXX	-	-	-	-
Glen Innes			XXXX	XXXX	XXXX	-	-	-	-	-
	Straw kept at Cowra									
Bathurst	3.8.22	19.9.22	XXXX	XXXX	XXXX	XXXX	XXXX	31.11.22	7.12.22	
Cowra			XXXX	XXXX	XXXX	XXXX	XXXX	XXX	-	-
Glen Innes			XXXX	XXXX	XXXX	XXXX	-	XXX	-	-

As regards the identity of the teleutospore material derived from the various sources, uredospore tests made with rust from Cowra and Grafton in that season showed the presence there of only Form 43. At Glen Innes the rust was Form 46, and at Bathurst a mixture of Forms 43 and 46. The difference in the teleutospore germinations is then not to be correlated with differences in the specialisation of the fungus.

An unexpected observation was the germination as early as July. McAlpine (105) states that teleutospores begin to germinate on warm days about the end of September. But in the July test - the first made - the germinations were abundant.

Another test was made this year with heavily rusted straw sent from Pusa, India. There was not sufficient material for this rust to be included in the series of tests just referred to. When tested on the 15th September germinations occurred, although they were not abundant. A month later the germinations were very abundant. Barberries were inoculated with these as indicated under the heading of "Aecidial Stage".

Results in 1923

In the succeeding year further tests were made. Wheaten straw showing heavy teleutospore infections was collected at Hawkesbury Agricultural College in December 1922. The rust present in this plot of wheat was examined in the uredospore stage and only Form 43 was isolated. The rusted straw was kept out in the open on the ground at Hawkesbury Agricultural College by Mr. T. H. Harrison and portions of it submitted from time to time for examination. The first test was made on the 16th April 1923 and germinations were definite, though not abundant. It had been considered that a mid-April commencement would have been quite early enough to give negative results as a starting point.

After the mid-April germination test, another was made on the 20th May and thereafter they were made at fortnightly intervals. The tests prior to the 3rd July gave negative results in every case, but on that date a few definite germinations were found. The same thing occurred on the 16th July. The test on the 31st July gave numerous germinations, and similar positive results were obtained at fortnightly intervals up to the 9th October. The last batch gave very abundant germinations and was used for an extensive series of barberry inoculations.

The teleutospore germination tests were continued at fortnightly intervals. On the 23rd October no single germination was observed. After such abundant germinations a fortnight previously, this was very striking. Further tests were made at intervals until the end of November with negative results in each case.

The net result of the 1923 teleutospore germination tests was, therefore, that positive results were obtained in April, followed by a period up to July when no germinations were secured, and a further period from July until October when positive results were the rule.

Results in 1924

In 1924 further germination tests were carried out. Rusted straw was collected at Hawkesbury Agricultural College in December 1923. In this case it was known that Form 43 was present in the plot from which the material was taken. Fortnightly germination tests were made. Circumstances arose which made it impossible to begin these until 25th June 1924. Then germinations were sparse, but definite. Similar results were obtained at intervals until September when they became very abundant. On 17th October they were again sparse. On the 29th October they were rare, and in November and December the results were negative.

Further work was done with teleutospore material derived from other sources in this year. In April, heavily rusted straw of "Little Joss" wheat was received from the State Research Farm, Rutherglen, Victoria. It had been left lying on the ground since the harvest in December 1923. In April, germination tests showed that the teleutospores were viable. Cultures from uredospores collected from the same spot in December 1923 had shown that Form 46 was present.

Another batch of rusted straw was received from Devonport, Tasmania, in March, 1924. Uredospores present in the material gave cultures of Form 46. The straw with its heavy teleutospore infections was exposed to winter conditions and tested at the end of September. The teleutospores were then viable.

Inoculations of barberries were made from both these batches of rust, as described under the heading "Aecidial Stage."

Results in 1925

In December 1924 a survey of the rust plot at Hawkesbury Agricultural College revealed the presence of Forms 43, 45 and 46. Six varieties growing in row tests were selected, viz. Bobs, Haynes' Blue Stem, Marquis, Alaska Branched, American Club, and Cleveland. Uredospore tests showed that in the first three, Form 43 was present, whilst in the last three varieties, the rust present was Form 45. Straw of all six with abundant teleutospores present was saved separately and exposed to weather conditions at Hawkesbury Agricultural College and at Epping, N.S.W. Tests for viability were made periodically. The results are shown in Table 2.

It will be seen that even as early as the middle of March germinations occurred. This was quite unexpected. No frosts were experienced during the period of exposure, which in the main was hot. Germinations were observed at fortnightly intervals until early November, with one gap at the middle of June. After November there was a sudden failure to germinate. Whilst it is true that the teleutospores germinate throughout so many months, it is evident that the most abundant germinations take place in September and October, i.e. in the spring. The germination capacity shown by Form 43 is not notably different from that shown by Form 45.

Further Results

Tests in 1926 were made on a small scale. The main aim was to utilise the rust for barberry inoculations. The material used was collected at Sydney University, Grafton, and Western Australia. This in the uredospore stage was proved to be Forms 43, 45 and 34 respectively. The straw in each case was collected early in 1926 and exposed at H. A. College throughout the winter. Tests made at the end of September gave positive results in each case. Further reference to this is made under the heading of "Aecidial Stage".

In 1927 a fairly extensive series of tests was planned with a comprehensive set of teleutospore material representing six physiologic forms. Accidental interference with the frames containing the straw was responsible for the bulk of the material being ruined. The little that was left comprised teleutosori of Forms 34, 44, 45, Indian teleutospores, and P. graminis avenae 2. Germinations of all were abundant at the end of September and a little of the material was available for barberry inoculations as reported under the heading "Aecidial Stage". The opportunity of making comparative germination tests, however, was lost.

In 1928 further tests were made with spores of known physiologic forms in order to produce infections on the barberry. When the first test was made on the 6th September good germinations were obtained. The results of this work on the barberry have been reported elsewhere (165).

Summary

It is clear from these studies that germinations of the teleutospores of P. graminis tritici take place much sooner after their formation than was formerly supposed. Whilst September and October are the months when the germinations are most abundant, the spores have been found to give sparse germinations as early as March, continuing thereafter to give somewhat capricious germinations up to the peak period. After November positive results are not to be expected. These results refer to material kept under natural conditions. By utilising cold storage the range could be widely extended. There has been no notable difference in the germination capacity shown by teleutospores of different physiologic forms.

THE UREDOSPORE STAGES OF P. GRAMINIS AND P. TRITICINA.

There can be no doubt but that this stage is the important one in Australia. It is by means of uredospores that dissemination of rust throughout the crop takes place. Specialisation studies involve the use of this stage mainly, although differences between physiologic forms may be discernible in the other stages of the life history. As might be expected then, attention has been devoted mainly to the studies involving this stage.

SPECIALISATION STUDIES.

HISTORICAL.

The occurrence of specialisation in Puccinia graminis Pers. and in other rust fungi has long been known and is a phenomenon of outstanding importance. Dietel (35) recorded a certain amount of morphological and physiological variation in rust fungi. Eriksson (38) showed that there was distinct specialisation of parasitism in P. graminis Pers., and divided the species into five "formae speciales". All produced aecidia on the barberry. Hitchcock and Carleton (70) in U.S.A. obtained results of a confirmatory nature. The fact of specialisation in the rusts was still further confirmed by Magnus (89), Rostrup (124), Klebahn (81), Marshall Ward (158A), and others.

Later studies have revealed a much more complex state of specialisation. Stakman and Piemeisel (137) in 1917 found that P. graminis tritici E. & H. was not a simple organism, but that there were at least two strains of it. In 1918 Melchers and Parker (100) discovered another strain of the rust. Levine and Stakman (83) in the same year recorded a further strain, and began to make use of certain varieties of wheat as differential hosts to effect a sorting out of the physiologic forms. As early as 1919 these workers had deter-

mined twelve distinct physiologic forms of the fungus. Since then the work has progressed to a remarkable degree. A standard technique developed by Stakman and Levine in U.S.A. has been adopted and is being applied in other important wheat-producing countries. The latest announcement from Minnesota shows that 55 physiologic forms of P. graminis tritici have so far been determined. In Australia it appears that no previous work has been done on this problem. McAlpine (105) was familiar with the phenomenon of specialisation, but reports having had no opportunity of carrying out investigations concerning it.

Present day methods of investigation have been mainly developed by Stakman and Levine (144) in dealing with P. graminis, and by Mains and Jackson (96) in the determination of the forms of P. triticina. These workers with characteristic courtesy have made available to the writer cultures of their differential hosts, and have thus made it possible to study specialisation here in such a manner as to obtain results comparable with theirs.

Method of determining physiologic forms.

To indicate the method that is used, the procedure followed in the case of P. graminis tritici may be set out. It has been developed by Stakman and Levine (144), also Levine (87), and is briefly as follows. Twelve standard varieties of wheat have been selected as a result of much careful work. They are shown in the following table.

Table 3. The set of differential hosts selected by Stakman and Levine for the determination of physiologic forms of Puccinia graminis tritici E. & H.

<u>Species of Triticum</u>	Varietal Name	Cereal Investigation Number.
T. compactum	Little Club	C.I. 4066
T. vulgare	Marquis	C.I. 3641
"	Kanred	C.I. 5146
"	Kota	C.I. 5878
T. durum	Arnautka	C.I. 4072
"	Mindum	C.I. 5296
"	Arnautka (Spelmars)	C.I. 6236
"	Kubanka	C.I. 2094
"	Acme	C.I. 5284
T. monococcum	Einkorn	C.I. 2433
T. dicoccum	Vernal Emmer	C.I. 3686
"	Khapli	C.I. 4013

Grain of these standard differential hosts was kindly supplied in 1921 by Dr. E.C. Stakman and Dr. M.N. Levine, and has since been grown in pure lines for use in the determinative work.

Seedlings to the number of about fifteen are grown in small pots. The first seedling leaf is moistened with water and by means of a flat sterile needle, the uredospores of the rust under examination are placed in this water adherent to the leaf. The pots are then incubated in a saturated atmosphere for 48 hours. They are afterwards placed on well-lighted benches in the plant house to allow the rust to develop. Notes are taken on the rust development twelve to sixteen days later, depending upon the prevailing weather conditions.

The types of infection shown by each host are recorded by a simple notation. Arabic numerals from "0" to "4" indicate the order of severity of infection. "0" indicates immunity, and "4" complete susceptibility. Fluctuations between these types are indicated by plus and minus signs. A sixth type is represented by X. This indicates that a heterogeneous reaction occurs on the one plant. A single leaf may show all classes of reaction ranging from "1" to "4", and further simplification of these reactions is impossible.

The explanation of these symbols is set out here-
under.

Resistant Class.

Type "0". - Host immune.

No uredosori are developed, but sharply defined hypersensitive flecks may be present, indicated then as "0".

Type "1". - Host very resistant.

Infection is very light; uredosori are minute and scattered, and surrounded by sharp, continuous, necrotic areas.

Type "2". - Host moderately resistant.

Infection is light; uredosori are small to medium in size. Hypersensitive areas in the form of necrotic halos are present. The pustules occur in green, but slightly chlorotic islands.

Susceptible Class.

Type "3". - Host relatively susceptible.

Infection is moderate; uredosori are medium in size and show a tendency to coalesce. True hypersensitiveness is absent excepting when cultural conditions are unfavourable.

Type "4". - Host completely susceptible.

Infection is normal and heavy. Uredosori are large and generally confluent. Hypersensitiveness is absent excepting when cultural conditions are unfavourable.

Indeterminate Class.

Type "X". - Host intermediately susceptible.

Infection is of an heterogeneous nature. Uredosori are very variable and apparently include all types and degrees of infection on the one leaf; no mechanical separation is possible since spores from small and from large uredosori alike produce the same heterogeneous infection.

These classes of reaction are illustrated in Plate II.

When the class of reaction on the standard differential hosts has been determined, the results are tabulated. From these tabulated results a dichotomous key has been prepared, and by using this it is easy to determine the physiologic form of the rust under examination.

In the case of the determination of P. triticina, a different series of wheat varieties is used, whilst in dealing with P. graminis avenae, a particular set of oat varieties has been selected. But in other respects the methods are essent-

ially the same. In taking notes on the reactions given by these rusts, it has, of course, to be remembered that complete susceptibility to P. triticina means that a pustule much smaller than that produced by P. graminis tritici is found. Similarly in dealing with P. graminis avenae, in very susceptible oats the large "4" reaction is commonly surrounded by a somewhat chlorotic area. But in the main the same system of note-taking holds

PURITY OF DIFFERENTIAL HOSTS.

Attempts to grow rust fungi on artificial media have so far failed. The only method of cultivating them is to make use of living host plants. Were it possible to utilise synthetic media, the rust problem would be greatly simplified.

Variation amongst living organisms is universal. To determine the variation - specialisation - within the fungus causing stem rust, living host plants must be used. These in turn are subject to variation. The extent of this variation it is important to investigate. The twelve varieties have already been enumerated. It is clear that if the determination of physiologic forms by this means is to be effected and the results are to be comparative, every care must be exercised in order to keep the differentials pure and true to name.

The need for this became apparent early in the studies. These have been possible only through the courtesy of Dr. Stakman and Dr. Levine in supplying grain of their standard differentials. It was expressly stated that in certain cases the sample given was known not to be pure. Results obtained with this grain prior to its being multiplied in Australia indicated that this was the case, and single plant selection has been practised from the beginning of the studies in 1921. Studies were made of some of the aberrant types.

The results of some of these studies of the variation shown by the differentials are of interest.

Arnautka.

Grain of this variety as received from Minnesota was observed to give seedling reactions which were sometimes abnormal. Pots of seedlings inoculated with Form 43, known to normally produce flecks on Arnautka, gave occasional plants, varying from 2% to 5% of those tested, which showed a fully susceptible reaction.

In 1926, six seedlings derived from Minnesota-grown grain and showing the characteristic flecks produced by Form 43, were transplanted to an open bed and grown to maturity. At the same time, four showing the aberrant "4" type of reaction were similarly taken. At maturity, all the resistant plants were of the "Arnautka" type, as were three of the four susceptibles. But the fourth plant proved to be a "vulgare" wheat conforming to Triticum vulgare erythrosperrum Körn. Clearly admixture of foreign grain with that of "Arnautka" had taken place.

In 1927, grain derived from each of the ten plants was sown and tested with Form 43 before the seedlings were transplanted to the open and grown to maturity. The progeny of the six resistant plants all gave resistant seedlings, whilst those of the four susceptibles gave all susceptible seedlings. At maturity a number of random selections, varying from three to six, were made from each family.

In 1928, the seedlings of the selections were again tested with Form 43, with the confirmatory result that progeny of the resistant plants were resistant, and those of the susceptibles were susceptible.

A scrutiny of the susceptible durumms in these tests

failed to reveal any morphological or marked cultural differences between them and the normal Arnautka types. Whilst the mixing of grain of a "vulgare" wheat with that of Arnautka could be readily discerned and corrected, the occurrence of two apparently identical types showing susceptibility in one case and resistance in the other might well lead to confusion and error in the results.

Mindum.

A state of affairs similar to that reported for Arnautka was found to occur with Mindum, and was also studied. In 1926 three seedlings derived from Minnesota grain which showed the expected flecking with Form 43 were grown to maturity, as were also two which showed a "3" type of reaction. The latter proved to be "vulgare" wheats again conforming to T. vulgare erythrosperrum Körn. Tests in 1927 of progeny of all five families showed that they bred true for their rust reaction, and this result was confirmed by a comprehensive series of tests in 1928 made with further random selections in each of the families.

In this case the aberrant results were due to impurity of the grain.

Kanred.

In the earliest studies made with grain kindly provided in 1921 by Dr. E.C. Stakman, as well as those in later years when grain was forwarded by Dr. M.N. Levine, departures from the normal reaction on this host were noted. For example, pots of twelve seedlings inoculated with Form 43 frequently showed that eleven were completely immune, and one completely susceptible. Summation of a number of these

results shows a frequency of from 5 to 8% of these susceptible plants. Inoculation of a fresh pot of Kanred with inoculum from a susceptible Kanred would give a similar result, practically all the seedlings being immune.

A study was commenced in 1926 with grain received from Minnesota in that year. Eleven plants conforming to the Kanred type at maturity were taken at random, and progeny of each was tested with Forms 33, 43 and 46. Kanred normally is susceptible to Form 33 and immune to Forms 43 and 46.

The grain of each of the eleven plants was divided into three portions which were sown in pots for the seedling tests. The results showed that there were three classes of families. In the first class there were six families which each showed susceptibility represented by a "3" reaction to Form 33, and immunity (no reaction whatever) to Forms 43 and 46. In the second there were three families which showed the susceptible reaction known as "3" to Form 33 and the moderately resistant "2" type of reaction to Forms 43 and 46. In the third class, there were two families which were each susceptible to Form 33 and also susceptible to Forms 43 and 46, giving a "3" reaction throughout. These seedling results were borne out by field observations. At Hawkesbury Agricultural College where Minnesota-grown grain of Kanred was sown and where Forms 43 and 46 were present, it was observed that the majority of the Kanred plants were quite free of rust, but occasional plants showed a severe attack of stem rust. Form 33 is a culture which is being kept under control in the plant house, not having occurred naturally in Australia.

In 1927 three out of the six families belonging to the first class were taken at random and the rest discarded. From each of these three families as well as from the three families belonging to the second class and from the two belonging to the third class, ten single plants were taken and their

progeny subjected to seedling tests with the three rust forms. In all cases they bred true to their class reaction. The only departure from a normal state of affairs occurred in one of the ten plant progenies belonging to the third class, where two seedlings from a pot of fifteen revealed the "grass tuft" habit. Further studies concerning this occurrence are in progress.

In 1928 further pedigree tests with 41 progenies of plants from each of the three classes were made. Again it was found that the seedling reactions were true to their class.

In 1929 a still further generation was tested, comprising families from each of the three classes. The reactions given were characteristic of the respective classes.

In Kanred, therefore, there was a mixture of strains indistinguishable morphologically, but showing wide divergence in their rust reactions.

Kubanka.

The case of this differential has been complicated by reason of the effect which altered environmental conditions apparently have upon the host-parasite relationship. In the earlier days of the investigation, before this effect was recognised and when attempts were being made to sort out pure lines of the variety, considerable difficulty was met with.

As far back as 1921, seedling results with grain kindly supplied by Dr. Stakman showed the mixed results similar to those already described for other differentials. At the harvest of 1922, eight apparently identical single plants were selected, and have been grown in pure lines since. A very great number of tests have been made from time to time with these selections.

From the outset, marked differences were shown between the eight families. Thus in 1923, the reactions given by Form 27 were of the "4" type with one of the families and of "0" type with another. An exact reversal of these reactions was given when these same two families were tested with Form 46. That is to say, the reaction on Kubanka given by each of these forms might have been listed as completely susceptible or completely resistant, depending upon the particular strain used in the test. This sort of divergent result was obtained in an extensive series of tests which utilised 55 different rust cultures representing six different stock physiologic forms.

In following years tests have been extended and show clearly that the eight plants taken at random in 1922 were not all genetically alike as regards rust resistance, although all appear to conform to the Kubanka type. They are divisible into three groups on the basis of the reactions shown in a series of tests made simultaneously under summer conditions to Forms 43 and 46. Five of the eight were susceptible to 43 and resistant to 46, one was resistant to 43 but susceptible to 46, and two were susceptible to both these forms. Further references to these results are made in the consideration of variations induced by environmental changes.

Other strains of Kubanka derived from other sources were also subjected to test. For example grain of Kubanka C.I. 2094, received from Dr. M.N. Levine in 1926, behaved like the first group of five families listed above, as did also grain of "Kubanka C.I. 8". Grain of "Kubanka C.I.10" gave results similar to those of the second group. Grain of "Kubanka" supplied by Mr. J.T. Pridham of Cowra, showed under summer conditions that the seedlings were equally susceptible to every one of the ten physiologic forms which were available for test.

The urgent necessity for scrupulous care in maintaining

the same pure lines if work at different centres is to be comparative is clearly brought out in this work with Kubanka.

Occurrence of Natural Crosses.

Natural crossing in pedigree families may occur and seriously complicate results. Several cases have come to light.

In a row test of "Indian 12" at the University of Sydney, two natural crosses occurred. The grain used for sowing came from a single ear of a typical plant grown at H.A. College in 1924. In the row test were noted two plants which were not fully bearded, but showed only tip-beards. Each was saved and its grain sown the following year. The progeny varied in regard to beard, showing gradations between slight tip-beard and full beard. There was also segregation for the smooth chaff and velvet chaff. There is little doubt but that natural crossing of "Indian 12" had occurred at H.A. College in 1924.

Another case dealt with a variety of Triticum sphaerococcum Perc. In 1924 several ears of two varieties of this wheat were sent from Cambridge, England, by Mr. F.T. Brooks. The grain of one of these ears was rubbed out and sown at the University of Sydney in a row test. One of the resultant plants was markedly different from the type, being a "vulgare" wheat with a tip-beard. This was saved and its grain sown the next year. Segregation occurred in regard to a number of characters, as illustrated in Plate IV. Clearly in this case the natural cross had occurred at Cambridge.

An instance in oats was very striking. Grain of "Richland" grown at Cowra in 1926, was sown at H.A. College the following year. A number of single plants were harvested. One of them had grain larger than the normal "Richland" grain.

A box of it was sown and the seedlings tested with P. graminis avenae l. This form gives a resistant reaction on "Richland". The test showed 103 resistant and 41 susceptible plants. This is a near approach to a 3:1 ratio, and indicates that probably at Cowra a natural cross between "Richland" and a susceptible variety like "Algerian" had taken place.

THE EFFECT OF VARYING ENVIRONMENTAL CONDITIONS IN THE PLANT HOUSE UPON RUST DEVELOPMENT.

The technique employed in the determination of physiologic forms has already been referred to (page 17 et seq.) and the statement made that after inoculation the pots of seedlings are placed on well lighted benches in the plant house to allow the rust to develop. Under the conditions which prevail in some seasons in U.S.A., sunlight is lacking. In N.S.W. the opposite is often the case. Here there are wide divergences between the conditions in the plant house under winter as compared with summer conditions. The differences in the temperatures are very considerable. Evidence has accumulated which shows that such a difference in the environmental conditions may profoundly affect the rust reaction upon particular hosts. Since the form determination is based upon the host reaction, this matter is of extreme importance.

It is unfortunate that no temperature control apparatus has so far been available for these studies. It has therefore only been possible to take advantage of the variations in the plant house conditions which have been induced by seasonal changes. The tests made have practically involved a comparison of results obtained under winter conditions with those found under summer conditions. In the early stages of the work, this variation in the results obtained was not recognised, with the consequence that a number of contradictory results

were obtained which were not then intelligible. So much evidence has now accumulated, that it is quite clear that determinative work must be done with a narrow range of fluctuation in the environmental conditions if the results are to have a value for comparative purposes. If it be remembered that the type of reaction which is taken note of in the determinative work is a measure of the host-parasite relationship, it is not surprising that the balance existing between the two should be liable to variation as environmental conditions change. It is not possible at this juncture to indicate the extent to which fluctuations in the specific environmental factors such as temperature and light bring about an alteration in the reactions, or of the degree of the response made by the host and by the parasite to these fluctuations. That marked effects in the rust reactions are induced will be seen to be indisputable.

Material and Methods.

A comprehensive series of tests was made to provide information regarding the behaviour of the Australian forms, as well as a comparison of their reactions with those of other valuable forms under varying environmental conditions. The following forms were used in the tests :-

Form 16. This was derived from aecidial infections of the barberry produced by inoculation with teleutospore material on wheat straw received from Pusa, India, in April 1927.

Form 27. This had its origin in aecidial infection of a barberry produced by inoculation with teleutospores on wheat straw received from Milton, Wales in May 1923 from Miss K. Sampson of Aberystwyth and Mr. A.D. Cotton of Kew.

Form 33. This form was supplied in the uredospore stage by Dr. M.N. Levine in 1922.

Forms 34, 43, 44, 45, 46, 54, 55. There were all obtained from collections of uredospore material made at different times from various localities in Australia.

Throughout the series of tests, the same culture of each of the forms was used. In no case was it a monosporous culture, but its reactions on the differential hosts were repeatedly checked. Of Form 45 two distinct cultures were used throughout. One reason was that one of them which originated from aecidiospores and is designated Form 45A, has always given a "2" reaction on "Kanred" whereas all other cultures of Form 45 have given a "0" reaction, and a search was being made to find a wheat which would serve as a wider differential of this culture.

These forms were tested on a number of the differential hosts. The results are described separately under the headings of these hosts.

Kubanka.

Reference has already been made to difficulties encountered with Kubanka as a differential, and to the fact that a series of single plant selections was made of several strains of this variety in founding pure lines. Tests made simultaneously with the one form on certain of these pure lines showed conclusively that there was extreme genetic diversity as regards rust resistance. A good deal of work was done in testing these pure lines in their reactions to known physiologic forms under varying environmental conditions, with interesting results.

The strains of Kubanka used were as follows :-

"Kubanka No. 1" to "Kubanka No.8". These 8 strains had their origin in grain labelled "Kubanka C.I. 2094" kindly

supplied by Dr. E.C. Stakman in 1921. This grain was sown in a small plot at the University of Sydney and at the harvest of 1922 eight single plants were taken at random and have since been grown in pure lines.

"Kubanka 2094". This line had its origin in a sample of grain sent by the Bureau of Plant Industry of the United States Department of Agriculture in 1919. From a row of it a random selection was made of a typical plant growing at H.A. College. It has since been grown as a pure line.

"Kubanka Minn." This line came from grain of "Kubanka C.I. 2094." kindly supplied by Dr. M.N. Levine in 1926, and since grown in pure line.

"Kubanka C.I. 8" The history of this is the same as that of "Kubanka 2094."

"Kubanka C.I. 10". This line came from grain supplied by Dr. E.C. Stakman under this name.

"Kubanka Cowra". In 1922 Mr. J.T. Pridham of Cowra Experiment Farm supplied grain which has since been pure lined. This strain has heads which are rather more compact than those of "Kubanka C.I. 2094," and is a rather heavier grain yielder under conditions prevailing at the University of Sydney and H.A. College.

There were, therefore, thirteen strains available for testing.

In the years 1924 to 1927 a number of tests were made with these strains at different times. Using the identical cultures which have been used throughout the studies to represent the particular forms, inoculations were made from time to time. The results were not identical. To illustrate the sort of variation found, the results obtained on "Kubanka 1" are set out in the following table.

Table 4. Reactions produced as a result of inoculating "Kubanka 1" with certain forms of P. graminis tritici at different seasons.

Form No.	Date of Inoculation				
	Oct. 1924	Oct. 1925	March 1926	May 1926	May 1927.
27	4	2	3	3	3
33	3	;	x	;	;
34			3	;& 1	;
43	x	x	x	;	;
45		2+	x	3	3
46	2+	1+	2	2	2
55	x	x	3 ^c	x	3+

It will be seen that a good deal of variation is shown in the reactions given by the same form in these tests. Uniformity in the results is lacking. But there are indications that the susceptibility is greater in those tests made in the hotter months.

Other tests utilising others of the strains of Kubanka gave results which were essentially similar.

To better investigate this variation in the reactions a more complete and comparative series of tests was carried out. The tests were made in pairs and were performed twice. For the first pair, a single plant of each of thirteen pure lines of Kubanka was used. Half of its grain was used for the "summer test", made in February 1928, and the balance in the "winter test" made in June 1928. For the second pair of tests, another single plant of each line was used, half the grain being used in the "winter test" made in July 1928, and the remainder of the grain from the same plant used in the "summer test" made in January 1929. An exception occurs with Form 11, and "New Form", which were tested in January 1929 and July 1929, not having been available earlier.

In all cases, every precaution was taken to ensure that the inoculum used was what it purported to be. Prior to the test a form determination on the standard differentials was made to ensure that no contamination of the culture had taken

place. The inoculum used was taken from an appropriate susceptible host. All the inoculations of any particular test were made on the same day and the pots of seedlings treated in the same way. The best possible conditions of light were given, all the pots being placed on a northern bench of the plant house. Note-taking on each test was done on the one day when the reactions were fully developed and the results checked two days later.

The following table sets out the results.

The results show clearly that there is great genetic diversity between the strains of Kubanka as judged by their rust reactions. There are numerous indications of this. For example consider the reactions to Form 46 given in the first of the series of tests, viz., "Summer 1928". "Kubanka 1" gives "1", "Kubanka 2" gives "4", and "Kubanka 5" gives "4". Form 27 in the same test gives on the same "Kubanka 1" "4^C", on "Kubanka 2" it gives flecks, and on "Kubanka 5" it gives "4^C".

The thirteen strains seem to fall into three groups within which the members somewhat closely resemble each other.

Group 1. Eight of the strains behave, in the main, in the same way. They are Kub. 1, 3, 4, 6, 8, 2094, Minn., and C.I.8. It is believed that this group represents the true Kubanka strain as used in the determinative work. It is bulk grain of the Kubanka Minn. which is used as the Kubanka standard in the determinative work on the physiologic forms. Tests made with this bulk grain have given results similar to those of Group 1.

Group 2. There are three of the strains which appear to be related, viz., Kub. 5, Kub. 7, and Kub. Cowra. All ten forms give reactions which in general agree on these strains.

Group 3. The remaining two strains, viz., Kub. 2, and Kub.C.I.10, show resemblances to each other. It was unfortunate that through an error the latter was omitted in the 2nd. series and only one year's comparative tests were made with Kub. C.I.10. But on this basis there is a general agreement between the two strains.

The agreement between the two summer and the two winter reactions is not absolute. In most cases the disagreement is slight. Again it is pointed out that differences in the conditions prevailing in the plant house may conduce to

this lack of agreement. In addition, the conditions prevailing at the times when the two summer tests were made were not identical, nor were they when the two winter tests were carried out. Higher temperatures prevailed in the summer test of 1929 than in the 1928 test. But in the main, there is a similarity between the two series of tests.

Within these three groups certain variations occur. Exact agreement is hardly to be expected. Major variations between the summer and winter reactions are shown in many cases. Thus Form 33 On Kub. 1, gives "3" in the summer and ";" in the winter. From other tests made during spring and autumn, evidence is available that intermediate reactions are to be expected then. In the plant house, particularly where a large series of tests are in progress at the one time, it is not possible to arrange all the pots of seedlings so that every one shall be under exactly the same environmental conditions. For example the degree of lighting and the amount of heat received from the sun vary considerably. This may result in a certain variation in the reaction shown. Only when adequate control apparatus is available can this point conclusively be cleared up. But within certain limits of variation the massing into the three groups would seem to be valid.

It is clear that certain forms show a much more marked variation between the summer and winter reactions than do others. Thus Forms 16, 27, 45, 46 and 55 do not exhibit a very marked difference between the summer and the winter reactions. On the other hand, Forms 33, 34, 43, 44 and 54 show, in most cases, a marked contrast between the reactions given under the two sets of conditions. This means that in the first case the host-parasite complex is not markedly affected by the range of seasonal variation, whereas in the

second case it is. It is to be emphasised that evidence is brought forward under other headings to support the contention that Forms 43, 44 and 54 are rather closely related, as are also Forms 45, 46 and 55.

Acme.

From the grain received from Minnesota in 1921, four typical plants were selected at random in 1922 when the Kubanka selections were made. Under the names Acme X, Acme 1, 2 and 3, they were pure lined. From the further supply of grain received from Dr. Levine in 1926, a further selection was made and designated "Acme Minn." A series of tests similar to those made with the selections of Kubanka was conducted with the same cultures on these five strains of Acme at the same times that the Kubanka tests were carried out.

The results are set out in the following table:-

Table 6 . Means of reactions on certain strains of Acme given by physiologic forms of Puccinia graminis tritici when tested under different environmental conditions.

Form No.	Acme X.		Acme 1.		Acme 2.		Acme 3.		Acme Minn.								
	Test		Test		Test		Test		Test								
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd							
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter							
11			3	X-			3	X=			3	X=					
16	3	3	4	3	3	3	4	3	3	3	3	3	4	3	4	3	
27	X+3	3+3			4	X	4	3	4	3	4	3 ^c	4	3	4	3	
33	3	3 ^c	3	X	3	3	3	3	3	3	3	3	3	X	3	3	3 ^c
34	X	3 ^c	3	X	3+X	3	3	X+X	3	X	X+X	3	X	3+X	3	3	
43	X-;	3+;			X-;	3+;			X-;	3-;	X;	3+;		X-;	3;		
44	3	X	3+X		4	X	3+X	4	X	3+X	X+X	3+X	3	X	3	X	
45	X	X	X-X-		X	X-3 ^c	X-	X	X	X	X	X	X	X	X	X	X
46	1	2-	1-2-		1	2=1	2-	1	2=1	2-	1	2=1	2-	1	2=;	2-	
54	X+X-	3	X		4	X	3+X-	4	X-	3+X-	3	X=3+X-	X	X=3+X-			
55	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
New Form			3	1+			3	1			3	1			3	1	

It will be seen that the variation that occurs between the strains is very slight. The difference between the summer and winter reactions is much less than in the case of Kubanka. Some of the forms do show a variation. This is notable in the case of Form 43 and Form 54, where the change from "3+" in the summer to a fleck in the winter is extreme.

Marquis.

When the series of tests were in progress with the selections of Kubanka and Acme, a pure-line of Marquis was also included. In previous work there was evidence that changes occurred in the reactions given on this host. The strain used came from a single plant selected in 1922 from grain supplied by Dr. Stakman.

The results are as follows :-

Table 7 . Means of reactions on a pure line of Marquis given by physiologic forms of Puccinia graminis tritici when tested under different environmental conditions.

Form No.	Marquis 1.			
	Test			
	1 st		2 nd	
	Summer	Winter	Summer	Winter
11			3+X-	
16	2	2-	3 ^c	2
27	2+2		4 ^c	2
33	2+1		3	2≡
34	4	3	3+3	
43	3	3	3	3
44	3+3		4	4
45	2	2=	2+2	
46	4	2-	4+2	
54	4-3		4	3
55 New Form	4	2	4+X=	
			3+2	
	.	.	.	

Again it is seen that marked variations occur in the reactions given by certain forms under summer as compared with winter conditions. This is notable in the case of Forms 46 and 55, in which an extreme range of variation occurs. A change from a "4" to a "2-", or from a "4+" to an "X=" reaction is one of major importance and again liable to be very upsetting in determinative work. Thus Form 55 is separated from Form 45 on the basis of the reactions on Marquis; this host gives "4" with the former and "2" with the latter. That there is a distinct difference between these forms is shown by other tests elsewhere reported.

There is evidence that intermediate degrees of resistance (or susceptibility) may be shown under the intermediate environmental conditions prevailing between summer and winter.

Thus in a test made in February 1928 it was found that five cultures of Form 45 - including the stock culture used throughout the work - were giving an "X" reaction on some Marquis plants in addition to a "3+" reaction on other plants in the same pots. In each case, from the "X" reactions cultures were started from the "2" and from the "3+" pustules on the same leaves, as well as cultures from the straight-out "3+" reactions. From these fifteen cultures on Federation comparative tests on the standard differential sets were made in April. The form in each case was Form 45, the reactions of Marquis varying from "1=" to "2". The stock culture of Form 55 was included in this test for comparative purposes. It gave the typical reactions, including the "4" on Marquis.

VARIATION IN FORM 34 ON THE DURUM DIFFERENTIALS.

Further evidence of extreme variability in the rust reactions on account of altered environment is available in connection with Form 34. This form was first found in

Western Australia in 1925. The next year it occurred in New South Wales and since then has been the predominant form in Australia. Changes in its reactions on three of the durum differentials have again and again taken place. In the summer the normal susceptible reactions which are listed for 34 are given. But in the winter, an entirely different set of resistant reactions is obtained. When these winter reactions were first observed it was considered that a different form was concerned. They occurred so frequently that the reactions were listed and forwarded to Dr. Stakman, who allocated a number to the form. It is now known that it was Form 34, and that transitions between the two occur.

The first case occurred in an isolation from wheaten straw collected at Bathurst. This was tested in the usual way in April 1927. Variation from the normal reaction given by Form 34 was shown on the durums. Instead of susceptible reactions throughout, the results were Arnautka "4", Mindum "0", Spelmars "0", Kubanka "X", and Acme "3". A repetition using inoculum from Little Club gave the same result, excepting that there was now only one Arnautka plant in the pot which showed a "4" reaction, whilst three others showed flecks. A further test was made, the result being the production of flecks on Arnautka, Mindum and Spelmars, "X" on Kubanka, and "3" or sometimes "X" on Acme. Three further sets of differentials were inoculated from different hosts in August and again the same resistant series of reactions was given. The culture was kept by sub-culturing on Federation until January 1928 when it was cultured on the set of differentials. It gave the susceptible reactions on the durums characteristic of Form 34. In March 1928 the same normal Form 34 reactions were shown. In May 1928 the durums again gave the resistant reactions. Illustrations are shown in Plate V.

Another culture from material collected at Wagga

came up for test in June 1927. It gave the resistant reactions on the three durums. Inoculum from six of the hosts, viz., Little Club, Marquis, Kanred, Kota, Kubanka and Acme was used to inoculate six further sets. In all cases the result was the same. Three of these subcultures were used early in September on three further sets of differentials, and again the resistant reactions were shown. At the end of November these cultures were again used to inoculate sets. This time the normal susceptible reactions for 34 were given.

Eight other newly received isolations from various localities were dealt with in the same way and gave the same result.

One of the two stock cultures which had been obtained in 1925 and since kept in culture was placed on a set in June 1927. The resistant reactions were shown on the durums.

The principal stock culture of Form 34 which had been isolated in December 1925 and had since been used in repeated tests, was tested in the same way. In June 1927 the resistant reactions were given. In October 1927, two tests gave the susceptible reactions. The same result was obtained in January and March 1928. On 24th. April one set was inoculated and kept in the warmer and sunnier plant house, and another set treated in the same way was kept in the cooler and shadier plant house. In the former, the reactions varied in the pots of durum seedlings. A typical result from a pot of sixteen seedlings was that five showed "4", six showed a mixture of "3", "2" and flecks, and four showed flecks only. In the set kept under the cooler conditions no case of simple "4" reactions occurred on the durums. On the same leaf were found flecks, "2" and "3" reactions, the latter occurring much less frequently than the flecks and "2" reactions.

Another pair of sets was inoculated and incubated under the same conditions. In the warmer situation, the

mixture of flecks, "2" and a few "3" reactions on the same leaves was shown. In the cooler position only flecks occurred.

On 28th May two further sets were treated in the same way. In both cases only flecks were shown. The next inoculation made on 2nd July, gave the mixture of "0", "2", and "3" in the warm situation, and flecks in the cool place. An inoculation on 22nd. August gave the same result. On the 10th September the results were some simple "4" reactions and others with the mixture of "0", "2" and "3" in the warm situation, and only ";", "2" and "3" in the cool location. On the 8th October the result was the normal "34" susceptibility in the warm situation, whilst in the cooler house there were "4" reactions on some leaves and the mixture of ";", "2" and "3" on other leaves in the pots. In December 1928, the normal "34" reactions were given in both locations.

It is clear that the same culture showed considerable variation in its reaction depending upon its environment. The transition was followed from susceptibility to resistance and back to susceptibility.

Lacking the temperature control facilities, another method of attack was followed. Early in January 1928 a large amount of heavily rusted wheat straw and a second batch of similar barley straw both in a green condition were collected at H.A. College from two plots which had repeatedly shown the presence of only Form 34. The straw was cut into short lengths and allowed to partially dry out during two days exposure in the laboratory. Each batch was then divided into 25 equal packets of rusted straw. One of each was used to inoculate a set of differentials. The other packets, thanks to the courtesy of Peters' American Delicacy Co. Ltd., were kept in their ice-house. At intervals of about a fortnight a packet of the wheaten and one of the barley straw were withdrawn. Inoculum from the straw was scraped into specimen tubes

containing distilled water, and the mixture of spores transferred to the moistened leaves of the differential hosts directly up to the set used on the 17th April. Thereafter, owing to lessened viability it was inoculated on to large pots of Federation seedlings and thence on to the sets of differentials.

The initial inoculation made on the 4th. January gave in each case the normal 34 reactions. Each of these cultures has been kept alive since by subculturing on Federation. An inoculation of sets made on 25th. June 1928 gave from each the resistant reactions on the durums. Tested again in November 1928 they gave the complete susceptibility.

Withdrawals made 24th January, 11th. and 27th February, 15th. March and 2nd. April gave the normal susceptibility on the durums. The set inoculated on the 17th April gave the mixture of "0;", "2" and "3" reactions on the same leaf. Inoculum from the next withdrawal made on the 30th April was not available in sufficient quantity for a set until 6th June. It then gave only "0;" on the durums. The same applied to withdrawals made on the 14th. and 28th. May and 11th. June. Withdrawals on the 26th. June and 9th. July gave inoculum for sets on the 13th. August and in both cases gave the "4" reaction on some leaves, and the "0;", "2" and "3" reactions on others in the same pots. Both of these particular cultures were again put on to sets in November and gave the fully susceptible reactions. Further withdrawals made on the 23rd. July, 6th. and the 23rd. August, and 4th. September, failed to give any infections of the Federation seedlings. But it was considered very satisfactory to have kept the uredospores in this viable condition for six months.

This test showed clearly that the normal susceptible reactions given by Form 34 on the durums alter during

the winter months to resistant types.

Another test was made. This time a batch of heavily rusted wheat straw was obtained from Cowra in July 1928 from an area where only Form 34 had been determined. It was made up into equal packets which were stored in the ice-house and tested at intervals.

The initial test made on the 30th July gave only flecks on the three durums. This initial culture was kept until November 1928 and again put on the set. It gave complete susceptibility on the three durums. On the 23rd. August and 4th. September there were "0;", "2" and "3" reactions. On the 24th. September some leaves showed "4" others the mixture of "0;", "2" and "3". Withdrawals were made on the 2nd., 16th., and 30th. October, and 13th. and 27th. November. They gave the complete susceptibility. Further withdrawals on 11th. and 27th. December 1928 failed to give any infections.

This series of tests showed that the material from which the resistant reactions were obtained during the winter gave the susceptible type during the summer.

Apart from this significant variation in the reactions on the durum varieties, some difference was discernible on the other differentials. On Little Club, Marquis, Kanred and Kota, the summer reactions of "4+", "4-", "4", and "4=" became respectively "3+", "3", "3-", and "3-". On Einkorn and Khapli, the summer reactions of "1=" in each case became "1++" in each case. Vernal Emmer showed no appreciable change. Variation of this order has little significance.

VARIATION IN FEDERATION AND KHAPLI.

Form 46 has been in culture since 1924 and has been repeatedly tested on the differential sets. That alterations occur in the reactions given on Kubanka and Acme when summer and winter tests are compared, has already been shown. A further striking variation has repeatedly been noted and is illustrated in Plate V.

For the purpose of subculturing the various wheat stem rusts, Federation seedlings are used. On them the reaction given by Form 46 during the warmer part of the year is "4+ ". During the winter this falls to a reaction approximating to a "1+ ". That is to say, very small isolated ure-doseri occur on highly chlorotic and somewhat necrotic areas. In the winter of 1927 when this weakening in the reaction on Federation was overlooked, three subsidiary cultures of 46 were lost through an insufficiency of inoculum for subculturing.

It has been noticeable that Form 27 which was obtained from teleutospores collected in Wales behaves in the same way, giving a very weak reaction on Federation during the winter. Just the opposite has frequently occurred on Khapli. On this host, the summer reaction is a very sharp "1". But on many occasions in the late autumn and the early spring, the reaction has been a "4". It was thought that this might have been due to the grain used being something other than true Khapli. Such "susceptible" plants grown to maturity have, however, been typical of Khapli, and have given grain which in the summer seedling tests have been quite resistant. Illustrations of these Khapli reactions are shown in Plate V.

EFFECT OF SEASONAL VARIATION IN THE PLANT HOUSE UPON REACTIONS OF P. graminis avenae ON OAT SEEDLINGS.

Single plants of "Joanette", "White Tartar", and of "Richland" oats were taken. Part of the grain was tested under summer conditions (February 1928) and part of the same grain tested under winter conditions (June 1928). Two different cultures of each of the three forms available were used. The results were as follows :-

Form No.	<u>Joanette</u>		<u>White Tartar</u>		<u>Richland</u>	
	Summer	Winter	Summer	Winter	Summer	Winter
1	3+	;	2++	2-	2+	2 =
2	3+	3	2++	2-	2+	2 =
7	3+	3	4	4	2+	2

It will be seen that the only significant change took place in Form 1. Here a complete change on Joanette is shown from susceptibility in the summer to resistance in the winter. A differentiation between Forms 1 and 2 under summer conditions is therefore impossible.

Several cultures of this Form 1 have shown on Joanette a very definite "X" reaction in spring and autumn tests. Isolations have been made from "1" pustules occurring in the "X" reaction and also from the "3" pustules, and these cultures compared later under summer and winter conditions. The results have been from each the "3+" in summer and the ; in winter. Illustrations are given in Plate VI.

A further test was made to confirm this result. In July 1927 a batch of heavily rusted oaten straw was collected at Cowra. It was cut into short lengths, partially dried out in the laboratory, and then divided into 25 equal packets for storage in the ice-house.

The first inoculation of a set of oat differentials was made on the 7th August. The reactions were those of P. graminis avenae 1, Joannette giving "2" reactions. This initial culture has since been kept alive. It was tested on the differentials again in January 1928 and gave the expected reactions for P. graminis avenae 1, excepting that Joannette gave "4" reactions.

Packets were withdrawn from the ice-house at intervals of approximately a fortnight and tested in the usual way. Withdrawals on the 23rd August, 4th. and 24th. September, gave the normal reactions for Form 1. Withdrawals on the 2nd., 16th., and 30th. October varied inasmuch as Joannette gave an "X" reaction. Withdrawals on the 13th. and 27th November gave a "4" reaction. Thereafter no uredospore germinations occurred although withdrawals were tested.

Forms 1 and 2 are valid forms under low temperature, i.e. winter conditions for testing. Sets inoculated simultaneously with them and treated in the same fashion throughout have given flecks on Joannette in the one case and "4" reactions in the other.

VARIATION IN REACTION OF Puccinia triticina.

In studies dealing with the leaf rust of wheat, the Farrar wheat "Thew" was found to be resistant to one of the two forms that have been discovered. It was noticed that tests in the winter months showed the sharpest possible flecks, whilst in the summer the reaction was "4^G". A series of tests was carried out using part of the grain of single plants in January 1928 as a "summer test", and grain of the same plants in June 1928 as a "winter test". In each case two cultures of each of the two forms was used. In addition to "Thew", there were included cultures of "Webster", "Hope", and "Iunillo", received from Drs. E.C. Stakman and M.H. Levine, and also the series of

differentials used by Dr. E.B.Mains in his determinative work and kindly supplied by him.

The results are shown in the following table :-

Table 8. Results of Summer and Winter Tests with two physiologic forms of Puccinia triticina on certain varieties of wheat.

Variety	Australian Form 1		Australian Form 2	
	Summer	Winter	Summer	Winter
Thew	4 ^c	;	4	4
Webster E.C.S.	X	X	X	X
Hope	4	X	4	X
Iumillo	4	X	4	X
Malakoff 4898-4	;	;	;	;
Hussar 4843-2	4	X	4	X
Norka 4377-2	;	;	;	;
Kawvale 5274-1	X	X	X	X
Democrat 3384-1	X	X	X	X
Webster 3780-8	X	X	X	X
Med. 3322-3	X	X	X	X
Unnamed 3747-5	X	X	X	X
Unnamed 3756-4	4	X	4	X
Unnamed 3778 Montana	4	4	4	4
Unnamed 3779-5	4	4	4	4

It will be seen that apart from "Thew", which serves to differentiate between the two Australian forms of leaf rust, any differences shown by Form 1 are also shown by Form 2. Some of the varieties are alike fully resistant under summer and winter conditions whilst others are fully susceptible under both sets of conditions. In 4 of the cases viz. Hope, Iumillo, Hussar, and Unnamed 3756, there is a noticeable difference. In the summer the reaction is fully susceptible i.e. "4" whilst in the winter it is the indeterminate type "X".

EFFECT OF VARYING PLANTHOUSE CONDITIONS UPON RESISTANCE
TO *Puccinia simplex*.

Working with a culture of the organism obtained from Warrabee, tests were carried out to determine whether a change from a resistant to a susceptible reaction could be detected.

The first indication that this might be the case came from an attempt made in mid-summer to illustrate heterozygosity in certain F₃ families of crosses between "Manchuria", and other susceptible varieties like "Cape" and "Skinless" which had previously given a normal ratio of 3 resistant to every 1 susceptible plant. These results had been obtained during the autumn and winter months. The summer results from 10 different families were quite different; the sharp segregation into the expected groups of plants giving resistant flecks and susceptible "4" reactions did not occur. A repetition of the work gave the same result. It was therefore decided to make a comparative test under summer and winter conditions.

In studies on the inheritance of resistance to this rust (164) it had been determined that certain varieties were fully susceptible in the plinthouse, giving a "4" type of reaction, others were moderately resistant, giving a "2" reaction whilst some were resistant giving flecks. Of the susceptible varieties, the following were taken :-

six of *Hordeum vulgare*, two of *H. intermedium*, four of *H. distichon*, and two of *H. deficiens*. All available varieties showing moderate resistance and a random group showing complete resistance were used. A single plant of each was used for the test, part of the grain being sown in summer (February 1928) and part in the winter (July 1928). The rust culture used was the one which had been used in the previous investigation.

The results are shown in the following table :-

Table 9: Comparison of reactions to a strain of Puccinia simplex shown by various barleys tested under summer and winter conditions.

Barley used	Time and Result of Test	
	Summer	Winter
<u>Susceptible Class</u>		
<u>Hordeum vulgare nigrum typica</u>	4	4
<u>H. vulgare atrum typica</u>	4	4
Manchuria Sel. C81.	4	4
Manchuria Sel. C225	4	4
Cape	4	4
Skinless	4	4
<u>H. intermedium haxtoni typica</u>	4	4
<u>H. intermedium mortoni typica</u>	4	4
<u>H. distichon angustispicatum typica</u>	4	4
<u>H. distichon nudum typica</u>	4	4
Kinver	4	4
Pryor	4	4
<u>H. deficiens deficiens typica</u>	4	4
<u>H. deficiens steudelli typica</u>	4	4
<u>Moderately Resistant Class</u>		
Californian Feed	4	2 and X-
Psaknon	4	2+
Locride	4	2 and X-
Sahara 3770	4	2+
Orge Fourragère	4	2 and X
Minn. I 16.13 Lion	4	4 ^c
Minn. II 20.10B	4	4 ^c
Minn II 21.14	4	2+

Barley used	Time and Result of Test	
	<u>Summer</u>	<u>Winter</u>
<u>H. vulgare aethiops typica</u>	4	;
<u>H. vulgare horsfordianum typica</u>	4	;
Minn. II 21.15	4	;
Minn. II 21.17	4	;
Minn. II 21.18	4	;
Manchuria Sel. C163	4	;
Manchuria Sel. C168	4	;
Manchuria	4	;

It will be seen that there is no difference as between the summer and the winter reactions given by the susceptible varieties. The fluctuations that occurred in the environment did not appreciably affect the susceptibility.

In the case of the moderately resistant types, an appreciable change was apparent. Varieties which showed moderate resistance under winter conditions were completely susceptible under summer conditions. A change from a "2" or an "X" to a "4" is significant.

The resistant types gave a very important result. Under winter conditions they gave flecks, but under the summer conditions they gave the completely susceptible "4" reaction. This was a very unexpected change. It explains why there was a failure to secure the expected segregation during the summer test in the crosses of "Manchuria" with "Cape", "Skinless" and "Kinver". The supposedly resistant "Manchuria" is not resistant under the summer conditions. Illustrations are given in Plate VII.

These results were obtained from only one year's test. A repetition of it is being made.

Summary.

It has been shown that important differences occur in the reactions of hosts to rust attack in the plant house. These differences may be extreme. Complete susceptibility shown by a rust under summer conditions may change to complete resistance in the winter. Since the determination of physiologic forms of rust is based upon the susceptibility or resistance shown on seedlings in the plant house, control of the environmental conditions becomes imperative. An intensive study of this change may give valuable information regarding the complex problem of the nature of rust resistance. No definite field observations have been made which indicate that in mature plants susceptibility may be shown under certain climatic conditions and resistance under others, but this happening may have an important bearing upon field practice, and epidemiology.

SPECIALISATION IN PUCCINIA GRAMINIS TRITICI

IDENTITY OF PHYSIOLOGIC FORMS OF P. GRAMINIS TRITICI
IN AUSTRALIA.

The preceding results have shown that major differences in the rust reactions may be brought about by altering the environmental conditions under which the tests are made. Complete susceptibility under summer conditions may change to complete resistance under winter conditions. This has not been occasioned on all the differentials. Nor do all physiologic forms react to such changes in the same degree.

This does not make invalid the determination of physiologic forms. But it makes it imperative that uniform conditions should prevail throughout the tests. Under identical environmental conditions different cultures of rusts behave quite differently on the differential hosts and can be sorted into distinct physiologic forms. The reactions given under normal plant house conditions, i.e. not extremes of winter or of summer, have been used in the designation of the forms. The ranges do not include those given under these extremes.

Studies involving these determinations have been in progress at the University of Sydney since 1921. The kindness of Drs. Stakman and Levine in making available grain of their differential hosts has made it possible to carry out this work. The arrival of Form 33 in the uredospore stage from Dr. M. H. Levine, and the occurrence of Form 16 from Indian material and Form 27 from English teleutospore material have helped in checking the grain of the differentials. Many officers of the Department of Agriculture and others interested in plant disease work have forwarded rusted material which has been used in the investigations.

In the following table are given the forms which have been met in the work. It should be clearly borne in mind

that only seven of these have occurred naturally in Australasia, viz. 34, 43, 44, 45, 46, 54 and 55. Of these all but 34 have so far been recorded only in Australasia. The six purely Australasian forms exhibit a number of features of interest.

TABLE 10

Behaviour of physiologic forms of *P. graminis tritici* as shown by the type of infection produced on the differential hosts.

Physiologic Form	Mean reactions on differentials.											
	Little Club C.I. 4066	Marquis C.I. 3641	Kanred C.I. 5146	Kota C.I. 5878	Arnautka C.I. 4072	Mindum C.I. 5296	Arnautka (Spelmars) C.I. 6236	Kubanka C.I. 2094	Acme C.I. 5284	Minkorn C.I. 2433	Vernal Banner C.I. 3686	Knapli C.I. 4013
11	4	4	3 ⁺⁺	3	4	4	4	3 ⁺⁺	3 ⁺⁺	3	1 ⁼	1 ⁼
16	4 ⁼	2 ⁼	0	1	4	4	4	3 ⁺	3 ⁺⁺	1 ⁼	1 ⁼	1 ⁼
27	4 ⁻	2	0	0	0;	0;	0;	4	3 ⁺⁺	1 ⁼	3 ⁺⁺	1 ⁺
33	4	2	3 ⁺⁺	2	0;	0;	0;	4	3 ⁺⁺	3	0;	1
34	4	4	3 ⁺⁺	3 ⁺⁺	4	4	4	3 ⁺⁺	3 ⁺⁺	1 ⁼	0;	1 ⁼
43	4	3 ⁺⁺	0	0;	0;	0;	0;	X	1	3	1	0;
44	4	3 ⁺⁺	0	0;	0;	0;	0;	3 ⁺	3 ⁺	3	1	0;
45	4	2	0	2 ⁻	4	4	4	X	X	3	3	1
45A	4	2	2	2 ⁻	4	4	4	X	X	3	3	1
46	4	3 ⁺⁺	0	2 ⁻	4	4	4	1	1	3	3	1
54	4	3 ⁺⁺	0	0;	0;	0;	0;	1	3 ⁺	3 ⁺	1	0;
55	4	4	0	2 ⁻	4	4	4	X	X	3	3	1
New Form	4	3 ⁺⁺	3 ⁺⁺	3	0;	0;	0;	3	3	0;	0;	0;

The six Australasian forms fall into two groups. The three members of each group show marked interrelations. Forms 43, 44, and 54 vary only in their reactions on Kubanka and Acme. But that differences between them do occur is further shown by

the studies of the reactions of other wheats to these forms. Similarly Forms 45, 46, and 55 show resemblances to each other but are separable on Marquis, Kubanka and Acme. The first group of three has always given sharp resistance on Arnautka, Mindum and Spelmars, whilst in the second group these wheats are completely susceptible. It has been repeatedly noticed in warm weather inoculations of stock cultures on Federation, which is susceptible to all forms, that uredosori on the second group break through the epidermis and give crops of uredospores in a shorter time than do members of the first group. This of course refers to pots treated in the same fashion. Furthermore, members of the second group give large "4" pustules on Federation which as they age are often quite necrotic round the edges. This does not occur with members of the first group.

COLOUR DIFFERENCES BETWEEN PHYSIOLOGIC FORMS

Differences in the colour of the uredo pustules of the different forms seemed to occur. To test the point, cultures of each form on Federation seedlings were kept under the same conditions in the plant house at a season when these approached the optimum. The colours of the pustules were directly compared with the Ridgway Colour Standards. The following was the result:-

Form 11	Sanford's Brown	of Plate 11.
" 16	Burnt Sienna	" "
" 27	" "	" "
" 33	Antique Brown	" 111.
" 34	Sanford's Brown	" 11.
" 43	Burnt Sienna	" "
" 44	Sanford's Brown	" "
" 45	" "	" "
" 46	Antique Brown	Plate 111.
" 54	Sanford's Brown	Plate 11.
" 55	Antique Brown	Plate 111.
New Form	Raw Sienna	" "

That is to say the "new form" is unlike any of the others. Forms 11, 34, 44, 45 and 54 are alike. Forms 16, 27, and 43 are alike, and Forms 33, 46, and 55 are alike. It will be seen that this grouping does not accord with that made on the basis of the specialisation and uredospore size of the forms.

An interesting case in connection with colour of the uredo pustules came under notice. In the determination of specialisation of a culture of rust from H. A. College, the normal reactions of Form 34 were found. But on Marquis, one pustule was of a different colour from the others. It was much paler. A culture was obtained from this pustule as well as one from the ordinary darker pustules on the same host. Determinations on the differential sets showed that each was Form 34, but throughout the hosts the lighter colour was apparent in the one culture. When compared with the Ridgway Colour Standards it was found to be "Mars Yellow" of Plate 111, whilst the original culture was "Sanford's Brown" of Plate 11. When the wheat straw from which the culture was first started was examined, no trace could be found of a colour difference in the rust present. It is considered that this light-coloured strain of Form 34 arose as a mutation. It has since been kept in culture and frequently tested on the differentials without showing any departure from the original colour and reactions, excepting that like Form 34, it responds strikingly to differences in the summer and winter conditions as shown by the reactions on the durums. A case of colour mutation in *P. graminis tritici* is recorded by Newton and Johnson (110.) The occurrence of mutations in stem rust may have an important bearing on the origin of physiologic forms of the organism.

DISTRIBUTION OF THE FORMS

Since the commencement of the studies in May 1921, efforts have been made to secure samples of rusted material as frequently as possible and from as many sources as possible.

The ideal method of paying frequent personal visits to different localities in order to collect specimens has not often been practicable, although a number of centres have been visited from time to time. The survey, therefore, has been mainly dependent upon the kindness of helpers in submitting rusted samples for study. Officers of the N.S.W. Department of Agriculture have very largely contributed to this work.

The results are summarised hereunder, grouped into periods of each one year. Empirically the end of March has been taken as closing each period of twelve months. Early sowing of crops takes place in April, and this month has been selected as the commencement of each of the periods set out.

MAY 1921 - MARCH 1922

The first stem rust received and subjected to test came from Hawkesbury Agricultural College and proved to be Form 43, From Cowra in September the same form came to hand, and this particular culture has been kept growing ever since as the standard Form 43. During the period, 41 different isolations were made from material which was received from 34 different Australasian localities. Five of the physiologic forms were represented in the collections.

The details of these are set out in the following table.

TABLE 11. - Results of investigations of uredospore material received from various sources in 1921- 1922.

Form No.	Total No. of isolations	<u>No. of different localities from which material came.</u>						
		N.S.W.	Vic.	Qld.	S.A.	W.A.	Tas.	N.Z.
43	20	15		1				
44	2	2						
45	3	1			1			
46	14	5	1	1	1		3	1
55	2	2						
TOTALS	41	25	1	2	2		3	1

The only State not represented is W. Australia. Considering a grouping of the forms as already suggested it will be seen that Group 1. was represented in 22 of the isolations, and Group 2 in 19. The distribution of the latter is much the wider.

In addition to these isolations it may be mentioned that Form 27 was isolated as the result of inoculating a barberry with sporidia from teleutospore material kindly forwarded from Milton, Wales, by A. D. Cotton Esq., of Kew.

The different places of collection were as follows:-

Form 27. Wales.

Form 43. H.A.College, Cowra, Packham, Coonamble, Trangie, Nyngan, Nowra, Yanco, Grafton, Temora, Wagga, Bankstown, University of Sydney Plots, Glen Innes, Bathurst, Gatton, Q.

Form 44. Camperdown, Nemingha.

Form 45. Bathurst, Roseworthy, S.A.

Form 46. Coonamble, Glen Innes, Wagga, Wellington, Newstead, Gatton, Q., Roseworthy, S.A., Werribee, Vic., Devonport, Tas., Central Tasmania, Stn. Tasmania, Longford, N.Z.

Form 55. Lumberah, Glen Innes.

2nd. PERIOD, APRIL 1922 - MARCH 1923

Only 15 isolations in all were made, very little material coming to hand from the various States. Three forms were represented, as shown in the following table.

Table 12. Results of investigations of uredospore material received from various sources in 1922 - 1923.

Form No.	Total No. of isolations	No. of different localities from which material came.						
		N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
43	10	5		1		1		
44	4	2						
54	1				1			
Totals.	15	7		1	1	1		

In addition, Form 33 was received from Dr.M.N.Levine of Minnesota during this period.

The different places of collection were as follows :-

Form 33. Minnesota, U.S.A.

Form 43. H.A. College, Bathurst, Glen Innes, University of Sydney Plots, Wagga, Pittsworth, Q., Chapman, W.A.

Form 44. Glen Innes, Inverell.

Form 54. Roseworthy, S.A.

3rd. PERIOD, APRIL 1923 - MARCH 1924.

In this period, 25 isolations were made from Australasian material representing four States as set out in the following table.

Table 13. Results of investigations of uredospore material received from various sources in 1923 - 1924.

Form No.	Total No. of isolations.	No. of different localities from which material came						
		N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
43	10	6				1		
46	15	2	2				1	
Totals	25	8	2			1	1	

In addition there were two isolations of Form 27 from teleutospore material collected at Milton, Wales, by Miss K. Sampson of the University of Wales.

The different places of collection were as follows :-

Form 27. Wales

Form 43. H.A. College, Cowra, University of Sydney Plots, Wagga, Leeton, Bathurst, Chapman, W.A.

Form 46. Cowra, Bathurst, Rutherglen, Vic., Werribee, Vic., Devonport, Tas.

4th. PERIOD, APRIL 1924 - MARCH 1925

More material came to hand during this period. It comprised 100 isolations in which were represented five forms as shown in the following table :-

Table 14. Results of investigations of uredospore material received from different sources in 1924 - 1925.

Form No.	Total No. of isolations	No. of different localities from which material came.						
		N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
43	55	24	3	1	1			
44	1		1					
45	15	4	1		1			
46	24	10		1			4	
55	5	3						
Totals	100	41	5	2	2		4	

Here again it will be noticed that if the forms be grouped, Group 1. is represented in 56 of the isolations and Group 2. in 44.

The different places of collection are as follows :-

Form 43. H.A.College, Cowra, Manilla, Delungra, Kelvin, Grafton, Trangie, Tamworth, Duri, Inverell, Coonamble, Parkes, Colinroobie, Bathurst, Illabo, Wagga, University of Sydney Plots, Roseville, Narromine, West Wyalong, Urana, Kocrawatha, Mendialla, Young, Gatton, Q., Dookie, Vic., Werribee, Vic., Euchareena, Vic., Roseworthy, S.A.

Form 44. Werribee, Vic.

Form 45. H.A.College, Illabo, Wagga, Cowra, Werribee, Vic., Roseworthy, S.A.

Form 46. H.A.College, Kelvin, Manilla, Tamworth, Duri, Parkes, Henty, Wagga. Peak Hill, Eugowra, Gatton, Q. Westbury, Tas., Wesley Vale, Tas., North Downs, Tas. Devonport Tas.

Form 55. Roseville, Eumungerie, Cowra.

5th. PERIOD, APRIL 1925 - MARCH 1926

Only 28 collections were available, covering five forms as set out hereunder.

Table 15. Results of investigation of uredospore material received from various sources in 1925 - 1926

Form No.	Total No. of isolations.	No. of different localities from which material came						
		N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
34	4							
43	15	4		1		2		
44	3	2		1				
45	5	3						
46	1	1						
Totals	28	10		2		2		

It is important to note the advent of Form 34. The material from Western Australia was forwarded by Mr. W.M. Carne and consisted of a large representative sample of rusted straw from the wheat and barley crops. The actual culture obtained has since been grown in the plant house and is the one used as the type culture of Form 34.

The different places of collections were as follows :-

Form 34. Ejading, W.A., Northam, W.A.

Form 43. H.A. College, University of Sydney Plots, Roseville, Grafton, Gatton, Q.,

Form 44. Glen Innes, Bathurst, Gatton, Q.,

Form 45. H.A. College, University of Sydney Plots, Grafton.

Form 46. Grafton.

6th PERIOD, APRIL 1926 - MARCH 1927

In this year, 89 isolations were made. They included all the known seven Australian forms as shown hereunder.

Table 16. Results of investigations of uredospore material from various sources in 1926-1927

Form No.	Total No. of isolations.	No. of different localities from which material came.						
		N.S.W.	Vic.	Qld.	S.A.	W.A.	Tas.	N.Z.
34	18	6			2	3		
43	14	8		1	1			
44	30	14		1	2			
45	17	9			1			1
46	6	5						1
54	3	3						
55	1	1						
<u>TOTALS</u>	89	46		2	6	3		2

On the system of grouping the six forms other than Form 34 into the two groups of each three forms, it will be seen that there are 47 isolations of the first group and 24 of the second.

The different places of collection were as follows:-

Form 34. Curlewis, Manilla, Cowra, Wagga, Loomberah, Bathurst, Bordertown, S.A., Waite, S.A., Lake Grace, W.A., Salmon Gums, W.A., Chapman, W.A.

Form 43. H. A. College, Oberon, Bathurst, Wagga, Narromine, Grafton, Goonoo Goonoo, Curlewis, Gatton, Q., Mt. Gambier, S.A.

Form 44. H. A. College, Grafton, Curlewis, Tamworth, Armidale, Yanco, Narrandera, Beddon, Curban, Cowra, Currabubula, Coonamble, Bathurst, Roseville, Gatton, Q., Lucindale, S.A., Millicent, S.A.

Form 45. Curlewis, Yanco, Narrandera, Griffith, Beddon, Curban, Tooraweenah, Cowra, Bathurst, Waite, S.A., Ashburton, N.Z.

Form 46. Tullamore, Balladoran, Gilgandra, Canowindra, Chandler, Nelson N.Z.

Form 54. Dubbo, Balladoran, Tooraweenah.

Form 55. Cowra.

7th PERIOD , APRIL 1927 - MARCH 1928

In this period there were 189 isolations. All the States and New Zealand are represented. As in the preceding year, all seven forms were represented in the collections, which are set out in detail hereunder.

Table 17. Results of investigations of uredospore material from various sources in 1927-1928.

Form No.	Total No. of isolations	No. of different localities from which material came.						
		N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
34	152	31	1	2	1	4	6	1
43	21	6					1	
44	6	2		1			1	
45	5	3					1	
46	2	2						
54	1	1						
55	2	1		1				
TOTALS	189	46	1	4	1	4	9	1

In this period Form 16 was also isolated from teleutospore material kindly forwarded from Pusa, India, and used to infect a barberry.

The actual places of collection were as follows:-

Form 16. Pusa, India.

Form 34. H.A. College, Glen Innes, Bathurst, Raleigh, Gowrie, Trangie, Grafton, Trundle, Botfield, Darrobelgie, Bogan Gate, Cowra, Quandong, Parkville, Warrah Creek, Quipolly, Quirindi, Duri, Dubbo, Young, Quaker's Hill, Ben Lomond, Sunnyside, Guyra, Armidale, Lecton, Carcoar, Currabubula, Werris Creek, University of Sydney Flots, Roseville, Gatton Q., Darling Downs, Q. Werribee, Vic., Monarulo, Sth. S.A., Kwolyin, W.A., Chapman, W.A. Dindilon, W.A., Merridin, W.A., Sassafras, Tas., W. Devonport, Tas., Mth. Down, Tas., Barrington, Tas., Scotsdale, Tas., Stonor, Tas., Nelson, N.Z.,

Form 43. H. A. College, Bathurst, Glen Innes, Raleigh, Peak Hill, Grafton, Barrington, Tas.

Form 44. Glen Innes, Bathurst, Gatton, Q., Mth. Down, Tas.

Form 45. Glen Innes, Cowra, Warrah Creek, Barrington, Tas.

Form 46. Quandong, Carcoar.

Form 54. Gowrie.

Form 55. Trangie, Darling Downs, Q.

8th PERIOD, APRIL 1928 - MARCH 1929

There were 156 isolations in the year. They came from numerous localities. Only Form 34 was present. The details are as follows.

Table 18. Results of investigations of uredospore material from various sources in 1928-1929

Form No.	Total No. of isolations	No. of different localities from which material came.						
		H.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
34	156	36	7	4	3			1

Material came to hand late in the season from Western Australia but the uredospores had unfortunately lost viability. It is believed, however, that it was Form 34.

During this period two other forms were added to the collection, viz. Form 11 and the "New Form" (165).

The different places of collection were as follows:-

Form 34. H. A. College, Bathurst, Lewis Ponds, Cowra, Boggabri, Trangie, Yanco, Pinecliff, Chatswood, Henty, Duri, Coonamble, Wyalong, Wagga, Brocklesby, Loomerah, University of Sydney Plots, Jindara, Berrigan, Grafton, Toogong, Gunnedah, Currabubula, Tamworth, Dubbo, Wellington, Narromine, Barellan, Lockhart, Forbes, Oaklands, Corowa, Peak Hill, Gowrie, Glen Innes, Inverell, Gatton, Q., Freestone, Q., Loch Lomond, Q., Gladfield, Q., Werribee, Vic. Dockie, Vic., Howlong, Vic. Rutherglen, Vic. Port Frairie, Vic., Shepparton, Vic., Willaura, Vic., Waite, S.A., Glencoe West, S.A., Furner, S.A. Nelson, N.Z.

The whole of these results are summarised in the following tables.

Table 19. Summary of the uredospore investigations showing the number of isolations and different localities from which they came during the several periods.

Form No.	Season ending March of																Total Number of Isolations
	1922		1923		1924		1925		1926		1927		1928		1929		
	Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities	
34									4	2	18	11	152	46	156	51	330
43	20	16	10	7	10	7	55	29	15	5	14	10	21	7			145
44	2	2	4	2			1	1	3	3	30	17	6	4			46
45	3	2					15	6	5	3	17	11	5	4			45
46	14	12			15	5	24	15	1	1	6	6	2	2			62
54			1	1							3	3	1	1			5
55	2	2			24	15	5	3			1	1	2	2			10
	41	34	15	10	25	12	100	54	28	14	89	59	189	66	156	51	643

If the grouping of the six forms into the two groups of each three forms be considered, it is seen that of Group 1. comprising Forms 43, 44, and 54, there were in all 196 isolations. Of Group 2. comprising Forms 45, 46, and 55, there were 117 isolations. The remaining 330 isolations were of Form 34.

Table 20. Summary of the uredospore investigations showing the different localities each season from which the forms came.

Form No.	Total No. of Isolations	Different localities from which material came.							Total Number of Localities
		N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.	
34	330	73	8	6	6	9	6	2	110
43	145	73	3	5	2	2	1		86
44	46	22	1	3	2		1		29
45	45	20	1		3		1	1	26
46	62	25	1	2	1		8	2	39
54	5	4			1				5
55	10	7		1					8
TOTALS	643	154	14	17	15	11	17	5	303

Here again it is instructive to group the forms into the two groups of each three forms. The first group came from 120 different localities, the second group from 73 different localities, and Form 34 from 110 different localities.

In considering these results attention is again directed to the limitations imposed upon the collecting of specimens, and to the fact that in the main, dependence had to be placed upon agents not directly concerned in these studies. The number of isolations for the various seasons cannot therefore be taken as a true index to the amount of rust present in the crops. In 1928 and 1929 rust was very prevalent in many areas, a fact brought out by the numerous isolations that were made. But in certain of the other periods when only few specimens came to hand, it is known that rust was present in the crops to a considerable extent.

DISTRIBUTION OF THE FORMS

Form 43.

It has already been stated that the first form which was determined was Form 43. With the exception of Form 34, this form turned up more frequently than any other during the eight years. It also came from more localities than any other form excepting 34. Furthermore it was determined during each of the periods with the exception of 1928-1929, when only Form 34 occurred. All the Australian States have provided isolations of this form, but it has not been recorded from New Zealand.

Form 44.

This form, which is allied to Form 43, also appeared in the early stages of the work, and occurred fairly frequently during the investigations excepting during the 1923-1924 period when so few isolations were made. It came from four of the Australian States.

Form 45

This is a member of the second group, and appeared fairly frequently. It was isolated during the first period, and only dropped out during the 1923 and 1924 periods when only a few collections were available. Queensland and Western Australia have not yielded isolations of it, but the other States and New Zealand have.

Form 46.

This was the second form to be isolated in Australia, occurring early in the investigations and showing very marked differences from the first form that was found, viz. Form 43. It is a member of the second group of forms. In frequency of isolation, as well as in the number of localities from which it came, it ranks next to Form 43. It was found in each period up to 1928 with the exception of 1923 when so very

little material came to hand, With the exception of Western Australia, all the Australian States and New Zealand have harboured this form.

Form 54.

This is a member of the first group of forms and has been isolated only five times during the periods 1923, 1927, and 1928. New South Wales and South Australia are the only States in which it has been found.

Form 55.

This member of the second group has only been isolated ten times, and has come from New South Wales and Queensland.

Regarding these six forms on the basis of their grouping, it will be seen that members of Group 1. were isolated 196 times and members of Group 2 117 times. Members of Group 1 came from 119 localities, whilst those belonging to Group 2 came from 73 localities.

Form 34.

This form markedly differs from the other six forms. They are essentially Australian, not having been recorded from other countries. Form 34 has been recorded from several other continents. It is a very much more virulent form than the other six.

It will be seen that during the first four periods of the investigations, Form 34 did not once show up. It was first found in November 1925 after the investigation had been in progress for $4\frac{1}{2}$ years. During that time, 193 determinations of other forms had been made. The material containing it was heavily rusted wheat and barley straw sent by Mr. W. M. Carne from Western Australia. It was not found again until just a year later, when Mr. Carne again forwarded a batch of Western Australian material. At about the same time

(Nov. 1926) Form 34 was isolated from rusted wheat sent from several localities in New South Wales and South Australia. At the same time forms belonging to one or other of the two groups were coming to hand. In some cases the one collection of straw yielded Form 34 together with one of the other forms. Gradually the latter became fewer. The 1928 period shows that from 189 isolations, Form 34 occurred 152 times. The localities from which it came also showed a remarkable extension. Every Australian State as well as New Zealand gave isolations of this form. Even more striking is the position revealed in the 1929 period. From 156 isolations only Form 34 was obtained. There was no appearance of any one of the other forms during this period. Form 34 completely dominated the situation. Tasmania failed to submit rusted material, but all the other States as well as New Zealand sent rusted straw carrying Form 34 and only this form. The last isolation of a form other than 34 occurred in January 1928 from rusted material obtained from Tasmania.

In the collections examined there were numerous cases in which more than one form of rust were present. Leaf rust and stem rust frequently appeared together; the most convenient host for separating them was Khapli. As many as three distinct physiologic forms of stem rust were found in the one collection. It has been a very common occurrence to find both the forms of leaf rust present in a batch of material and sometimes on the same leaf of a plant.

The advent of such a form as 34 profoundly affects the rust situation. It has been found that few varieties of wheat of commercial value are resistant to it, and its attack on cross-bred wheats known to be resistant to the other forms which had been isolated was very upsetting.

OCCURRENCE OF P. GRAMINIS TRITICI ON OTHER HOSTS

In addition to attacking wheat, P. graminis tritici is known to occur on other plants. Whenever possible, these other hosts have been examined, with a result indicated in the following section.

BARLEY

This is a crop which is not very extensively grown in Australia. To date 57 cultures of barley stem rust have been examined. They came from all the States. Of the seven physiologic forms known to occur in Australia, five occurred in these barley isolations. Form 54 and 55, which were not represented, have only been found in all on five and ten occasions respectively. Seedling tests have shown that the commercial varieties of barley are susceptible to these two forms. Had more extensive collections been available it is likely that natural infections with them would have been discovered.

The results of the collections are summarised hereunder.

Table 21. Number of times isolations of the different physiologic forms were made from barley.

Form No.	Number of isolations.
34	28
43	13
44	7
45	3
46	6
TOTAL	57

The numbers dealt with are small, but a comparison of these figures with the total number of isolations made of each of the forms shows a general agreement.

RYE

Whenever it has been possible, rye crops have received special attention. Stem rust was first found on rye at Glen Innes in 1922. Fresh uredospores were inoculated on to seedlings of the same variety ("Black Winter") without giving any result. The next collection was from H. A. College in 1924 when a similar failure was encountered. Two years later a further sample came to hand from Grafton. Seedlings of wheat, oats, barley and rye were inoculated. Infections were obtained on the wheat and barley alone. Tests on the standard differentials showed that the rust was P.graminis tritici 44. Shortly afterwards another collection of rust on rye from Queensland proved to be P.graminis tritici 43. Thereafter stem rust on rye has occasionally been collected from various sources in New South Wales, Victoria and Queensland. Fourteen samples of viable material have been available and have yielded one isolation of Form 43, two of Form 44, and eleven of Form 34. The last mentioned have all turned up since November 1927.

On several occasions there have been opportunities for scrutinising crops of rye nearing maturity. Stem rust is uncommon in the crop. In coastal areas there have been numerous cases of leaf rust occurring on the sheath and on the stem itself. As showing the paucity of stem rust, in 1927 a careful search through a field of Slav rye at Cowra in a season when rust was plentiful in wheat and oats, yielded only one plant infected with stem rust. The grain was at the dough stage. It was collected and the grain allowed to mature whilst a culture of the rust (Form 34) was kept growing. Tests with seedlings derived from this infected plant gave two susceptible and 39 resistant plants. The two susceptibles and representatives of each of the resistant types were grown to maturity and selfed. No viable grain was obtained so the experiment lapsed.

This work is being carried further forward and promises to yield interesting results. Efforts have been made to find rye plants infected with stem rust and to secure grain from them. In this work valuable help has been rendered by Mr. G.S. Gordon of Werribee, Mr. J. T. Pridham and Dr. A. R. Callaghan of Cowra, and Mr. W. H. Darragh of Grafton. In a few cases, rusted rye plants were found sufficiently early in the season to make it possible to bag some heads prior to flowering and thus secure a little selfed grain in addition to the open-pollinated grain. There were three of these plants. The yield of grain from these bagged heads was very light, numbering only 12, 2, and 9 grains respectively.

The grain of the individual plants was tested with Form 34, which was the attacking form, and after note-taking, the seedlings were planted out into open beds where they are being grown to maturity. The object is to obtain selfed grain of plants belonging to each class of rust reaction, and to test the progeny for resistance.

The results of these G1 tests are shown in the following table :-

Table 22. Means of reactions given by rye seedlings inoculated with Form 34.

Acc. No.	Source	Variety	Mode of Pollination	0	;	1	2	X	3	4
624	Cowra	Slav	Open	17	22	2		2	26	
955A	Cowra	Black Winter	Open	38	50	2	5	1	26	
955A	Cowra	Black Winter	Selfed	3	1	-	1		7	
984	Bathurst	do.	Open	9	10	4	10	2	6	
987A	Grafton	do.	Open		3		1		2	
B	do.	do.	do.	1	1	1		1		
C	do.	do.	do.	5	5	2	8			
D	do.	do.	do.	8	3					
1015M1	Werribee	March	do.	26	30		4	2	7	
B1	do.	Black Winter	do.	20	42	19	23	14	40	
B2	do.	do.	do.	27	29	8	13	6	11	
B3	do.	do.	do.	5	38	8	10	4	12	
B5	do.	do.	do.	22	6	4	4	1	15	
P1	do.	Petkus Rug	do.	43	9		5		12	
	do.	do.	Selfed				1		1	
P2	do.	do.	Open	14	20	13			18	
	do.	do.	Selfed						9	
Total of open-pollinated plants				235	268	63	83	33	149	

It will be seen that the seedlings derived from selfed grain of susceptible plants were mostly susceptible. One of the plants produced 9 seedlings which were all susceptible. In the aggregate, of the 23 selfed grains derived from these three susceptible plants, 17 were susceptible and 6 were resistant. Until further results are available it will not be possible to give an adequate explanation of the happenings. Is it a coincidence that when the sum total of the reactions given by the open pollinated plants is taken, there are 649 resistant to 182 susceptible plants, a near approach to a 3 : 1 ratio? The results obtained from the tests on the next generation plants should give an answer.

GRASSES AS HOSTS OF P. GRAMINIS TRITICI

It has long been recognised that grasses may play a part as hosts of the cereal rusts. Numerous investigators have given attention to the problem. Stakman and Piemeisel (138) summarise the results of previous work and report the results of their extensive studies. They conclude that grasses are unquestionably important in the cereal rust problem.

In Australia McAlpine (105) has recorded the occurrence of Puccinia graminis on a number of grasses, but was unable to carry out infection experiments with them. It is therefore uncertain whether wheat stem rust was present or whether it was some other variety of P. graminis.

Collections of rusts on grasses have been examined wherever possible during the present investigations. So far 24 cultures of wheat stem rust have been studied, occurring on five different grasses. The determinations of the physiologic forms concerned have shown that five out of the seven physiologic forms known in Australia have occurred naturally on the grasses. Forms 54 and 55, which were not represented, have been obtained in all only five and ten times respectively in the course of the studies. It is therefore hardly surprising that they were not found on grasses.

After the determinations on the standard differentials of the physiologic forms concerned, it has been the practice to inoculate the initial grass host of the particular rust with the culture taken from the wheat differential hosts. By this means the susceptibility of the grass to the particular form has been checked.

The results are summarized in the following table:-

Table 23. Number of times certain grasses were found infected with physiologic forms of Puccinia graminis tritici.

No. of collections	Form	Form 34		Form 45		Form 44		Form 46.		Form 45.	
		No. of collections	Grass	No. of collections	Grass	No. of collections	Grass	No. of collections	Grass	No. of collections	Grass
6		1	<u>Hordeum murinum</u>	1	<u>Hordeum murinum</u>	1	<u>Hordeum murinum</u>	3	<u>Hordeum murinum</u>	3	<u>Hordeum murinum</u>
1		3	<u>Hordeum maritimum</u>	1	<u>Agropyron scabrum</u>	1	<u>Agropyron scabrum</u>	1	<u>Agropyron scabrum</u>	1	<u>Agropyron scabrum</u>
2		1	<u>Agropyron scabrum</u>	1	<u>Echinochloa ovatus</u>	1	<u>Arrhenatherum avenaceum</u>				
1		1	<u>Arrhenatherum avenaceum</u>	1	<u>Arrhenatherum avenaceum</u>						
10		6		3		1		4		4	

It will be seen that Barley Grass, Hordeum murinum, was found eleven times infected with P. graminis tritici. Four out of the five physiologic forms which occurred on grasses were found on it. Taking into account the fact that P. graminis avenae also commonly occurs on barley grass, its importance as a rust host becomes evident. Its widespread occurrence throughout the wheat-belt further emphasizes this. Although it dries off in the early summer, it may function in the early part of the season in infecting the growing crops of wheat and oats.

Hordeum maritimum infected with rust was found only once. It was carrying both wheat and oat stem rusts as shown in the tables.

Agropyron scabrum bearing rust was collected on eight occasions. All five forms occurring on grasses were isolated from it. Like Barley grass, it is also naturally attacked by P. graminis avenae.

Arrhenatherum avenaceum in a rusted state was examined three times. A different physiologic form was present in each instance.

Echinopogon ovatus infected with rust was found once. The rust present was Form 43. It also serves as a host for P. graminis avenae.

OTHER GRASS RUSTS.

The stem rusts which have occurred on grasses other than cereals have already been listed. In addition to these, there have been collections of other grass rusts which have been subjected to test. The practical practice has been to use the material for inoculating pots of wheat, oats, barley and rye. Where infections were obtained, specialisation studies were made and then the rust on the cereal seedlings used to inoculate the grass host on which it first occurred.

Rusts from the following grasses have failed to give any cereal infections:-

<u>Alopecurus geniculatus</u>	<u>Festuca Hookeriana</u>
<u>Andropogon pertusis</u>	<u>Heteropogon contortus</u>
<u>Aristida vagans</u>	<u>Imperator arundinacea</u>
<u>A. behriana</u>	<u>Lamarckia aurea</u>
<u>Bromus mollis</u>	<u>Microloena stipoides</u>
<u>B. racemosus</u>	<u>Panicum sanguinale</u>
<u>Cynodon dactylon</u>	<u>Stipa flavescens</u>
<u>Danthonia bipartita</u>	<u>S. pubescens</u>
<u>D. semiannularis</u>	

Several other grass rusts which produce a certain degree of infection of some of the cereal seedlings are being studied. When the results are complete they will be separately reported. In this work there are numerous indications that successful infections of the cereals takes place in the plant house at the high temperature of summer, whereas no effect is produced during the winter.

PLANT HOUSE INVESTIGATIONS WITH GRASSES FOR SUSCEPTIBILITY
TO RUST.

In addition to the work dealing with the rusts which occurred naturally on grasses, studies have been made of grasses inoculated in the plant house with known forms of various rusts. The practice has been to use stock cultures for the inoculation, and where the result has been successful to then inoculate the appropriate cereal seedlings with the rust on the grass and prove its identity. The stem rusts have received chief attention. Many of the tests have yielded negative results.

As far as the work has gone the results may be summarised as follows:-

GRASSES TESTED WITH *Puccinia graminis tritici*.Susceptible.

Aegilops divaricata
 A. ovata
 A. triticoides
 A. ventricosa

Andropogon scabrum
 Bromus tectorum
 B. hordeaceus
 B. arenarius
 B. racemosus
 Hordeum murinum
 H. maritimum

Slightly attacked.

Aegilops comosa
 Avena elatior
 Bromus maximus
 B. uniloides

Briza major
 B. minor
 Ehrharta longifolia
 Festuca bromoides
 Koehleria cristata

Immune.

Andropogon tenerum
 Agrostis exarata
 Anthoxanthum odoratum
 Alopecurus pratensis
 Bromus mollis
 B. erectus
 B. inermis
 Festuca ovina
 Holcus lanatus

Hordeum jubatum
 H. bulbosum
 Lolium temulentum
 L. perenne
 L. italicum
 L. subulatum
 Lamarckia aurea
 Poa pratensis
 P. annua
 Phleum pratense.

In most of these tests all 13 physiologic forms which are available have been used. Some of the results show that the grass is susceptible to all the forms. In other cases there are differential reactions.

GRASSES TESTED WITH *Puccinia triticina*.

Susceptible.

Aegilops divaricata
A. *ovata*
A. *triticoides*
A. *ventricosa*
Hordeum murinum
H. *maritimum*

Slightly attacked.

Aegilops comosa
Agropyron scabrum
Avena elatior
Bromus maximus
B. *racemosus*
Briza minor
Festuca bromoides

Immune.

Agropyron tenerum
Agrostis exarata
Anthoxanthum odoratum
Alopecurus pratensis
Bromus unioloides
B. *arenarius*
Briza major
Ehrharta longiflora
Festuca ovina
Hordeum jubatum
Koehleria cristata
Lolium temulentum
L. *perenne*
L. *italicum*
L. *subulatum*
Poa annua

Both the known physiologic forms have been used in these years tests. Only in the case of *Aegilops ovata* was a differential reaction shown. Aust.1. showed resistant and Aust.2. susceptible reactions on this grass.

GRASSES TESTED WITH *Puccinia graminis avenae*.

Susceptible.

Bromus maximus
B. hordeaceus
B. tectorum
Briza minor
B. major
B. arenarius
B. racemosus
Festuca bromoides
Hordeum murinum
H. maritimum
Koehleria cristata
Bechmannia eruciformis

Slightly attacked.

Aegilops ovata
Avena elatior
Bromus maximus
B. unioloides
Ehrharta longiflora
Lolium temulentum
L. perenne
L. subulatum

Immune.

Aegilops comosa
A. divaricata
A. triticoides
A. ventricosa
Agropyron scabrum
A. tenerum
Agrostis exarata
Aira caryophyllata
Anthoxanthum odoratum
Alopecurus pratensis
Bromus erectus
B. inermis
Festuca ovina
Lolium italicum
Phleum pratense
Poa annua

All the available physiologic forms have been used in these tests.
In a few cases differential reactions have been shown by the forms.

GRASSES TESTED WITH Puccinia simplex.

Susceptible.

Hordeum marinum

Slightly attacked.

Aegilops ventricosa
Briza minor
Hordeum maritimum

Immune.

Aegilopá divaricata
A. ovata
Agropyron scabrum
Avena elatior
Bromus maximus
B. uniloides
B. arenarius
B. racemosus
Briza major
Ehrharta longiflora
Festuca bromoides
Holcus lanatus
Hordeum bulbosum
Koehleria cristata
Lolium temulentum
L. perenne
L. subulatum

GRASSES TESTED WITH *Puccinia coronata*.

Susceptible.

Aegilops ovata
Briza minor
Bromus racemosus
Hordeum maritimum

Slightly attacked.

Aegilops divaricata
A. triticoides
A. ventricosa
Bromus uniloides
Festuca bromoides
Hordeum murinum
Lolium temulentum

Immune.

Agropyron scabrum
Bromus maximus
B. arenarius
Briza major
Ehrharta longiflora
Holcus lanatus
Lolium temulentum
L. perenne
L. subulatum
Poa annua

GRASSES ATTACKED WITH Puccinia dispersa.

Slightly attacked.

Aegilops ovata

Immune.

Aegilops divaricata
A. triticoides
A. ventricosa
Avena elatior
Bromus maximus
B. uniloides
B. racemosus
B. arenarius
Briza minor
B. major
Ehrharta longiflora
Festuca bromoides
Hordeum murinum
H. maritimum
Holcus lanatus
Lolium temulentum
L. perenne
L. subulatum
Poa annua

SPECIALISATION IN Puccinia graminis avenae

Studies of the organism causing stem rust of oats have revealed in other countries the occurrence of five physiologic forms. In the present investigations some attention has been paid to this rust, thanks to the kindness of Drs. Stakman and Levine in forwarding grain of their differential hosts. In 1921 grain of Victory, Ligowo, White Tartar, Green Mountain and Richland were received. Joannette was sent in 1925. Therefore determinations prior to this date were imperfect. They showed that the Form present was either Form 1, 2, or 5, since in no case was White Tartar or Richland susceptible. These determinations are consequently not included in the results recorded.

The following tables summarise the results obtained:-

Table 24. Summary of investigations of uredospore material of P.graminis avenae derived from different sources in Australasia.

Form No.	Total No. of Isolations	Period ending March of									
		1925		1926		1927		1928		1929	
		Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities	Isolations	Localities
1	50	29	17	3	3	2	2	8	6	8	4
2	81	1	1			19	11	30	18	31	14
6	8							2	2	6	2
7	11							7	5	4	3
	150	30	18	3	3	21	13	47	31	49	23

Table 25. Summary of investigations of uredospore material of *P.graminis avenae* showing distribution in Australasia.

Form No.	Total No. of Isolations	Total No. of Localities	Different localities from which material came.						
			N.S.W.	Vic.	Q.	S.A.	W.A.	Tas.	N.Z.
1	50	32	23	4	2	1	1		1
2	81	44	34	2		4	1	2	1
6	8	4	3	1					
7	11	8	3					5	
	150	88	63	7	2	5	2	7	2

It will be seen that 150 isolations were studied. They came from all the Australian States and New Zealand, although the great majority came from New South Wales.

The different localities from which material came are as follows, taking the season as ending in March of the year stated.

1925. Form 1. H.A.College, Cowra, Epping, Trangie, Dubbo, Inverell, Coonamble, Tamworth, Eugowra, Wagga, Glen Innes, Woniora River, Gatton, Q., Werribee, Vic., Dookie, Vic., Adelaide, S.A., Christchurch, N.Z.

Form 2. Cowra.

1926. Form 1. Bathurst, Glen Innes, Gatton, Q.

1927. Form 1. H.A.College, Currabubula.

Form 2. Willow Tree, Yanco, Grafton, Cowra, Coonamble, Bathurst, Oberon, Waite, S.A., Allandale, S.A., Merredin, W.A., Ashburton, N.Z.

1928. Form 1. H.A.College, Glen Innes, Cowra, Roseville, Werribee, Vic., Chapman, W.A.

Form 2. H.A.College, Glen Innes, Cabramatta, Bathurst, Wagga, Cowra, Coramba, Dubbo, Roseville, Ben Lomond, Armidale, Glenfield, Leeton, Yanco, University of Sydney Plots, Monarto, S.A., Barington, Tas., Tunnack, Tas.

Form 6. H.A.College, Grafton.

Form 7. Barrington, Tas., Sheffield, Tas., Parattah, Tas., Loyetea, Tas., South Preston, Tas.

1929. Form 1. Cowra, Wagga, Roseville, Werribee, Vic.

Form 2. H.A.College, Mathoura, Cowra, Canowindra, Yanco, Parkes, Gourie, Bathurst, University of Sydney Flots, Grafton, Glen Innes, Dookie, Vic., Port Prairie, Vic., Waite, S.A.

Form 6. Cowra, Dookie, Vic.

Form 7. Kentucky, Cowra, Ganmain.

Form 1.

This was recorded in each of the five years dealt with, and was almost certainly present in the material examined during the preceding three years. It was most abundant during 1925. With the exception of Tasmania, all the Australian States and New Zealand yielded cultures of this form. The only material received from Queensland proved to be Form 1. Clearly it is widespread in Australasia.

Form 2.

This was isolated more frequently than any of the other forms, and came to hand from New Zealand and all the States except Queensland. The number of localities represented is also greater than in the case of Form 1. Excepting in 1926 when only three cultures were available for examination, it occurred in each of the seasons dealt with.

Form 6.

Only eight isolations of this form were made. They occurred in 1928 and 1929. The material was collected at four places, three of them in New South Wales and one in Victoria.

Form 7.

This form also occurred only in 1928 and 1929, and was uncommon. The 11 isolations cover material received from three localities in New South Wales and five in Tasmania.

Since the compilation of these results, there has come to hand from Glen Innes, N.S.W., a batch of oaten straw from which Form 3 has been isolated. This makes five naturally occurring forms of P. graminis avenae in Australasia.

In addition to these, at least two forms have been isolated as a result of infecting barberries with teleutospore material collected in Ireland. As tested on the oat rust differentials, one of these gives the reactions for Form 1. and the other for Form 7. Both of these cultures differ markedly in colour from the Australian rusts. A strictly comparative test was made, using cultures on a fully susceptible variety kept under identical conditions. Comparing the colour of the pustules with those given in Ridgway's Colour Standards, all of the Australian forms were "Morocca Red" of Plate I, whilst the Irish rusts were "Xanthine Orange" of Plate III. This is a very striking difference. There is another significant difference between the Australian Form 1 and the Irish Form 1 when the uredospore measurements are biometrically studied. Furthermore, the Irish rust has shown a remarkable tendency to produce teleutospores on the seedling oats. On varieties which are susceptible as well as on those which are resistant, abundant teleutosori appear within a month of inoculation as a frequent occurrence. Exactly the same thing is found on grasses, e.g. Festuca bromoides, which are susceptible to the rust.

GRASSES AS HOSTS OF P. GRAMINIS AVENAE.

In the studies 35 isolations of P. graminis avenae were made from grasses other than cereals. This does not include isolations from Avena fatua or other species of Avena which occur as "Wild Oats." These have been very commonly found attacked with stem rust. On account of their near relation to the oats which are cultivated, these isolations from wild

oats are not included here but have been included in those from "oats" already listed.

To date eleven different grasses have been found attacked by oat stem rust. Determinations have shown that three out of the five forms known in Australia occurred on these grasses. One of these forms has only been found on eight occasions altogether, so it is not surprising that it has not been found on a grass.

The results are shown in the following table :-

Table 26. Number of isolations of certain physiologic forms of P. graminis avenae made from certain grasses.

<u>Form 1.</u>		<u>Form 2.</u>		<u>Form 3.</u>	
No. of collec- tions.	Grass Host.	No. of collec- tions.	Grass Host.	No. of collec- tions.	Grass Host.
7	<u>Festuca bromoides</u>	5	<u>Festuca bromoides</u>	1	<u>Phalaris minor</u>
2	<u>Echinopogon ovatus</u>	1	<u>Calamagrostis aemula</u>		
1	<u>Calamagrostis aemula</u>	2	<u>Koehleria cristata</u>		
1	<u>Koehleria cristata</u>	3	<u>Hordeum murinum</u>		
1	<u>Agropyron scabrum</u>	1	<u>H. maritimum</u>		
1	<u>Hordeum murinum</u>	3	<u>Phalaris minor</u>		
1	<u>Phalaris minor</u>	2	<u>Dactylis glomerata</u>		
1	<u>Dactylis glomerata</u>	1	<u>Bromus arenarius</u>		
		1	<u>Briza minor</u>		
15		19		1	

Festuca bromoides was found twelve times, sometimes infected with Form 1 and sometimes with Form 2. Extremely heavy infections involving practically all above-ground parts of the plant were encountered in the wheat-belt. This particular grass is a worthless introduction and it would be a good thing if the oat rust exterminated more of it. It is notable for its appearance early in the spring. At the time when the oat crop is not far advanced, Festuca bromoides is in head and often severely rusted. Infections of the oat crop may thus be initiated by the rust on this grass. With the exception of one isolation from the Waite Institute in South Australia when it was infected with Form 2, all collections examined came from New South Wales.

Phalaris minor showed an even wider degree of infection. All three forms which have been found on grass occurred on this host. In all, five isolations were examined. Each collection came from N.S.Wales. The collection from which Form 3 was derived was made at the Cowra Experiment Farm. A number of infected plants were gathered at the same time, and from them Forms 2 and 3 were separated out. It is not known whether the two forms were present upon the one plant. At the time of collection, the oat crop in this locality was nearing maturity and was heavily infected with Forms 1, 2 and 3.

Hordeum murinum was found rusted on four occasions. All collections came from N.S.Wales. Forms 1 and 2 were determined on it. It is a striking thing that on all four collections the oat rust was associated in the same material with one or two forms of wheat stem rust. When it is remembered that barley grass is most widespread in the wheat belt, and that it occurs early in the season before the wheat and oat crops approach maturity, it will be realised that this host may be important in producing early infections of the crops.

Hordeum maritimum bearing rust was found on only one occasion. It occurred at Cowra, N.S.Wales, and was infected

by both wheat and oat stem rust.

Koehleria cristata carrying rust was collected three times. Extremely heavy infections with Forms 1 and 2 of oat stem rust were observed. Remarks made concerning Festuca bromoides also apply here.

Dactylis glomerata yielded three collections which were either Forms 1 or 2. Infections involving the "glumes" as well as the other above ground parts have been common. One of the three sets of material came from Tasmania and was of this nature.

Calamagrostis aemula was twice examined and yielded Forms 1 and 2. It falls into the same category as Festuca bromoides.

Echinopogon ovatus was twice found in the coastal district of N.S.Wales infected with Form 1.

Agropyron scabrum collected at H.A.College was found to be infected with two forms of P. graminis tritici as well as with Form 1 of oat rust.

Bromus arenarius was found only once. It was growing at Cowra and was heavily rusted. In addition to Form 2, one form of P. graminis tritici was found on it.

Briza minor was once found infected by stem rust which proved to be Form 2.

SPECIALISATION IN PUCCINIA TRITICINA.

Leaf rust occurs frequently in the wheat crops, and takes a heavier toll than is generally recognised. In coastal areas particularly it often does much damage. Frequently material of "stem rust" which was submitted was found to consist of a mixture of stem and leaf rusts. Sometimes it was really leaf rust only.

In the earlier years of the work, a number of cultures received from various sources were tested on the standard set of differentials as well as on a number of other wheats. No indication of specialisation was found on these hosts. But in 1926 a culture from Hawkesbury Agricultural College gave an unusual result. The customary thing was to find that Farrer's wheat known as "Thew" was resistant to the leaf rust. In this case the inoculation resulted in a mixture of heavy susceptible pustules together with sharp flecks which on ageing produced "1" reactions. A separation of the two was made, and it was clear that a second physiological form had turned up. Thereafter each culture of P. triticina that became available was tested on Thew and recorded as Aust."1" or Aust."2", depending upon the resistance or susceptibility respectively shown on this host.

There have been striking proofs that these seedling leaf reactions obtained in the plant house are to be directly correlated with the reactions exhibited in the field. In Plate VII. are shown illustrations of the upper leaves of wheat varieties grown at H.A. College under natural conditions when both forms of rust were present. In Plate VII (e) are shown leaves of "Federation" which is susceptible to both forms of leaf rust. In Plate VII (f) are illustrated leaves of "Thew". The mixed "4" and "fleck" reactions on these mature leaves are clearly shown. Cultures were made from the susceptible "4"

reactions and gave only Form 2. Similar isolations from tiny "1" reactions gave only Form 1. It was found that the same state of affairs existed on "Japanese Bearded" and other varieties which also behave as differentials.

In 1927 Dr. E.B. Mains kindly forwarded grain of his differential hosts. These have been several times tested with the two Australian forms. Several cultures representing each of the two forms have been used from time to time. The result is that the reactions of the two forms are identical on this set of differentials. Although separation by these differentials is not possible, it can readily be accomplished by "Thew" and certain other varieties. The series of reactions shown by the Australian rust on Mains' set of differentials is given in the following table alongside those listed by Mains for his Form 12, which most closely of the 12 forms listed, approximates to ours.

Table 27. Reactions shown by each of the two Australian forms of *P. triticina* on the differential hosts used by Mains and Jackson.

Variety	C.I. Number	Each of the Aust. Forms.	U.S.A. Form 12.
Malakoff	C.I. 4898-4	;	0
Norka	C.I. 4377-2	;	0
Unnamed	C.I. 3756-4	4	4
do.	C.I. 3778	4	4
Webster	C.I. 3780-8	;	1
Unnamed	C.I. 3747-5	;	0 - 1
do.	C.I. 3779-5	4	4
Mediterranean	C.I. 3332-3	;	4
Hussar	C.I. 4843-2	4	4
Democrat	C.I. 3384-1	;	4
Kawvale	C.I. 5274-1	1	-

These reactions are shown in the same manner as those given by Mains (96). The variety listed as "Turkey 47" has not been available, and an additional one, "Kawvale" was sent by Dr. Mains.

It will be seen that the Australian rust differs from any of the twelve recorded by Dr. Mains. The nearest approach is perhaps to his Form 12, although marked differences occur even here.

Owing to the inability of these hosts to differentiate the Australian forms, it has become the practice since 1926 to test the leaf rusts on "Thew". Prior to that year the tests were not made. The results are summarised hereunder.

Table 28. Results of examination of isolations of P. triticina obtained from different sources.

Form No.	Total No. of Isolations.	Different localities in Australian States from which material was collected.				
		N.S.W.	Vic.	S.A.	Q.	W.A.
		<u>Period 1926 - 1927.</u>				
Aust. 1	3	3				
Aust. 2	3	3				
Total	6	6				
		<u>Period 1927 - 1928.</u>				
Aust. 1.	52	17			1	
Aust. 2.	70	19			1	1
Total	122	36			2	1
		<u>Period 1928 - 1929.</u>				
Aust. 1.	60	25	3	2		
Aust. 2.	71	27	3	2	2	
Total	131	52	6	4	2	
		<u>Totals for the 3 periods.</u>				
	259	94	6	4	4	1

Table 29. Summary of results of examining collections of P. triticina.

Form No.	Total No. of Isolations.	Period ending March of					
		1927		1928		1929.	
		Isolations.	Different Localities.	Isolations.	Different Localities.	Isolations.	Different Localities.
Aust.1	115	3	3	52	18	60	30
Aust.2	144	3	3	70	21	71	34
Totals	259	6	6	122	39	131	64.

It will be seen that in each of the three years under consideration both forms were present. In the aggregate, Form 2 was a little more abundant than Form 1. So far Form 1 has not turned up in material from Western Australia. From the other States, both forms have been isolated.

The actual localities from which the collections were made are as follows:-

Period 1926 - 1927.

Form 1. H.A. College, Cowra, Curlewis.

Form 2. H.A. College, Wagga.

April 1927 - March 1928.

Form 1. H.A. College, Cabramatta, Wollongbar, Grafton, Trundle, Cowra, Quandong, Grenfell, Bathurst, Parkville, Warrah Creek, Quaker's Hill, Ben Lomond, Sunnyside, Armidale, Glen Innes, Currabubula, Gatton, Q.

Form 2. H.A. College, Wollongbar, Trangie, Grafton, Griffith, Bathurst, Gowrie, Trundle, Botfield, Cowra, Quandong, Grenfell, Parkville, Warrah Creek, Quaker's Hill, Armidale, Glenfield, Glen Innes, University of Sydney Plots, Gatton, Q., Chapman, W.A.

April 1928 - March 1929.

Form 1. H.A. College, Bathurst, Lewis Ponds, Cowra, Boggabri, Trangie, Chatswood, Wagga, Uranquinty, Mummybla, Coolamon, Coonamble, Berrigan, Brocklesby, Finley, Katoomba, Wollongbar, Corowa, Nyngan, Mathoura, Lockhart, Oaklands, Jindera, Toogong, Corowa, Rutherglen, Vic., Werribee, Vic., Dookie, Vic., Waite, S.A., Mallala, S.A.

Form 2. H.A. College, Cowra, Bathurst, Boggabri, Trangie, Pinecliff, Chatswood, Wagga, Grafton, Glenfield, Parkes, Mummybla, Coolamon, Coonamble, Berrigan, Brocklesby, Finley, Wollongbar, Corowa, Nyngan, Mathoura, Lockhart, Oaklands, Jindera, Toogong, Parkes, Corowa, Gatton, Q., Darling Downs, Q., Rutherglen, Vic., Dookie, Vic., Werribee, Vic., Waite, S.A., Mallala, S.A.

BIOMETRICAL STUDIES OF THE MORPHOLOGY OF SPORE FORMS

Specialisation of the rust fungi is shown by the differential physiologic reactions exhibited on certain selected host plants. Morphological differences have also been shown to exist in some cases. Levine (85) (87) made extensive studies of the comparative morphology of various cereal stem rusts. By altering the cultural conditions, pronounced morphological differences were obtained in the uredospores of an individual form. But the differences in the size and shape of the uredospores of different forms grown under identical conditions were in many instances considerably greater than those induced by alteration of the cultural conditions. Definite parallelism between the differences in parasitism and in morphology was not found. It was concluded that although there is a real morphologic distinction between physiologic forms of P. graminis tritici when developed under uniform conditions, the forms are best identified by their parasitic behaviour on standard differential hosts.

Homma (71) has shown that there are significant differences between forms of Erysiphe graminis D.C.

It would seem, therefore, that a biometrical study of the spores may serve as a useful tool in the rust identification work. In Australia determinations of physiologic specialisation have been made only in recent years. In the past when severe epidemics occurred, no determinations were made of the forms concerned. But if biometrical studies can establish the identity of the forms, then from a study of herbarium material it may be possible to determine the form or forms which caused the damage and perhaps trace the changes in specialisation which have occurred.

Methods and Material

Efforts were made to ensure that the spores measured were truly representative and that the collections were uniform.

In the case of the aecidiospores, the infected barberry

shoots were lightly shaken over slides faintly smeared with albumen fixative. A 50% watery solution of lactic acid was used as a mounting medium. From the same shoots further lots of spores were taken and used to inoculate susceptible cereal hosts. The specialisation of the rust concerned was then determined by culturing on the standard differentials.

Uredospores were taken from fully susceptible hosts. A set of differentials together with a pot of "Federation" wheat or of "Algerian" oats was inoculated in the early summer. The pots of Federation and Algerian oats were kept in similar locations on a well-lighted bench of the plant house and given the most favourable conditions possible for rust development. After taking notes on the sets of differentials and making certain that the form used for measurement was what it purported to be, uredospores were obtained from the Federation and Algerian plants by lightly shaking the infected leaves over smeared slides and mounted in 50% lactic acid.

The teleutospores were scraped from straw which had been collected whilst some uredospores were present. The latter were used to determine the specialisation of the rust concerned. The straw in each case was later exposed in wire frames throughout the winter to weather conditions at H.A.College. The spores were taken in the following summer for measurement. Lactic acid was again used as the mounting medium.

No one of the cultures used was of monosporous origin. To date over 250 single uredospores of P. graminis tritici have been isolated and used to inoculate susceptible Federation wheat plants. In no case has an infection been obtained, and therefore no monosporous culture is at present available. Levine's studies indicate that there is no significant difference between the results of measuring random samples of uredospores of monosporous as compared with composite cultures of the same physiologic form.

The measurements were all made with the same Zeiss microscope, standardised and calibrated in the ordinary way.

An eyepiece micrometer was used of which each division under the existing conditions measured 1.86 μ . Measurements were made to this degree of accuracy, and hence its occurrence throughout the work. The same light intensity derived from an artificial source was used throughout. The spores in each case were mounted in a 50% solution of lactic acid in water. Every effort was used to avoid bias in regard to the particular spores measured; each spore encountered as the slide was moved from one side of the stage to the other was taken. The greatest length and greatest width were taken in the case of aecidiospores and uredospores. The length of the teleutospores was measured from the exterior of the apex to the point of attachment of the pedicel, and the width taken across the septum separating the two cells. The shape was determined by calculating the ratio of length to width of each individual spore measured; the figures thus obtained were used in the calculations of the constants.

It should be stressed that these conditions of working differ from those employed by Levine. Whilst strict comparisons of the American and Australian results are therefore not justifiable, a general agreement may be looked for.

AECIDIOSPORES

Puccinia graminis

As indicated already, the aecidial stage is uncommon in Australia. Only three collections of aecidiospores have been used for measurement. Two were forms of P. graminis tritici and the other a form of P. graminis avenae. Aecidiospores of P. graminis tritici 46 were obtained in 1921 when only 50 were measured (162). P. graminis tritici 11 was obtained in 1928 under artificial conditions as reported elsewhere (165). In this case 100 spores were measured under conditions as nearly as possible identical with those obtaining in 1921.

The result of adding together the measurements of these two forms of P. graminis tritici is given immediately following the individual results of each form. The aecidiospores of P. graminis avenae 1 were obtained as a result of infecting a barberry with teleutospore material sent by Miss K. Sampson from Ireland.

The following table shows the results of these measurements.

Table 30 . Variations and constants for length, width, and shape of aecidiospores of Puccinia graminis.

Form.	Spore classes in microns.											Size Limits	Constants.		
	11.16	13.02	14.88	16.74	18.60	20.46	22.32	24.18	26.04	27.90	29.76		Mean.	Standard Deviation	Coefficient of Variability
	<u>LENGTH.</u>														
P.gr.tr. 46			3	6	24	10	6	1				14.88-24.18	19.08±.199	1.95±.131	10.45±.71
P.gr.tr. 11			2	4	22	38	24	8	1		1	14.88-29.76	20.68±.153	2.26±.108	10.96±.53
,, 46 & 11			5	10	46	48	30	9	1		1	14.88-29.76	19.15±.126	2.29±.089	11.95±.48
P.gr.av. 1			1	6	10	41	26	11	4	1		14.88-27.90	21.18±.155	2.29±.11	10.85±.52
	<u>WIDTH.</u>														
P.gr.tr. 46				18	19	11	2					14.88-20.46	16.63±.162	1.54±.104	9.49±.64
P.gr.tr. 11		5	10	28	52	5						13.02-20.46	17.52±.115	1.71±.082	9.71±.465
,, 46 & 11		5	28	47	63	7						13.02-20.46	17.12±.098	1.78±.069	10.40±.41
P.gr.av. 1	2	4	25	41	23	5						11.16-20.46	16.58±.129	1.91±.091	11.49±.53
	<u>SHAPE.</u>														
	Ratio of length to width.														
	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0				
P.gr.tr. 46	14	17	10	3	3	3						1.0 - 1.5	1.146±.0155	.148±.0099	14.15±1.025
P.gr.tr. 11	13	40	22	10	10	1	1	2	1			1.0 - 1.8	1.187±.0106	.153±.0073	12.88±.625
P. ,,46 & 11	27	57	32	13	13	4	1	2	1			1.0 - 1.8	1.17 ±.0085	.154±.006	13.18±.53
P.gr.av. 1	2	26	26	16	15	7	6	1			1	1.0 - 2.0	1.274±.0107	.158±.0073	12.73±.618

Comparisons of the results obtained are summarised
in the following table.

Table 31 . Summary of differences between the means of the dimensions of aecidiospores of Puccinia graminis.

Types compared.	Length.		Width.		Shape.	
	Difference in means in .microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.
<u>P. graminis tritici</u> 11 and <u>P. graminis tritici</u> 46.	1.60±.25 2.0	6.4	.89±.20	4.4	.041±.019	2.2
The total of <u>P. graminis tritici</u> 11 and 46 and <u>P. graminis avenae</u> 1.	2.03±.20	10.1	.54±.162	3.3	.104±.016	6.3
The total of <u>P. graminis tritici</u> 11 and 46 and <u>P. graminis tritici</u> recorded by Levine.	.67±.226	2.96	1.46±.14	10.4		
<u>P. graminis avenae</u> 1 and <u>P. graminis avenae</u> recorded by Levine.	2.56±.25	10.5	1.89±.16	11.8		

It will be seen that there is a significant difference in the length and the width of the aecidiospores of the two forms of P. graminis tritici. Form 46 has spores which are shorter and narrower than those of Form 11. The difference in the shape of the two is therefore not significant, the difference divided by the probable error being only 2.2. For purposes of further comparison the constants for all the 150 measured spores of these two forms were determined and are used as representing those of P. graminis tritici.

Levine reports that of the different varieties of P. graminis, the aecidiospores of P. graminis tritici have the largest arithmetical mean for both length and width. In the cases under consideration it will be seen that the aecidiospores of P. graminis avenae l. are significantly longer than those of P. graminis tritici 11 & 46 and probably they are significantly wider. The difference in the shape is significant. Assuming that the differences in the methods employed do not preclude a general comparison of the results, it will be further seen that there are differences between the measurements of spores of P. graminis tritici as recorded by Levine and those herein recorded. In length the difference is probably not significant, but the Australian aecidiospores are significantly wider. Again in the case of the aecidiospores of P. graminis avenae the measurements given by Levine show that the spores obtained in Australia are significantly longer and wider than those of the American spores.

UREDOSPORES

The variety of P. graminis which occurs on rye and known as P. graminis secalis has so far not been determined in Australia. In all cases examined the stem rust on rye has proved to be one or other of the well known forms of P. graminis tritici. On barley the stem rust is P. graminis tritici.



Oats commonly are attacked by P. graminis avenae. On cereals in Australia, then, the only stem rusts known are P. graminis tritici and P. graminis avenae. Other varieties of P. graminis occur on certain grasses, but these are not included in these measurements.

McAlpine (105) records the rust on these plants and gives the measurements of uredospores of Puccinia graminis Pers. as a composite species. He records a length of 20 - 36 μ and a width of 14 - 18 μ . It will be noted that these figures are unlike those found for the variety P. graminis tritici as a whole, but are in fair agreement with those given by P. graminis avenae as a whole.

Of P. graminis tritici and P. graminis avenae, Levine reports that the former has the larger uredospores in regard to both length and width. Taking the totals of the spores measured it will be seen that the same thing applies to the Australian rusts.

A comparison of these two varieties of Puccinia graminis with those recorded in U.S.A. is of interest.

(a) P. graminis tritici

In all, thirteen physiologic forms were available for measurement. The results are set out in the following tables in which after the measurements of the individual forms, are given the dimensions for the total of the eight forms which have occurred naturally in Australia, and then the sum total of all thirteen forms used.

Table 32 . Variations and constants for length of uredospores of physiologic forms of Puccinia graminis tritici.

Form No.	Spore classes in microns.													Size Limits	Constants.			
	20.46	22.32	24.18	26.04	27.90	29.76	31.62	33.48	35.34	37.20	39.06	40.92	42.78		44.64	Mean	Standard Deviation	Coefficient of Variability
43	1	6	6	12	13	23	21	13	3	2					20.46-37.20	29.39±.24	3.58±.17	12.10±.58
44	1	2	3	8	9	28	20	13	9	6	1				20.46-39.06	30.79±.24	3.56±.17	11.58±.56
54		3	6	12	21	30	13	9	5	1					22.32-37.20	29.29±.21	3.08±.15	10.05±.48
34		1	1	6	14	26	28	22	1	1					22.32-37.20	30.62±.17	2.58±.12	8.21±.39
11	1	1	9	14	15	37	13	7	3						20.46-35.34	28.96±.21	3.09±.14	10.7 ±.51
33		3	5	11	11	18	19	20	7	4	2				22.32-39.06	30.62±.25	3.73±.18	12.18±.6
46		2	2	9	9	11	17	21	15	9	4	1			22.32-40.92	32.09±.27	4.09±.19	12.72±.62
45			1	3	4	9	13	34	15	15	5	1			24.18-40.92	33.55±.21	3.18±.16	10.65±.56
45A		1	1	6	7	14	14	25	18	7	3	2	1		22.32-42.78	32.68±.25	3.73±.18	11.4 ±.5
55		1	2	8	8	18	18	28	10	5	1	1			22.32-40.92	31.64±.24	3.39±.16	10.70±.52
27		1	3	5	5	9	21	30	12	11	2	1			22.32-40.92	32.53±.27	3.51±.17	9.26±.44
16			2	3	6	13	15	26	21	9	3	1		1	24.18-44.64	33.05±.23	3.48±.17	10.5 ±.51
New Form Total			4	25	33	26	9	3							24.18-33.48	28.37±.14	2.08±.09	7.36±.35
Aust. Sum	2	16	22	64	85	159	144	165	77	46	14	5	1		20.46-42.78	31.25±.09	3.68±.06	11.75±.20
Total	3	21	45	122	155	262	221	251	120	70	21	7	1	1	20.46-44.64	31.04±.07	3.70±.05	11.90±.16

Table 33 . Variations and constants for width of uredospores of physiologic forms of Puccinia graminis tritici.

Spore-classes-in-microns.

Constants.

Form No.	Spore classes in microns.							Size Limits	Constants.		
	11.16	13.02	14.88	16.74	18.60	20.46	22.32		Mean	Standard Deviation	Coefficient of Variability
43				6	53	31	10	16.74-22.32	19.44±.09	1.40±.07	7.21±.34
44				9	69	17	5	16.74-22.32	18.94±.08	1.21±.06	6.42±.31
54			3	3	40	38	16	14.88-22.32	17.56±.13	1.93±.09	11.0 ±.55
34			4	15	48	23	10	14.88-22.32	18.97±.12	1.77±.08	9.34±.44
11			2	24	60	14		14.88-20.46	18.43±.08	1.23±.06	6.67±.32
33				14	56	26	4	16.74-22.32	18.33±.09	1.34±.06	7.3 ±.35
46			29	33	36	2		14.88-20.46	16.95±.11	1.58±.08	9.30±.44
45		1	10	47	42			13.02-18.60	17.30±.09	1.28±.06	7.37±.35
45A	2	10	37	29	22			11.16-18.60	15.98±.13	1.85±.09	11.59±.56
55			13	30	48	6	3	14.88-22.32	17.78±.11	1.67±.08	9.4 ±.45
27		1	15	25	51	7	1	13.02-22.32	17.69±.11	1.68±.09	9.05±.43
16		1	16	28	54	1		13.02-20.46	17.45±.10	1.48±.07	8.50±.41
New Form			1	3	40	47	9	14.88-22.32	19.72±.09	1.37±.07	6.92±.33
Total Aust.	2	11	96	172	358	117	44	11.16-22.32	18.14±.05	2.01±.03	11.02±.188
Sum Total	2	13	130	266	619	212	58	11.16-22.32	18.25±.04	1.88±.03	10.3 ±.14

Table 34 . Variations and constants for shape of uredospores of physiologic forms of Puccinia graminis tritici.

Form No.	Ratio of length to width.										Size Limits	Constants.		
	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0		Mean	Standard Deviation	Coefficient of Variability
43	15	17	40	22	6						1.2-2.0	1.57±.015	.22±.011	14.0 ±.68
44	5	13	38	25	16	3					1.2-2.2	1.69±.015	.23±.011	13.60±.66
54	12	29	36	16	6	1					1.2-2.2	1.55±.015	.22±.011	14.18±.69
34	1	16	31	40	9	2	1				1.2-2.4	1.70±.014	.21±.010	12.35±.60
11	5	17	45	22	9	2					1.2-2.2	1.74±.015	.22±.011	12.63±.61
33	6	15	28	31	17	1	2				1.2-2.4	1.69±.017	.25±.012	14.8 ±.77
46	2	2	16	16	38	14	6	4	2		1.2-2.8	1.98±.021	.31±.015	15.65±.77
45		3	11	22	29	15	12	5	3		1.4-2.8	2.03±.021	.31±.015	15.25±.75
45A		2	12	18	21	21	10	6	6	4	1.4-3.0	2.11±.026	.38±.018	18.09±.87
55	3	6	17	35	28	5	3	2	1		1.2-2.8	1.84±.019	.28±.013	15.2 ±.74
27	1	6	18	31	21	17	4	2			1.2-2.6	1.88±.019	.28±.013	14.89±.73
16		4	11	25	31	14	9	4	2		1.4-2.8	1.99±.021	.31±.015	15.58±.76
New Form	3	50	36	11							1.2-1.8	1.51±.010	.15±.007	9.94±.47
Total Aust. Sum	38	88	201	194	153	61	32	17	12	4	1.2-3.0	1.79±.0081	.339±.0057	18.90±.33
Total	53	180	339	314	231	95	47	23	14	4	1.2-3.0	1.77±.0061	.324±.0043	18.25±.25

A comparison of types is shown in Table 35 .

Table 35.

Summary of differences between the dimensions of the means of uredospores of Puccinia graminis tritici.

Types compared.	Length.		Width.		Shape.	
	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.
<u>P. graminis tritici</u> at large as recorded by Levine and the total of the 13 forms measured in Australia.	1.36±.20	6.8	1.54±.07	22.0		
<u>P. graminis tritici</u> at large as recorded by Levine and the total of the 8 forms naturally occurring in Australia.	1.15±.21	5.5	1.65±.08	20.6		
Total of all 13 forms measured in Australia and the total of the 8 forms naturally occurring in Australia.	.21±.11	1.9	.11±.06	1.8	.02±.01	2.0
<u>P. graminis tritici</u> 27 recorded by Levine and the same form isolated from English straw in Australia.	2.59±.39	6.7	1.84±.14	13.1	.324±.026	12.5
<u>P. gr. tr.</u> 43 compared with <u>P.gr.tr.</u> 46.	2.70±.36	7.5	2.49±.14	17.8	.41 ±.026	15.8
<u>P. gr. tr.</u> 45 compared with <u>P.gr.tr.</u> 45A.	.87±.33	2.6	1.32±.15	8.8	.08±.033	2.7

It will be seen that the results of measurements of all thirteen forms when compared with those given by Levine show that the local types are shorter and narrower than those recorded in America. In length the difference is significant though not so positive as is the width. The same thing applies in the comparison of the American results with those obtained from the eight naturally occurring Australian forms.

Of the individual forms, the only one common to the two sets of measurements is Form 27. Levine compared two isolations of this form, one from India with another from California. He found an appreciable difference in the width of the spores from the two sources, but considers that there is no conclusive evidence of them having different morphological identities. He remarks that this Form 27 was the most variable, as regards shape, of all his stock cultures. The measurements herein reported when compared with Levine's show very significant differences as regards length, width, and shape. Apart altogether from the differences in methods of measuring, there may be difference in the genetic constitutions of the rusts obtained from the different sources. Furthermore the form measured locally was obtained from aecidial infections produced on barberries from Welsh wheaten straw. It is known that this stage may give rise to the occurrence of new physiologic forms under certain conditions. The genetic constitution of the form used may be different from the isolations dealt with in U.S.A.

Taking the eight naturally occurring Australian forms, some interesting facts come to light. It has elsewhere been pointed out that Forms 43, 44 and 54 show rather marked similarities in parasitic capabilities. The separation of Forms 44, and 54 is not easy. An examination of the uredospore measurements of these three forms shows that there are striking similarities in length, width and shape. But morphology alone cannot be taken as a guide to specialisation. The contrast in parasitism between these three forms of Form

34 -- which at present is the predominant form in Australasia -- is most marked. And yet Form 34 conforms closely to the measurements given for the Forms 43, 44, and 54. The artificially produced Form 11 which is parasitically rather similar to Form 34, and was derived as a hybrid between it and Form 43 differs but slightly from it biometrically. And yet again the rather weakly parasitic Form 33 obtained in the uredospore stage from Dr. Levine agrees in measurements with these two virulent forms, 11 and 34. These six forms together constitute a group with biometric similarities.

Turning next to the other four forms which occur naturally in Australia, viz. Forms 46, 45, 45A, and 55, it will be seen that they show similarities in their measurements. As a group they are longer and narrower than the first group. This is strikingly reflected in the measurements of the shapes. As previously reported (163) this difference may be noted in a casual examination of the uredospores. They are much more variable in shape, and much longer and narrower. The actual differences between Forms 43 and 46 are set out and are very significant.

Conforming to this group of forms in which the uredospores are relatively long and narrow are the overseas Forms 16 and 27 derived from India and Wales respectively. The longest spore measured came from the lot of Form 16, being over 44μ in length.

Of special interest is the remaining form, designated the "New Form." It may be remembered that this was obtained from aecidia on a barberry as a hybrid between Forms 34 and 43. The uredospores are the most uniform of those met. They are the shortest of any of the forms measured, having a mean length of only $28.37 \pm .14$. They are shorter than those of any form recorded by Levine. Amongst the Australian measurements, they approximate most closely to those given by Form 11 which had its origin on the barberry at the same time and in much the same fashion as the New Form. Not only is it shorter, but at

the same time its mean width is the greatest of the forms measured. From this it follows that in shape it is also unique, the mean ratio of length to width being 1.51 : 1 .15.

(b) P. graminis avenae

Five forms of this rust have been determined from material collected in Australia. One further isolation has been made from aecidiospores produced on a barberry infected by teleutospores present on oaten straw collected in Ireland. Unfortunately, before the measurements were made one of the five Australian forms was lost. The two stock cultures of it died during very hot summer conditions. The measurements of the others are given in the following tables, together with the dimensions for the total of the Australian forms, and then for the sum total of all five forms measured.

Table 36. Variations and constants for length of uredospores of Puccinia graminis avenae.

Form No.	Spore classes in microns.											Size Limits	Constants.		
	20.46	22.32	24.18	26.04	27.90	29.76	31.62	33.48	35.34	37.20	39.06		Mean	Standard Deviation	Coefficient Standard of Variability
1(Aust.)		1	8	20	22	28	12	9				22.32-33.48	28.68±.18	2.67±.13	9.33±.45
1(Irish)			1	10	10	17	21	26	8	4	3	24.18-39.06	31.53±.22	3.26±.16	10.35±.50
2		1	9	16	28	25	12	7	2			24.18-37.20	30.52±.18	2.71±.13	8.90±.42
3	1	4	7	24	25	24	10	5				20.46-33.48	27.81±.18	2.72±.13	9.75±.47
7				13	19	23	19	16	7	2	1	26.04-39.06	30.51±.21	2.97±.14	9.75±.47
Total Aust	1	5	16	66	82	103	66	42	14	4	1	20.46-39.06	29.41±.10	2.99±.07	10.14±.24
Sum Total	1	5	17	76	92	120	87	68	22	8	4	20.46-39.06	29.99±.10	3.14±.07	10.52±.23

Table 37. Variations and constants for width of uredospores of Puccinia graminis avenae.

Form No.	Spore classes in microns.						Size ...Limits	Constants.		
	.13.02	14.88	16.74	18.60	20.46	22.32		Mean	Standard .Deviation	<u>Coefficient</u> of Variability
1 (Aust.)		2	15	66	16	1	14.88-22.32	18.58±.09	1.25±.06	6.75±.32
1 (Irish)		4	13	60	23		14.88-20.46	18.64±.09	1.38±.07	7.38±.35
2	1	15	37	40	7		13.02-20.46	17.43±.11	1.59±.08	9.13±.44
3		3	19	57	21		14.88-20.46	18.53±.09	1.34±.06	7.23±.35
7		1	27	49	23		14.88-20.46	18.49±.09	1.37±.07	7.40±.35
Total Aust.	1	21	98	212	67	1	13.02-22.32	18.26±.05	1.46±.04	8.00±.19
Sum Total	1	25	111	272	90	1	13.02- 22.32	18.33±.05	1.59±.03	8.67±.18

Table 38. Variations and constants for shape of uredospores of Puccinia graminis avenae.

Form No.	Ratio of length to width.									Size Limits	Constants.		
	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6		Mean	Standard Deviation	Coefficient of Variability
1 (Aust.)		5	22	40	30	3				1.2 - 2.0	1.61±.010	.151±.007	9.37±.45
1 (Irish)			14	30	34	13	7	1	1	1.4 - 2.6	1.75±.016	.242±.012	13.72±.66
2			10	22	30	20	12	4	2	1.4 - 2.6	1.88±.019	.28 ±.013	14.8 ±.72
3		8	24	45	16	6	1			1.2 - 2.2	1.58±.014	.22 ±.010	12.92±.63
7			14	41	27	12	5	1		1.4 - 2.4	1.71±.015	.218±.010	12.75±.62
Total Aust.		13	70	148	103	41	18	5	2	1.2 - 2.6	1.69±.009	.25 ±.006	14.51±.35
Sum Total		13	84	178	137	54	25	6	3	1.2 - 2.6	1.70±.008	.24 ±.005	14.20±.31

A number of comparisons may be usefully made as shown in the next tables.

Table 39.

Summary of differences between the means of the dimensions of uredospores of Puccinia graminis avenae.

Types compared.	Length.		Width.		Shape.	
	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.
Types of all forms measured in Australia and the U.S.A. type recorded by Levine.	1.49±.18	8.3	1.61±.09	17.9		
Types of all Australian forms and the form derived from straw from Ireland.	2.12±.23	9.2	.38±.10	3.8	.06±.018	3.3
A form derived from oaten straw from Ireland and a similar form obtained from Australian straw.	2.85±.34	8.5	.06±.13	.46	.142±.019	7.5

Table 46 . Summary of differences between the means of the dimensions of uredospores of Puccinia graminis avenae.

Types compared.	Length.		Width.		Shape.	
	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns	Difference divided by P.E.
<u>P. graminis avenae</u> 1. and <u>P. graminis avenae</u> 2.	1.84±.255	7.2	1.15±.133	8.6	.27±.021	12.8
P. ,, ,, 1. and P. ,, ,, 3.	.87±.255	3.3	.05±.12	.6	.03±.037	.9
P. ,, ,, 1. and P. ,, ,, 7.	1.83±.28	6.5	.09±.125	.7	.10±.018	.55
P. ,, ,, 2. and P. ,, ,, 3.	2.71±.255	10.6	1.10±.14	7.9	.30±.024	12.5
P. ,, ,, 2. and P. ,, ,, 7.	.01±.28	.03	1.06±.14	7.6	.17±.024	7.1
P. ,, ,, 3. and P. ,, ,, 7.	2.70±.28	9.7	.04±.129	.3	.13±.021	6.5

If a comparison be allowable with the dimensions of P. graminis avenae at large as recorded by Levine, it will be seen that uredospores of the rust as measured in Australia are significantly shorter and narrower. The same applies to the total of the Australian measurements in comparison with the type from Ireland. Even though strict comparison between the Australian measurements and those given by Levine be not permissible, such comparisons can be made between the Australian measurements and those of the Irish rust; these were all made under identical conditions. That the uredospores of the Australian rust are smaller than those of the overseas type is further borne out by a comparison of the stock culture of P. graminis avenae l. which originally came from Werribee, Vic. and the Irish culture of P. graminis avenae l. The differences in width are not significant, but they are certainly significant in regard to both length and shape of uredospores. It has already been pointed out that as regards colour of pustule, and a striking tendency to quickly produce teleutospores on seedling leaves of oats and grasses, this Irish rust is obviously different from the stock culture of P. graminis avenae l.

Taking next the forms naturally occurring in Australia, comparisons are instructive. Form 1. is significantly different from Form 2. as regards length, width, and shape of uredospores. Parasitically these two forms are markedly different. Very striking differences are also seen to exist as between Forms 2. and 3. But as tested on the standard differentials the differences between them are comparatively slight. As in the case of P. graminis tritici, it is therefore clear that there is no definite parallelism between differences in regard to morphology and parasitism.

(c) Puccinia triticina

It has been pointed out that in Australia there are two physiologic forms. These cannot be differentiated on

the standard set of varieties used by Mains and Jackson, but on "Thew" the separation is absolute. Australian Form 1 produces flecks whilst Australian Form 2 produces "4" reactions.

Measurements of uredospores of the two forms are shown in the following table.

Table 47. Variations and constants for length, width, and shape of uredospores of two Australian physiologic forms of Puccinia triticina.

Form No.	Spore classes in microns.								Size Limits	Constants.			
	16.74	18.60	20.46	22.32	24.18	26.04	27.90	29.76		Mean Length	Standard Deviation	Coefficient of Variability	
Aust.1.									<u>LENGTH.</u>				
Aust.1.			9	18	35	31	7		20.46-20.90	24.35±.133	1.97±.094	8.1±.39	
Aust.2.				13	24	45	11	7	22.32-29.76	25.13±.120	1.77±.084	7.06±.34	
Aust.1.									<u>WIDTH.</u>				
Aust.1.	1	18	54	26	1				16.74-24.18	20.61±.093	1.375±.065	6.67±.316	
Aust.2.	6	46	41	7					16.74-22.32	19.51±.095	1.4 ±.067	7.17±.34	
Aust.1.									<u>SHAPE.</u>				
Ratio of length to width.													
	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7					
Aust.1.	4	38	26	19	12	1			1.0 - 1.5	1.20±.0074	.11 ±.0053	9.17±.43	
Aust.2.	1	8	20	30	25	5	10	1	1.0 - 1.7	1.33±.0074	.11 ±.0053	8.28±.39	

A comparison of the two forms is set out here-
under :-

Table 41 . Summary of differences between the means of the dimensions of uredospores of two Australian physiologic forms of Puccinia triticina.

Types compared.	Length.		Width		Shape.	
	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.
Australian 1 and Australian 2.	.78± .147	5.2	1.10± .13	8.5	.13± .01	13.0

It will be seen that the second form is significantly longer and narrower than the first, and as is to be expected, significantly different in shape.

McAlpine gives the measurements of the uredospores as 20-28 μ in length and 18 to 21 μ in width. It will be seen that as regards length, these limits agree with those found in Australian Form 1. In width there is not the same agreement, but the figures approach more nearly to those of Australian Form 2. It would seem probable therefore that both the forms were present when McAlpine carried out his work.

TELEUTOSPORES

The identity of the uredospores taken for measurement can be accurately determined, and so one can be certain that a particular form and only that form is being dealt with. Teleutospores are somewhat more difficult to identify with certainty. In the cases in point, the teleutospores measured were obtained from naturally occurring straw which had also carried uredospores whose identity was established. Only the one physiologic form was present in each case, and it is reasonable to suppose that this form is also represented by the teleutospores which were measured.

McAlpine gives the teleutospore dimensions for Puccinia graminis as a composite species as 35-63 x 14-25 μ , with an average of 52 x 18 μ . He states that in size the teleutospores are very variable. No indication is given as to whether the measurements of length were made from the exterior of the apex to the point of attachment of the pedicel, and for width across the septum separating the two cells. At the last mentioned spot, a constriction usually occurs and it seems probable that the maximum width of the spores was actually measured. Taking the average figures for the measured teleutospores of P. graminis tritici and P. graminis avenae together it will be seen that the length

ranges from 30 to 74 μ and the width from 11 to 22 μ , with an average of 45 x 16 μ .

(a) P. graminis tritici

Teleutospores of only three Australian forms were available for measurement. Their dimensions followed by those for the sum total are given in the following table :-

Table 42. Variations and constants for length, width, and shape of teleutospores of three Australian physiologic forms of Puccinia graminis tritici.

Form No.	Spore classes in microns.												Size Limits	Constants.			
	29.76	33.48	37.20	40.92	44.64	48.36	52.08	55.80	59.52	63.24	66.96	70.68		74.40	Mean	Standard Deviation	Coefficient of Variability
	<u>LENGTH.</u>																
34	1	4	13	12	20	15	10	10	9	3	2		1	29.76-74.40	47.89±.58	8.64±.42	17.67±.89
43		6	19	22	18	25	6	3					1	33.48-63.24	43.64±.41	6.07±.29	13.87±.69
46	1	11	25	25	19	12	3	2	2					29.76-59.52	41.66±.40	5.89±.28	14.10±.68
Total	2	21	57	59	57	52	19	15	11	4	2		1	29.76-74.40	44.32±.25	7.40±.20	16.70±.47
	<u>WIDTH.</u>																
	11.16	13.02	14.88	16.74	18.60	20.46	22.32										
34		8	28	32	21	8	3							13.02-22.32	16.78±.15	2.20±.11	13.10±.63
43	14	21	45	11	8	1							11.16-20.46	14.53±.14	2.79±.10	14.28±.68	
46	2	5	35	29	25	4							11.16-20.46	16.41±.13	1.95±.09	11.9 ±.58	
Total	16	34	108	72	54	13	3							11.16-22.32	15.90±.075	2.27±.063	14.25±.41
	<u>SHAPE.</u>																
	Ratio of length to width.																
	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5								
34	4	15	19	28	19	6	6	2	1			1.5 - 5.5	3.01±.056	.826±.039	27.53±1.38		
43	1	14	24	26	13	10	8	4			1.5 - 5.0	3.09±.056	.829±.039	26.80±1.37			
46	1	30	36	18	9	2	1	2	1			1.5- 5.5	2.35±.048	.709±.033	30.00±1.55		
Total	6	59	79	72	41	18	15	8	2			1.5 - 5.5	2.92±.027	.82 ±.022	28.12±.895		

- 114a -

114a

114a

Comparisons of the forms measured are set out in the following table :-

Table 44. Summary of differences between the dimensions of the means of teleutospores of Puccinia graminis tritici.

Types compared.	Length.		Width.		Shape.	
	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.
The U.S.A. type recorded by Levine and the total of 3 Australian forms.	7.48±.55	13.6	.77±.142	5.4		
Form 34 and Form 43.	4.25±.71	6.0	2.25±.21	10.7	.08±.079	1.0
Form 34 and Form 46.	6.23±.71	8.8	.37±.20	1.9	.66±.074	8.9
Form 43 and Form 46.	1.98±.57	3.5	1.88±.19	9.9	.74±.074	10.0

Comparing the total of the three Australian forms with the measurements given by Levine, it will be seen that the Australian rust is significantly shorter and narrower. The greater variability is striking.

Between the three Australian forms there are differences. Forms 34 and 43 differ significantly in length and width. Form 34 is longer and wider than 43. It is much more variable in length. In shape there is no appreciable difference between the two. Between Forms 34 and 46 there are again differences. Form 34 is significantly longer than 46, the shortest of the three forms. The difference in width is not appreciable, but in shape the difference is significant.

Comparing Forms 43 and 46 it is seen that Form 43 is significantly longer and narrower than Form 46. There is also a difference in shape. These are just the reverse of the results of the uredospore measurements which showed that 46 is longer and narrower than 43.

(b) P. graminis avenae

Two sets of material were available for measurement. One batch of oaten straw came from Cowra, N.S.W. Uredospores from this straw showed the form to be P. graminis avenae l. The other batch came from Ireland and was in part used to infect a barberry. From the acidiospores, uredospore cultures were obtained. The form present proved to be P. graminis avenae l. The results are given in the following table.

Table 45.

Variations and constants for length, width, and shape of teleutospores of an Irish and an Australian form of *Puccinia graminis avenae*.

Form	Spore classes in microns.										Size Limits	Constants.		Coefficient of Variability								
												Mean Length	Standard Deviation									
	.29	.76	33	.48	37	.20	40	.92	44	.64	48	.36	52	.80	55	.80	59	.52	63	.24	66	.96
	<u>LENGTH.</u>																					
Irish	2	6	13	28	18	13	8	10	1	1	29.76-66.96	45.19±.59	8.83±.42	19.50±.96								
Australian	1	5	5	12	22	24	19	8	3	3	29.76-66.96	46.95±.44	6.54±.31	13.93±.67								
	<u>Width.</u>																					
	Spore classes in microns.																					
Irish	11.16	13.02	14.88	16.74	18.60	20.46	11.16-20.46	15.94±.12	1.83±.087	11.45±.55												
Australian	2	8	41	31	16	2	11.16-20.46	15.27±.12	1.85±.088	12.10±.58												
	<u>SHAPE.</u>																					
	Ratio of length to width.																					
Irish	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	1.5 - 5.5	2.915±.051	.755±.036	25.90±1.32									
Australian	3	12	31	28	15	5	4	2	2	1.5 - 5.0	3.145±.046	.685±.032	21.85±1.09									

A comparison of types are given in the following

table :-

Table 46 . Summary of differences between the dimensions of teleutospores of Puccinia graminis avenae.

Types compared.	Length.		Width.		Shape.	
	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.	Difference in means in microns.	Difference divided by P.E.
An isolation of <u>P. graminis avenae</u> 1 from Ireland and an isolation of the same form from Australia.	1.76±.31	5.7	.67±.17	3.9	.230±.07	3.3
<u>P. graminis avenae</u> as recorded by Levine and the isolation of <u>P. graminis avenae</u> 1 from Australia.	.80±.31 .80±.62	1.29	.57±.17	3.3		
<u>P. graminis avenae</u> as recorded by Levine and the isolation of <u>P. graminis avenae</u> 1 from Ireland.	.96±.73	1.3	.10±.17	.59		

It will be seen that the results given by Levine are intermediate in length and width between those of the Australian and the Irish rusts. Statistically there is no significant difference between the Irish and the U.S.A. rusts, but the difference in width between the Australian and the U.S.A. types may be significant. Comparing the Australian and the Irish forms, the former is the longer. The difference in length is significant, and this probably is also the case in width and shape. The uredospore measurements showed that the Irish was significantly longer than the Australian rust.

Photographs of the various spore forms used in the measurements are shown in Plates XII and XIII. It is obvious that these cannot have as great a value as the biometrical measurements for identifying forms. As far as possible, truly representative spores of the various forms were photographed to illustrate the types, but a small group of spores considered alone may be misleading. Taken in conjunction with the measurements they are instructive.

OVER-SUMMERING OF RUST.

In the case of annual crops like the cereals, the mode of persistence of pathogens from season to season is a consideration of the utmost importance. Rust is no exception.

In the Northern Hemisphere, many stem rust studies have been made and much information accumulated. The aecidial stage on the barberry is of the greatest importance in Europe. It has been found that in places where this alternate host has been eradicated, stem rust has diminished or is altogether unknown. In North America there is abundant evidence to show that the aecidial stage is similarly important. At one time it was considered that in the Mississippi Valley, uredospores were capable of overwintering and were important agents in producing the first infections in the spring. More recently it has been stated (66) that too much emphasis has been placed on overwintering uredospores in that country.

It has already been pointed out (162) that the aecidial stage in Australia has not been found under natural conditions, although it has been artificially produced on numerous occasions. Barberries are not native, and although they have been planted as ornamental shrubs in places, they are not common. A further consideration is that in Australia the wheat crop is sown in the late autumn and early winter and is harvested in the early summer. It is, then, during the hot dry summer season that wheat is not cultivated. Instead of the rust fungus having to "over-winter" as in the Northern Hemisphere, it has here to "over-summer". This does not mean that the teleutospores in Australia could not function as resting spores as they do in the Northern Hemisphere. They remain viable throughout the hot dry summer. In the following late spring and early summer (October may be considered the peak month) they germinate and can infect the barberry. It is at this period when the aecidia may be produced that the

uredospore stage is already abundant in the crops. The aecidial stage in the Northern Hemisphere mainly functions in bringing about the early spring infections of the crop. From these are produced the abundant uredosori. It becomes evident that the alternate host in this country is not important and that there must be some means other than by aecidiospores whereby the organism oversummers in Australia.

McAlpine (105) considers that "volunteer" and second growth wheat probably harbour the uredospore stage of the fungus, and that the organism persists by this means. Evidence bearing upon this was reported (160) in 1920.

In the present studies, a good deal of further information has been obtained.

OVERSUMMERING OF *P. GRAMINIS TRITICI*.

Taking first the case of stem rust of wheat, the following table shows the position.

Table 47. Summary of the number of samples of rusted wheat examined during each month of the period.

Month	Season of collection, ending March of								Total
	1922	1923	1924	1925	1926	1927	1928	1929	
April					1		8	3	12
May	1	2					4	4	11
June		1		1		1		8	11
July	1						6	9	16
Aug.								6	6
Sept.	1				2	1	3	11	18
Oct.	12	1		20	9	17	10	27	96
Nov.	8	3	9	30	8	39	91	66	254
Dec.	7	4	4	35	5	8	43	11	117
Jan.	5	2	7	13		5	15	8	55
Feb.	3	2	3	1	1	4	7	2	23
March	3		2		2	14	2	1	24
Totals	41	15	25	100	28	89	189	156	643

The reason for the name "summer rust" being applied to the stem rust becomes at once apparent. November is the month when the rust is most abundant. Regarding the aggregate receipts for the various months, it is seen that samples have come to hand during each month of the year. In the earlier years of the study but little material was received. The more abundant collections during the last three years are in measure due to valuable assistance rendered by graduates appointed to country districts. The collections for these three years indicate clearly that rust in the uredospore stage is almost certainly present in wheat growing areas in a viable condition all the year round. The collections which are available under existing conditions are after all very scanty. A wider search would certainly have brought to light many more samples of rust. Since September 1927 there have been only two months when viable uredospore material has not been collected. This clearly points to the fact that the rust persists in the uredospore stage from season to season.

In this connection it should be pointed out that there is a very wide range in the conditions which prevail in the wheat-growing areas of Australia. Having regard solely to the south-eastern portion of the continent, this still applies. Wheat in coastal areas is sometimes sown in March for providing green-feed during the winter. Sowing in other areas continues throughout the ensuing months as late as July. Similarly there is a continuity in the harvesting operations. Early crops for hay may be taken off in October, and harvesting operations are in progress right through until even February in late districts. This means a great difference in the development and the amount of "volunteer" wheat, - both "second-growth" and "self-sown" - which is present in paddocks in the various districts.

Apart altogether from this occurrence of stem rust in different districts, evidence is not wanting that in a particular area, rust is present on volunteer wheat throughout

the year when conditions are favourable. Mr. R.E. Dwyer of Bathurst, kindly undertook a search at the Bathurst Experiment Farm. In February 1927 a first collection was forwarded, and thereafter until April 1928, in each month with one exception of August 1927, rusted wheat straw carrying viable uredospores of P. graminis tritici was forwarded. The absence of the collection in the one month of August may well have been due to the pressure of other duties limiting the search.

In any case six other collections from different N.S. Wales localities came to hand during that month. Some of these were on volunteer wheat, others on the growing crop, young as it was at that time. It was very striking to see heavy stem rust infections of the leaves of seedlings collected in the paddocks.

At H.A. College a series of collections points to the same state of affairs. Mr. T.H. Harrison has forwarded very many specimens from time to time, and though there are gaps in the monthly sequence, there would seem little doubt about the presence of viable uredospores on volunteer wheat the year round.

There is a practical bearing to this persistence of the uredospore stage on volunteer wheat. Ordinary cultivation methods cannot be expected to eliminate it, for the cultural practices at the Bathurst Experiment Farm and H.A. College for example, are good. Whilst viable uredospores are present in the wheat areas there will be the possibility of epidemics of rust in favourable seasons so long as susceptible varieties of wheat are grown. Safety in "muggy" seasons lies in the growing of varieties which are resistant to rust.

OVERSUMMERING OF P. GRAMINIS AVENAE

The results of the studies of this organism are summarised in the following table.

Table 48. Summary of the numbers of samples rusted oats examined during each month of the period.

Month	Season of collection, ending March of								Total
	1922	1923	1924	1925	1926	1927	1928	1929	
April							3	1	4
May							2	2	4
June						1			1
July	1			2				3	6
Aug.								2	2
Sept.	1							3	4
Oct.	5		1	5			1	5	17
Nov.	1		1	11	1	14	17	28	73
Dec.	2			11		3	11	3	30
Jan.	1		2			1	7	2	13
Feb.			1	1	1	1	2		6
March					1	1	4		6
Totals	11		5	30	3	21	47	49	166

In the aggregate, the relative abundance of oat stem rust in the various months throughout the year approximates closely to that shown by the wheat stem rust. Although fewer isolations were available, they indicate clearly that stem rust of oats is most abundant in the summer, but it is nevertheless present on "volunteer oats throughout the year when the crop is not being grown. An intensive search would almost certainly reveal the presence of abundant viable material continuously throughout a year. The widespread occurrence of "wild oats" is a further important factor in the case.

OVERSUMMERING OF P. TRITICINA.

The results of the collections are summarised in the following table.

Table 49. Summary of the numbers of samples of rusted wheat examined during each of the months of the period.

Month	Season of collecting, ending March of								Total
	1922	1923	1924	1925	1926	1927	1928	1929	
April								4	4
May	2						1	5	8
June							2	9	11
July							1	11	12
Aug.								17	17
Sept.	1		1				2	41	45
Oct.	6					1	16	23	46
Nov.	3					2	75	16	96
Dec.	1						15	4	20
Jan.	1							1	2
Feb.						1			1
March	1					2			3
Totals	15		1			6	112	131	265

Here again it appears that November is the "peak" month. But it is clear that as early as September the rust is abundant. Actually the occurrence of the peak in November is probably misleading. It should occur earlier. The studies herein reported have dealt primarily with stem rust. Collections forwarded from country centres have been, in the main, stem rust, and have been made because this particular rust has been present. The occurrence of leaf rust on the flag earlier in the season has not resulted in field workers forwarding specimens. Whenever leaf rust has been present on material in addition to stem rust, it has been cultured and subjected to study.

The indications are clear that leaf rust is also present on volunteer wheat the whole year round.

BREEDING FOR RUST RESISTANCE

INTRODUCTION

In the control of plant diseases many methods are used. Of these it is generally conceded that the practice known as "Immunisation" offers the greatest promise. When dealing with an annual crop plant cultivated as wheat is, other methods are difficult of application. Breeding for disease resistance is the main hope for the control of rust as well as the other diseases of wheat. Considerable success has attended work of this nature in many places, and a voluminous literature has accumulated. References to some of the important contributions are included in the bibliography. The phenomenon of specialisation offers one of the greatest complications. Controlled genetical studies help largely in overcoming this obstacle.

Naturally success in breeding work is not likely to be met with except as a result of many years of work. Any addition to the physiologic forms of the pathogen present in a country is going to seriously prolong the operation. This is well illustrated in the results of the work now to be recorded. These have not involved the production of a rust epidemic in the field. The danger of having valuable F₁ and other wheats killed or seriously affected by such an occurrence has made it unwise to produce an epidemic. With a "rust nursery" available, this would be done.

CROSSING TECHNIQUE

The methods adopted in the work may be briefly described.

Heads of the maternal variety were selected just as the anthers about one third the length of the head below the tip were turning yellow and ripening. The flowers were emasculated in the usual way. But instead of leaving only about three spikelets on each side of the rachis of the s

it has been the practice to leave double that number, and frequently many more. The emasculated heads were covered with glassine bags and allowed to mature for pollination.

A test was made to determine how long such heads ought to be left before being pollinated. The variety "Federation" which is botanically Triticum vulgare alborumbrum Körn, was selected as the ovule parent, and "Indian 12", which is T. vulgare pseudo hostianum Flaksb, as the pollen parent. The former has opaque grain and the latter corneous grain. Grain produced as the result of crossing the two is corneous, so it was possible to be assured that grain set on "Federation" was the result of crossing. Furthermore the F₁ plants are markedly different from each of the parents so that any plants not true crosses can be readily detected.

In the test that was made, fifteen heads were selected at the same stage of development, viz. with the ripest anthers just turning yellow. These were emasculated and bagged, and successively pollinated with "Indian 12" on different days, the operation being carried out in the early afternoon. For purposes of pollination, from the selected male parent an anther which at a touch was ready to dehisce was inserted in each emasculated flower. The pollinated heads were then covered again with the glassine bags and allowed to mature grain. The result of the test is shown in the following table:

Table 50. The result of pollinating stigmas of "Federation" of different ages with pollen of "Indian 12".

Cross No.	Date of Emasculation	Date of Pollination	Age of Stigmas in days	Number of Flowers Pollinated	Number of Grains produced	Percentage of Grain Set
23.31	13.10.23	13.10.23	0	24	11	45.8
32	"	14.10.23	1	28	21	75
33	"	15.10.23	2	28	22	78.6
43	"	17.10.23	4	26	16	61.5
54	"	22.10.23	9	28	3	10.7
69	"	24.10.23	11	24	1	4.2
72	"	26.10.23	13	28	0	0
74	"	27.10.23	14	20	0	0
76	"	30.10.23	17	32	0	0
83	"	31.10.23	18	26	0	0
92	"	1.11.23	19	23	0	0
96	"	2.11.23	20	24	0	0
100	"	5.11.23	23	24	0	0
103	"	6.11.23	24	28	0	0
106	"	7.11.23	25	26	0	0
109	"	8.11.23	26	26	0	0

Each of the grains was corneous. Confirmation of the fact that they were the result of the pollination specified was obtained by sowing them and noting their growth. All had tip-beards, velvet chaff which was bronze and coloured grain. These characters are of course quite different from those of "Federation" the ovule parent.

Clearly then, under the conditions prevailing at Sydney University, the best result was obtained by pollinating flowers 2 to 3 days after emasculation. After about 10 days a setting of grain can hardly be expected.

It was of interest to note that the one grain produced in cross No. II 23.69 was present in the lowest flower on the spike; this is of course the latest flower in the head to mature.

At Chico, California, Florell reports that a 26% successful pollination was obtained in 1920 in a case involving about 240 pollinations of emasculated flowers which had been left for 16 days. The different climatic conditions doubtless explain the divergent results.

To indicate the efficiency of the method the following results which were obtained may be quoted.

Table 51. Results obtained in crosses between varieties of the same species groups of wheat in 1925, showing the number of grains set.

Cross No.	Parents	Grains set	Pollinations made
1125.13	Bunyip x Early Bird	20	24
16	Gullen x Firbank	18	24
18	Bunyip x Gullen	14	26
19	Florence x Gullen	11	22
21	Riverina x Firbank	19	22
22	Gullen x Florence	17	24
24	Clarendon x Bunyip	12	24
27	Thew x Riverina	16	18
30	Indian F x Canberra	16	26
31	Cedar x Indian F.	20	26
33	Canberra x Gullen	20	24
34	Canberra x Bunyip	18	24
35	Canberra x Bunge	18	26
37	Indian F x Cedar	14	28
38	Gullen x Federation	11	18
40	Bunyip x Federation	15	24
43	Federation x Gullen	27	30
46	Waratah x Clarendon	23	24
47	Bena x Clarendon	28	30
48	Clarendon x Bena	21	26
49	Clarendon x Waratah	18	24
52	Federation x B 33/4	23	26
54	Federation x Canaan	27	30
55	Federation x College Eclipse	26	28
56	Federation x Aussie	16	26
57	Federation x White Federation	15	22
58	Indian F. x Jonathan	22	26
59	Canaan x Waratah	19	20
60	Canaan x Federation	30	32
65	Bobs x Federation	23	26
69	Canberra x Bobs	21	22
70	Jonathan x Bobs	24	26
71	Bathurst 7 x Bathurst 8	29	30
72	Indian F. x Bobs	17	22
75	Khapli x Glossy Huguenot	26	30
80	Federation x Jonathan	29	34
86	Khapli x Kahla	21	28
88	Federation x Barletta	21	22
100	Federation x Japanese Bearded	19	22
	Percentage of grain set 79%	781	986

Table 52. Results obtained in crosses between varieties of the same species groups of wheat in 1926, showing the number of grains set.

Cross No.	Parents	Grains set	Pollinations made
II 26.6	Florence x Gullen	18	22
9	Firbank x Florence	15	24
10	Gullen x Federation	13	24
14	Federation x Firbank	10	24
15	Federation x Florence	15	24
21	Federation x Thew	18	24
22	Bunyip x Gullen	16	22
26	White Federation x Federation	15	24
29	Hard Federation x White Federation	12	24
30	White Federation x Hard Federation	13	22
31	Federation x White Federation	11	32
38	Marshall's No. 3 x Thew	13	30
39	Federation x Roseworthy	24	26
40	Thew x Roseworthy	11	14
43	Exquisite x Thew	9	28
44	Wandilla x Federation	21	28
47	Bobs x Roseworthy	23	26
48	Roseworthy x Bobs	24	30
49	Thew x Marshall's No. 3	14	16
53	Glossy Huguonot x Kubanka 2094	25	32
56	Yandilla King x Gullen	17	22
57	1123.97.2 x 1123.97.4	10	22
58	Federation x Webster	20	22
65	Webster x Federation	12	24
66	Federation x Webster	15	18
71	Thew x Japanese Bearded	11	20
78	Hope x Federation	8	24
	Percentage of grain set 64%	413	648

Table 53. Results obtained in crosses between varieties of the same species groups of wheat in 1927, showing the number of grains set.

Gross No.	Parents	Grains set	Pollinations made
1127.12	Firbank x Florence	22	26
13	Florence x Firbank	17	26
14	Gullen x Florence	20	24
15	Florence x Gullen	17	26
16	Bunyip x Gullen	19	22
17	Gullen x Federation	20	28
19	Federation x Florence	21	30
20	Florence x Federation	21	26
21	Hard Federation x Florence	19	30
25	Thew x Canberra	23	24
28	Yandilla King x Thew	28	34
34	Hard Federation x White Federation	16	28
36	Yandilla King x Gullen	16	28
37	Federation x Yandilla King	20	26
43	Federation x Roseworthy	19	26
48	Federation x Marshall's No. 3	22	26
53	Federation x Rieti	14	22
80	Federation x Euston (Sel.)	21	24
81	Federation x Euston (Sel.)	25	26
82	Federation x Euston (Sel.)	20	24
87	Waratah x Webster	18	26
89	Clarendon x Webster	16	24
91	Marshall's No. 3 x Webster	12	14
93	Warran x Webster	16	26
94	1 17/4 x Webster	18	20
98	J 37/1 x Webster	14	18
102	H 27/2 x Webster	23	26
103	G 1/1 x Webster	14	20
110	Webster x Euston (Sel.)	13	16
111	Euston x Webster	14	18
117	Euston x Webster	20	26
123	Euston x Webster	17	22
127	Euston x Webster	19	22
128	Euston x Webster	15	18
	Percentage of grain set 76%	629	822

In many species crosses made between varieties of groups like T. vulgare and T. durum, where the chromosome numbers are different, poor setting of grain was of course obtained. Low results were also obtained, as was expected, in certain crosses in which it was known that the emasculated heads had been left longer than they should have been, or that the pollen used was unavoidably in a poor condition. These results have not been included in the tables. There was a noticeable falling off in the efficiency of the crossing in

1926 and in 1928 when abnormally dry spring and early summer conditions were experienced. But the results show that under ordinary conditions, an efficiency of 75% may be looked for.

GERMINATION AS AFFECTED BY THE AGE OF GRAIN

It was decided to see how soon after pollination under the prevailing conditions, viable grain was set. In a uniform plot of Federation wheat growing at Sydney University, a number of plants were selected on the same day when the first flowers of the most forward ear of each plant were just about to push up the anthers. All these ears at the same stage of maturity were tagged, and all but the two lowest flowers in each spikelet removed. Commencing on the third day from the first appearance of the anthers, i.e. the 16th of October, the stalk bearing the tagged head was cut off at ground level. This was done at daily intervals as far as possible. The harvested heads were suspended in a dry room for a little more than four months, when the grain was rubbed out and tested for germination by sowing in separate pots of garden soil. Pots of normal graded "Federation" grain were sown at the same time for comparative purposes. The results are shown in the following table:-

Table 54. Stage of development of wheat grain after pollination in relation to germination capacity Anthers first showing in each case on 13th October.

No.	Date Harvested	Age of Grain in days	Character of Grain	Character of resultant Seedlings.
1	16 Oct.	3	Nil	Nil
2	17 "	4	Nil	Nil
3	19 "	6	Very shrivelled	$\frac{11}{28}$ Very spindly
4	20 "	7	do.	$\frac{12}{26}$ do.
5	22 "	9	do.	$\frac{17}{26}$ do.
6	23 "	10	Shrivelled	$\frac{24}{24}$ Spindly
7	24 "	11	do.	do.
8	25 "	12	do.	do.
9	26 "	13	do.	do.
10	27 "	14	do.	do.
11	29 "	16	do.	do.
12	30 "	17	Somewhat pinched	Almost normal
13	31 "	18	do.	do.
14	1 Nov.	19	do.	do.
15	2 "	20	do.	Normal
16	3 "	21	do.	do.
17	5 "	23	do.	do.
18	6 "	24	do.	do.
19	7 "	25	do.	do.
20	8 "	26	Normal	do.
21	9 "	27	do.	do.
22	10 "	28	do.	do.
23	12 "	30	do.	do.
24	13 "	31	do.	do.
25	14 "	32	do.	do.

It was surprising to find that after only six days from pollination, 28 very shrivelled grains in an ear produced 11 very spindly seedlings. The seven day old ear gave 12 very spindly seedlings from the 26 very shrivelled grains formed, and the nine day ear, 17 from 26 grains. Thereafter although the grain was shrivelled, it was fully viable but gave spindly seedlings. An ear 17 days old gave almost normal seedlings. These results are illustrated in Plate VIII.

The result showed that it was perfectly safe to use this method of harvesting crossed heads of wheat and obtain a

normal germination of the crossed grain. On account of the danger from sparrow attack and from damage by trespassers, the method of harvesting the stalks carrying the crossed ears after lapse of a period of 3 to 4 weeks from pollination was adopted, and has proved fully satisfactory.

WIDE CROSSES

The possibility of obtaining resistant types of wheat from wide crosses has not been neglected. This was additional to considerations concerning the genetics of such crosses.

Wheat x Barley

The commercial variety of wheat named "Bobs" is reputed to have been derived from a cross of this nature. It was produced by the late William Farrer in 1896 and is stated to have originated from a cross between "Nepaul" Barley and a wheat named "Early Lambrigg". Starting with one shrivelled grain obtained from the cross, the variety was fixed in four years.

A second Australian variety known as "Canberra" is also stated to have originated in a cross of this nature. The parents in this instance are reputed to be "Federation" crossed with "Volga" Barley.

In the work that is in progress, both these varieties of barleys were used as the pollen parents, the main work being done with "Volga". Farrer's "Early Lambrigg" was not available as an ovule parent. A number of varieties of T. vulgare which flowered at the same time as the two barleys were used as the ovule parents. The work reported covered tests made in 1924, 1925, 1926, and 1927. This gave a range of seasons in case some special seasonal influence might contribute to successful crossing. The practice was to emasculate the flowers at the stage already described under the technique of crossing, and to leave them until the next day

for pollination. On the following day the pollinations of the same flowers were repeated. In a number of cases when it was practicable, yet another pollination was made on the next day. In the results given hereunder, this third pollination is not counted. Nor is any note taken of numerous further attempts made by senior students of various classes. The "pollinations made", totalling 1932, mean that 961 wheat flowers were actually used, being pollinated at least twice. The results are as follows:-

Table 55. Results of attempts to cross varieties of T. vulgare with 2 varieties of barley.

Parents of Cross	No. of grains set	No. of Pollinations made
Clarendon x Volga Barley	2	336
Hard Federation x "	0	80
Federation x "	0	104
Hurst's 11. x "	0	328
Bunyip x "	0	332
Early Bird x "	0	92
Sunset x "	0	48
Flowerman's No. 3 x "	0	52
Canberra x "	0	144
Firbank x "	0	52
Federation x Nepaul Barley	1	164
Canberra x "	2	144
Bobax x "	0	56
	5	1932

It will be seen that in all 5 grains were obtained. In each case they were sown the next season and grown to maturity. All proved to be normal wheat plants of the variety used as the pollen parent, viz. Federation, Canberra and Clarendon. In the succeeding generation no segregation occurred. Each was clearly the result of accidental self-pollination.

There would therefore seem to be doubt whether the claims that barley and wheat were successfully crossed to give "Bobs" and "Canberra" can be substantiated.

Wheat and Rye

It has long been known that crosses between these two cereals can be made. Reference has elsewhere been made to the fact that even in seasons when wheat was heavily attacked by P. graminis tritici 34, adjacent crops of rye showed but rare infections. For a long time it has been hoped that cross-bred material might be available for studies in rust resistance.

In the years 1921, 1922, 1924, 1925, 1926, 1927 and 1928, crosses have been attempted. The ovule parent in each case has been wheat. Federation has been mainly used, but in some cases Hard Federation has been substituted, and in fewer instances Thew. The pollen parent has in the main been "Rosen" rye, but "Black Winter" has also been used. As in the case of the wheat x barley crosses, pollinations of the same flowers were made on the 2nd. and on the 3rd days after emasculation. In these instances also, yet a third pollination was many times made, so the results given are conservative. Actually 1010 wheat flowers were used. Senior students made additional attempts which are not here included.

During the six years, 13 grains have been obtained from 2020 pollinations, 5 of the grains failed to germinate,

7 yielded Federation or Hard Federation plants which showed no departure from the normal behaviour of these varieties and were therefore the result of accidental self-pollination. In only one case to date has a true cross been obtained. This was in 1925 from a cross between Federation and Rosen rye. The F_1 plant tested with Forms 43 and 46 gave "flecks", whereas the ovule parent Federation at the same time gave "4" reactions. The seedling after test was transplanted and grown to maturity. A notable feature was its abundant stooling. This was welcomed as giving the possibility of making back crosses. One of the heads is illustrated in Plate VIII.

The hybrid plant proved to be completely sterile, though pistils and stamens were seemingly normal. A large number of attempts were made to effect crosses with each of the parents, and also to pollinate stigmas of the hybrid plant with pollen from the wheat and from the rye parents. In no case was the attempt successful. The hybrid was sterile and the attempt to obtain resistance accordingly failed.

From the crosses made in 1928 one plant is available from Canberra x Rosen rye. In the seedling test, this gave flecks with *P. graminis tritici* 46 which normally produces a fully susceptible reaction on Canberra. It is therefore a hybrid and is being grown to maturity under carefully controlled conditions.

It will therefore be seen that so far no success has attended the efforts to secure fertile progeny as a result of crossing wheat and rye.

INTERSPECIFIC WHEAT CROSSES

Khapl1 Crosses

Of the wheat varieties tested by different workers for resistance to stem rust, the variety of *T. dicoicum* Ajar,

Perc. known as "Khapli" is perhaps the most resistant. Of the physiologic forms recorded to date by Stakman and Levine, only one attacks Khapli. It is therefore not surprising that many efforts have been made to cross this resistant emmer with the commercial types of vulgare wheat which are susceptible.

Hayes and Stakman (61) report disappointing results from the attempts to cross Khapli with Marquis wheat. Hynes (76) reports a successful cross between Federation and Khapli.

Commencing in 1921 attempts have been made each year to obtain successful crosses between commercial vulgare wheats and Khapli. In the main Federation has been the variety used. Extending the work over a number of seasons like this has made it possible for various seasonal influences to operate.

In the work it has been the practice to pollinate the vulgare wheat with Khapli pollen on the day following the emasculation, and to repeat this the next day. In very many cases a third pollination was also effected. In tabulating the results only the two pollinations are taken into account. Actually 930 flowers were used for the pollinations. Senior students also made a number of attempts to secure results, and these are not taken into account. The figure 1860 is therefore a very conservative estimate of the pollinations made.

The results are summarised as follows:

Table 56. Results obtained in 7 of the years in attempts to cross certain vulgare wheats with Khapli emmer.

Year	Vulgare Parent	No. of Grains set	No. of Pollinations made.
1921	Federation	0	32
1922	Federation	1	40
	Hard Federation	5	34
1923	Hard Federation	9	96
1924	Federation	17	262
1925	Federation	13	96
	Canberra	7	52
1926	Federation	25	304
	Gluyas	6	48
	Gurka (Gullen)	9	44
1927	Federation	106	380
	Gluyas	52	240
	Gurka (Gullen)	7	64
	Bunyip	15	60
	Hurst's 11.	9	56
	Early Bird	5	52
TOTALS		286	1860

Table 57. Summary of the results of attempts to cross certain varieties of vulgare wheat with Khapli emmer.

Variety	No. of Grains set.	No. of Pollinations
Federation	162	1114
Hard Federation	14	130
Gluyas	58	288
Gullen	16	108
Bunyip	15	60
Hurst's 11.	9	56
Early Bird	5	52
Canberra	7	52
TOTALS	286	1860

Out of the 286 grains obtained, 2 germinated with the production of pure Federation plants. These 2 grains were plump and well filled. Clearly they were accidental self-pollinations. The 284 F_1 plants which were crossed thus show approximately a 15% setting of grain. In all instances the grain was much shrivelled. Much of it was very tiny. Typical results are illustrated in Plate IX.

The crossed grain was sown in pots or boxes under the best conditions for germination.

Prior to 1927, the F_1 seedlings were in part inoculated in the "first leaf" stage with Form 43, and in a later leaf stage with Form 46. In other cases this order was reversed. Form 46 being used first and Form 43 second. The mean of the reactions shown with Form 43 was "1" and with Form 46 "2". Simultaneous tests on Federation and Khapli gave respectively "4" and "0"; with Form 43, and "4" and "1" with Form 46. There is therefore dominance of resistance in the F_1 , although the dominance is not quite complete. In 1927 and 1928 the F_1 seedlings were inoculated with Form 34. Whilst the parental reactions were again as just stated, the F_1 reactions were of the "X" type. They are illustrated in Plate IX.

After the seedling tests, the F_1 plants were transplanted to open beds and given the best possible conditions for growth. In no case did any one of them attain to maturity. The plants were stunted and died before producing ears, - with two exceptions, - usually when about 10 inches high. It was observed that there was a difference in development depending upon the vulgare parent used. When Gluyas and Hurst's 11 were the parents, the smallest growth took place, the plants reaching only 6 inches in height. With Federation as the parent, the F_1 plants grew slightly larger, reaching 8 inches. The plants derived from the crosses using Bunyip and Early Bird, grew to 10 inches, being exceeded slightly by those derived from the Gullen crosses, which grew to 12 inches. Illustrations of these are given in Plate X.

The two exceptions above referred to occurred in the crosses of Khapli with Bunyip and Gullen. In each case one tiny ear resembling an emmer with a tip beard was

produced. Each ear comprised two lateral spikelets on each side of the spike. In one of the spikelets of one ear three small stamens were produced and in one spikelet of the other ear, a small pistil was found. In each instance an effort was made to cross back on to the Bunyip, Gullen and Khapli parents. The pistil of the F_1 in addition to receiving pollen from Bunyip, Gullen and Khapli, was also pollinated with one of the three small anthers produced by the other F_1 plant. No setting of grain occurred in any case.

In 1924 Mr. J. T. Fridham forwarded a quantity of grain stated to be an F_2 generation obtained from a cross between Canberra and Khapli. The grain was white and opaque and perfectly plump and well-filled. No shrivelled grains at all were present. A series of over 900 seedlings was tested with Forms 43 and 46. To these Canberra shows respectively resistance and susceptibility, whilst Khapli is strongly resistant to both. In all cases the seedlings showed resistance to Form 43 and susceptibility to Form 46. Nevertheless they were transplanted to open beds and grown to maturity because of the value of such cross-breds. All plants showed the typical characteristics of Canberra proving clearly that the F_1 plant or plants had resulted from accidental self-pollination of the female parent.

It is therefore believed that little is to be gained from attempts to directly cross vulgare wheats with Khapli. The fact that the F_1 plants of some vulgare parents showed greater vigour than others may make it worth while to preserve with tests of other vulgare varieties as parents.

The attempts to incorporate the Khapli resistance in vulgare wheat are being made in other ways. Khapli has been crossed with certain durum wheats which readily give fertile

progeny as a result. Some of these F_1 plants have been used for crossing with commercial vulgare wheats. In other cases, series of F_3 families of such crosses have been tested in the seedling stage for resistance and families homozygous for resistance of the "Khapli" type noted. These will be used for crossing with commercial vulgare wheats. These studies are still in their early stages but the general results may be indicated.

One of the crosses involves the durum wheat known as "Kahla". This is resistant to all the Australian forms except the second group, viz. 45, 46, and 55. "Khapli" is strongly resistant to all.

In the F_1 , six seedlings tested with Form 43 as representing the first group gave flecks. Tested with Form 46 they gave "1" reactions. There was dominance of resistance. At that time the troublesome Form 34 had not yet made its appearance.

A group of 190 F_2 seedlings was tested with Form 43. They showed strong resistance throughout. Another batch of 184 was tested with Form 46. There were 170 resistant; 14 susceptible plants. This approximates to a 15 : 1 ratio. On a 2 factor basis the expectancy for 184 individuals would be 172 resistant : 12 susceptible plants. This result is now being confirmed by F_3 tests.

A series of F_3 tests involving the use of Form 34 is in progress and points clearly to the operation of 2 factors.

Further crosses between "Khapli" and "Abyssinian" and between "Khapli" and "Glossy Huguenot" are giving essentially similar results.

VULGARE AND DURUM CROSSES

The durum wheats are reputedly rust-resistant in Australia. Tests of seedlings have shown that some varieties are quite susceptible. Others are strongly resistant to some of the physiologic forms, e.g. Arnautka, Mindum, and

and Spelmars of the differential set. It was decided to see the mode of inheritance of this sharp resistance in crosses with vulgare wheats.

Crosses were made in 1922 between Federation and each of these three durums. From 176 Federation flowers pollinated, 98 grains were secured. This approximates to a 50% setting of grain. They were somewhat shrivelled and in some cases produced very weakly F_1 plants which failed in the seedling stage. The F_1 plants were tested in the seedling stage with Forms 43 and 46 to which the durum parents are respectively strongly resistant and strongly susceptible. The reactions are flecks in the first case and "4" in the second. Federation is completely susceptible to both.

The F_1 plants gave "1" reactions to Form 43 and "4" to 46, indicating dominance of resistance. The F_1 plants at maturity were intermediate in characters between the two parents. Many flowers in each spike failed to produce any grain, and where grain was set much of it was very shrivelled.

F_2 tests were made with seedlings sown in boxes and inoculated with Form 43 in the usual way. The results are as follows:-

Table 58. Results in the F_2 generation of crosses between Federation and certain durum varieties tested with Form 43

Cross	Reactions and Frequency				
	0;	1	2	3	4
Federation x Arnautka	26	22	50	28	5
Federation x Spelmars	151	26	34	44	10
Federation x Mindum	13	9	3	11	
Mindum x Federation	38	37	9	3	21
TOTALS	228	94	96	86	36

Summating the resistant and susceptible classes, the ratio is 418 resistant : 122 susceptible. The expectancy on a single factor hypothesis is 405 resistant : 135 susceptible.

$$\frac{D}{P.E.} = \frac{13}{6.79} = 1.91$$

After completing the tests on the first leaf, the seedlings were carefully pruned and new leaves of each plant inoculated with Form 46. In 10 plants out of the 540 the reaction instead of being a "4" was "1". Cultures from these

tiny reactions were made and proved to be Form 46. Transgressive segregation had occurred. This series of tests was carried out in the spring and although the transgressive segregates were transplanted they failed to mature.

In regard to Form 43, there would seem to be a single dominant factor determining the resistance of these durum wheats when they are crossed with Federation. Complications from sterility in the progeny, may, however, upset this assumption.

A second cross made in the same year was between Marquis and Mindum and reciprocally. Form 43 gives completely susceptible and resistant reactions respectively on these parents. The grain setting was 29 grains from 46 pollinations.

The F_1 plants gave flecks with Form 43. There was a considerable degree of sterility in the F_1 plants.

The F_2 results were as follows:-

Table 59. Results of testing F_2 generation seedlings of crosses between Marquis and Mindum with Form 43

Cross	Reactions and Frequency				
	0;	1	2	3	4
Marquis x Mindum	35	18	48	16	16
Mindum x Marquis	52	4	20	22	1
TOTALS	87	22	68	38	17

Summating the resistant and susceptible groups, the ratio is 177 resistant : 55 susceptible plants. On a single factor hypothesis the expectancy would be 174 : 58.

$$\frac{D}{P.R.} = \frac{3}{4.45} = .68$$

Here again there are clear indications that a single dominant factor for resistance is present in the durum parent.

This work with the durums has not so far been carried beyond the F_2 generation. As pointed out later, from certain vulgare wheats the desired resistance should be obtainable, and will not involve the very wide segregation and sterility involved in the vulgare and durum crosses. But the work points to the resistance of the durums being due to one main dominant factor.

VULGARE CROSSES

CANBERRA x THEW

It has been pointed out that specialisation studies revealed that prior to 1926 there were six physiologic forms of stem rust in Australia. These six forms belonged to two divergent groups, each group comprising three forms. Tests with commercial varieties of wheat showed that amongst others, "Canberra" was strongly resistant to Forms 43, 44, and 54 which comprise the first group, but was completely susceptible to the second group comprising Forms 45, 46, and 55. The variety "Thew" showed reciprocal reactions. Crosses were therefore made with a view to selecting commercial types which would embody the complete resistance to all six forms. The discovery that "Thew" was also resistant to one of the two known Australian forms of P. triticina and to at least one form of Erysiphe graminis was not made until the work was well in progress. The initial aim was simply to obtain stem rust resistance from this cross.

INHERITANCE OF RESISTANCE TO STEM RUST

F₁ Results

A number of seedling tests of the F₁ plants has been made, using inoculations with Form 43 as representing the first group of forms, and with Form 46 as representing the second group. In some tests the first leaf was inoculated with Form 43, and a later leaf with Form 46; in others Form 46 was used for the first leaf and Form 43 for a later leaf. When tested with Form 43 the reaction was "0;" with Form 46 it was "2." Simultaneous tests with the parental varieties were made. "Canberra" with Form 43 gave "0;" and "Thew" with 46 gave "2." These tests were

carried out in the late autumn (May). Dominance of resistance is shown.

Certain of the F_1 grains were not tested but were kindly grown at the Cowra Experiment Farm by Mr. J.T.Pridham in order that a good yield of grain might be obtained. The F_1 seedlings which had been subjected to test were afterwards transplanted to open beds and grown to maturity. After harvest a comparison of the heads of the individual plant progenies was made in order to be certain that each plant was truly an F_1 plant and not the result of an accidental self-pollination. That this was really so was further proved by the segregation which occurred in the progeny. In all cases the F_2 progenies of individual F_1 plants were kept separate.

F_2 Results

Grain from single F_1 plants was sown in boxes and tested in the seedling stage. Illustrations are given in Plate XI. In some instances the first inoculation was made with Form 43, and after notes had been taken on this first leaf reaction, these leaves were carefully removed, the plants thoroughly sprayed with distilled water and the emerging leaf then inoculated with Form 46. In other instances the order of inoculation was reversed. Pots of each parent were similarly treated and used for comparison.

The results were as follows :-

Table 60. Results of testing F₂ seedlings of "Canberra" x "Thew" with two forms of P. graminis tritici.

Form of Stem Rust Used	Reactions and Frequency					Totals				D P.E.
						Observed		Expected		
	0	1	2	3	4	Resistant.	Susceptible.	Resistant.	Susceptible.	
Form 43	306	89	114	123	12	509	135	483	161	$\frac{26}{7.41} = 3.5$
Form 46	231	39	223	134	17	493	151	483	161	$\frac{10}{7.41} = 1.3$

From these results it will be seen that the F₂ analysis is explainable on a single factor hypothesis. There is a somewhat wide divergence in the case of Form 43, but it appears that one main factor determines the resistance to each of the forms.

F₃ Results

During the late autumn, winter, and spring, of 1924 and of 1926, two series of F₃ tests were carried out. Single F₂ plants grown at H.A. College were harvested separately, at least three good ears of each being taken. In promising agronomic plants more were harvested. Two pots containing about 20 grains of each family were sown and tested in the ordinary way with Forms 43 and 46. Pots illustrating the method are shown in Plate XI.

Table 61. Results of testing seedlings of F₃ families of a cross between Canberra and Thew.

Rust Used	Number of families present in each of the three classes					
	Homozygous Resistant		Heterozygous		Homozygous Susceptible	
	Observed	Expected	Obsvd.	Exptd.	Observed	Expected
Form 43	90	83	170	166	72	83
Form 46	74	76.25	148	153.5	83	76.25

In the pots tested with Form 43, there were, on an average, 24 plants per pot in the homozygous resistant class, and 23 per pot in the homozygous susceptible class. In the pots tested with Form 46, the numbers were 23 and 25 respectively. Taking the heterozygous classes, in the tests with Form 43 it was found that there were 3058 resistant and 993 susceptible plants. The expectancy on a single factor difference is 3038 : 1013. The average number of plants tested in each family was 23. In the tests with Form 46, the heterozygous class comprised 3565 plants of which 2638 were resistant and 927 were susceptible. The expectancy here on a single factor hypothesis is 2674 resistant : 891 susceptible.

It would seem therefore, that a single factor underlies resistance to each of these two forms. An investigation of the results was made to see whether the factor for resistance to Form 43 was inherited independently of the factor for resistance to Form 46.

Assuming "A" to represent the dominant factor for resistance to Form 43 and "a" its allelomorph for susceptibility, and "B" to represent the dominant factor for resistance to Form 46, and "b" its allelomorph, then "Canberra" can be represented as having the genotype AAbb and "Thew" as being aaBB. The F₁ would be AaBb, and in the F₂ there would be the usual 9 classes. On the basis of the F₃ reactions shown, the genotypes of the F₂ families was allocated and the results summarised as follows :-

Table 62. F₂ genotypic results as determined by the F₃ tests.

F ₂ genotypes									
AABB	AABb	AaBB	AaBb	AAbb	Aabb	aaBB	aaBb	aabb	
30	30	27	33	18	38	15	30	22	Observed
18.3	36.6	36.6	73.2	18.3	36.6	18.3	36.6	18.3	Expected

Table 63. F₂ Phenotypic results.

AB	Ab	aB	ab	
170	56	45	22	Observed.
165	55	55	18	Expected.

It will be seen that there is a fairly close agreement between the observed and the expected results, showing that the two factors for resistance are inherited independently.

Inheritance of Resistance to Leaf Rust

The facts that "Thew" shows marked resistance to one form of leaf rust designated as "Aust.1," whilst "Canberra" is fully susceptible, were also taken into account in this work.

F₁ tests with six seedlings showed that they gave flecks at a time when the resistant parent "Thew" was also showing flecks. Dominance of resistance occurs.

One series of 110 F₃ families after being tested with Form 46 was pruned and tested with this form of P. triticina. It was found that there were 26 homozygous resistant families, 55 heterozygous families and 29 homozygous susceptible families. This is a close approximation to the expectancy on a single factor hypothesis.

Work was done to determine whether the same factor determined resistance to Form 46 and the form "Aust.1." of P. triticina. In one instance a series of 12 pots of families heterozygous for resistance to Form 46 was taken and the resistant and susceptible plants separately tagged prior to inoculation with the Leaf Rust. There was no connection between the resistance to the two rusts.

This was further borne out by an examination of the results for all the families.

Calling the dominant factor for resistance to this form of P. triticina "C" and its allelomorph "c," "Thew" may now be represented as having the genotype "aaBBCC" whilst "Canberra" is AAbbcc.

On the basis of the F₃ results, the genotype was allotted to each of the 107 families fully tested. In dealing with this number of families on a three factor basis it is to be expected that a divergence from the genotypic expectancy should occur. On a phenotypic basis the approximation is close. The results were as follows :-

Table 64. Results of F₃ tests of seedlings of Canberra x Thew.

Genotype	Observed	Expected	Phenotype	Observed	Expected
AABBCC	3	2	ABC	45	45
AABBCc	6	3			
AABbCC	3	3			
AaBBCC	1	3			
AABbCc	5	7			
AaBBcC	1	7			
AaBbCC	9	7			
AaBbCc	17	13			
AABBcc	2	2			
AaBBcc	4	3			
AABbcc	4	3			
AaBbcc	4	7			
AAbbCC	0	2	AbC	16	15
AabbCC	5	3			
AAbbCc	7	3			
AabbCc	4	7			
aaBBCC	1	2	aBC	13	15
aaBbCC	1	3			
aaBBcC	4	3			
aaBbCc	7	7			
AAbbcc	2	2	Abc	5	5
Aabbcc	3	3			
aaBBcc	0	2	aBc	4	5
aaBbcc	4	3			
aabbCC	2	2	abC	7	5
aabbCc	5	3			
aabbcc	3	2	abc	3	2
Totals	107	107		107	107

It is clear, then, that with this independent assortment of the factors for resistance, homozygous types combining the complete resistance should be obtainable.

F₃ Field Results.

Of the same F₃ families which were tested in the plant house, seed residues of 132 taken at random were sown in row tests at H.A. College in 1924. Observations were made on stem rust attack naturally occurring. Forms 43 and 46 were known to be present in the area. The plant house tests of seedlings inoculated with the two forms had shown that 14 of these families were homozygous for resistance. The field observations indicated that in all but two cases, these families showed no infection. In the remaining two where rust was present, the attack was light and occurred on only a few of the plants in the rows. There were six additional field rows where no rust was noted. These families were all early maturing types and they had apparently escaped attack. The seedling tests in the plant house had shown heterozygosity for resistance in these families.

F₄ Results.

Of the F₃ families, 11 were carried into the F₄. They comprised 5 of the first series tested in 1924 which were shown to be homozygous for resistance to Forms 43 and 46, together with six other families which were heterozygous for resistance but were particularly promising agronomically in the row tests. Two of the five homozygous families were the most promising of all. They were heterozygous in the F₃ for chaff colour and tip beard, and one of the two was heterozygous for grain colour as well. The selections used in these F₄ tests totalled 44; they came from 11 of the F₃ families, varying from 1 to 7 per family.

Two pots, each of 20 grains, were used for plant house tests with Forms 43 and 46. The five families which had shown homozygosity for the double resistance in the F₃ tests were again homozygous for resistance. Further sowings

of the grain of these F_4 selections were made in the open at Sydney University and at H.A. College, and Mr. J. T. Pridham kindly made yet another sowing of them at Cowra Experiment Farm. He made selections for agronomic characters from these families, and these selections formed the basis of the subsequent work.

F_5 Results

These tests dealt with 14 selections tracing back to the two homozygous resistant F_3 families which were so promising. Each one showed homozygosity for resistance to Forms 43 and 46. Four were also homozygous for resistance to the form of *P. triticina* designated "Aust.1," one was homozygous for susceptibility, and the remaining nine were heterozygous to this leaf rust.

Sowings of all families were made at the Sydney University and H.A. College for field observations. At Cowra Mr. Pridham again grew them and made further selections.

At H.A. College Forms 43 and 46 were found to be present in the area this year. Thew and Canberra were each infected. Plant house tests showed the rusts to be Forms 43 and 46 respectively. Both Australian forms of leaf rust were also abundant in the crop. On the flag of Thew were pustules of leaf rust intermingled with sharp flecks and tiny "1" reactions. Illustrations of these occurrences are shown in Plate VII. Cultures from the pustules tested in the plant house showed that the rust was the "Aust.2." The flag of Canberra gave cultures of Aust.1 and Aust.2. A scrutiny of the (Canberra x Thew) selections revealed no stem rust attack. On the flag, leaf rust was present. The family which was homozygous for susceptibility gave cultures of both forms. The homozygous resistant families showed flecks and pustules on the leaves, and from these latter the form Aust.2. was determined.

There was, then, complete agreement between the plant house and field results at H.A. College.

At the same time that these results were obtained, further samples were grown by Mr. J.R.A. McMillan at the Gatton Agricultural College in Queensland, and by Mr. W.H. Darragh at Grafton, N.S.W. Mr. McMillan reported that at Gatton the wheats were sown on black soil and were irrigated during growth. As was to be expected, a very severe rust attack developed in the early summer as the wheats approached maturity. Samples of susceptible varieties submitted showed some of the heaviest infections ever met. Tests showed that Forms 43 and 44 were present.

Under these identical conditions the (Canberra x Thew) selections were reported to be "perfectly clean and free of rust."

At Grafton Mr. Darragh reported that rust was present in abundance on susceptible varieties of wheat. Tests showed that it consisted of Forms 43 and 44. The (Canberra x Thew) selections were rust-free as had been the case at Gatton.

F₆ Results.

The tests made in 1927 dealt with 41 selections. Homozygosity for resistance to Forms 43 and 46 was shown, but in some cases there was heterozygosity for resistance to the Form Aust.1 of P. triticina.

The 1927 field results with the F₆ material proved to be very interesting. An extensive series of selections which plant house tests showed to be homozygous for resistance to Forms 43 and 46 was sown at Sydney University, H.A. College, Cowra, Gatton, and Grafton. It will be remembered that the specialisation studies had shown that prior to 1926, only the two groups of forms represented by 43 and 46

respectively were present in N.S.W., and that in the summer of 1926, a few scattered isolations from western areas of N.S.W. revealed the advent of Form 34.

In July and September of 1927, rust samples sent by Mr. T.H. Harrison showed that Form 43 was present in the crops at H.A.College. On 31st. October a scrutiny of the plots showed that the (Canberra x Thew) cross-bred were perfectly clean, but rust was found on "Little Club," "Thew," and a number of other varieties. The collection made was a representative one of wheats and barleys. The rust proved in each case to be Form 43. Canberra was free. On 4th. November, Mr. Harrison forwarded a sample of rust on "Japanese Bearded" growing in the plot: it proved to be Form 43. A further visit to H.A.College was made on the 11th. November. Collections of rust on "Thew", a representative group of wheats, and a group of barleys were made. The (Canberra x Thew) wheats were still free of stem rust. Tests made with the three collections showed in each case a mixture of Forms 43 and 34. On the 18th. November another visit revealed a tremendous development of stem rust throughout the plot. Samples were collected from "Thew," "Canberra," numerous other wheats and groups of barleys. Stem rust was now present on the (Canberra x Thew) selections and was also sampled extensively. In every instance Form 34 and only this form was present. Since that date very numerous isolations of rust from H.A.College have been made. Excepting for an occurrence in December 1927 of Form 43 on Agropyron scabrum and one on "Glossy Huguenot" wheat when Forms 43 and 34 were together present, Form 34 alone has been present at H.A.College. This applies to wheat, barley, rye and grasses. The change in the rust flora was as complete as it was remarkable.

The leaf rust tests during this period at H.A.College revealed that forms Aust.1 and 2 were present on susceptible

varieties in the plot. On Thew, Japanese Bearded, and the (Canberra x Thew) selections, flecks and pustules occurred. In each case the pustules were those of Aust.2.

From Gatton, Q., Mr. McMillan forwarded representative rust samples of the commercial varieties twice in October 1927. In each instance a mixture of Forms 44 and 34 occurred. A fortnight later another representative sample was sent, together with a series of rusted specimens of the (Canberra x Thew) selections which were stated to now be showing rust. In each instance the only form present was Form 34.

The Grafton results were of the same nature. In October 1927, samples of wheat and barley showed that Forms 43 and 34 were present. Three weeks later similar samples showed that only Form 34 occurred in both the wheats and barley and that the (Canberra x Thew) selections were now attacked by this form. Leaf rust isolations from this locality again showed only Aust.2. on the (Canberra x Thew) selections.

At Cowra the same general result was obtained.

At Bathurst there was also a development of Form 34. In this year Mr. R.E. Dwyer was given a number of F_6 selections of (Canberra x Thew), many of them different from those tested at Cowra and Gatton. The only rust determined on the samples of these which were submitted was Form 34.

Thus it became abundantly clear that the "Euston" types needed resistance to more than Forms 43 and 46 and their cotypes. Further crossing of the "Euston" types became necessary in order to obtain resistance to Form 34. It was a fortunate circumstance that Dr. Levine of Minnesota at this time forwarded samples of his "Webster" and "Hope" varieties, both of which show resistance to 34. Crosses with them have been made, and some of the material is now in the F_3 generation. Various strains of "Euston" and of other commercial varieties have been used in this work.

F₇ Results

In 1928 a further series of plant house tests of the selected material was made, and homozygosity for resistance to Forms 43 and 46 determined. Now known as the variety "Euston," tests were made at Cowra on a field scale for the first time. In its trial as a late sown grain variety Euston headed the list with a computed yield of 23 bush. 15 lbs. per acre (98). At Bathurst, yield tests carried out by Mr. Dwyer with his types showed that one of the (Canberra x Thew) selections headed the list. Taking the yield given by the standard variety Waratah as 100, the best of these (Canberra x Thew) selections yielded 115.6%. In all, five families were included in this test and all except one exceeded 100%.

Conclusion

The results of this work show that plant house work in conjunction with field tests can lead to the development of useful varieties of wheat which are rust resistant.

INHERITANCE OF MORPHOLOGICAL CHARACTERS

When practicable the inheritance of certain obvious morphological characters has been traced. Canberra has tip beard, bronze chaff, and white grain. Thew is beardless, has white (golden) chaff and reddish grain.

In the F₁, intermediacy was found in the characters. A group of P₂ plants was analysed, with the following results :-

Tip Beard

By direct comparison with the parent types, the

generation was sorted into a group with tip beards similar to Canberra, a group that was beardless similar to Thew, and a third group that was intermediate in character. The ratio was

90 tip bearded : 191 intermediate : 106 beardless. This approximates to a 1:2:1 ratio, the expectancy being 97 : 193 : 97. Seemingly a single genetic factor determines the production of the tip beard.

Chaff Colour

The same F_2 generation was sorted into groups on the basis of the presence or absence of colour in the chaff. The ratio obtained was 298 coloured : 89 white-chaffed plants. The expectancy on a single factor basis is 290 : 97.

$\frac{D}{P.E.} = \frac{8}{5.75} = 1.4$. That is, a single factor also underlies the production of bronze chaff.

Grain Colour

The same F_2 generation was sorted, by direct comparison with grain of the parental types into the three groups, one coloured corresponding to Thew, one white corresponding to Canberra, and a third group comprising individuals intermediate in colour. There were 107 coloured : 195 intermediate : 85 white grained, the expectancy being 97 : 193 : 97. A single factor determines the production of grain colour in this cross.

Grass Tuft Habit

In the F_2 generation it was early noticed that a number of the segregates were dwarfed, resembling tufts of grass. These are illustrated in Plate XI. Counts of these have been made in all available F_2 generations of this cross.

The results to date show a total ratio of 1220 normal : 281 grass tuft plants, a very close approach to the 1219 normals : 282 grass tufts which would be expected on a 13 : 3 basis. A series of F_3 counts further confirmed this result. Clearly then an inhibiting factor operates. Further evidence will be presented to show that it is carried by "Thew."

These four characters were found to be inherited independently of each other, and also independently of the rust resistance or susceptibility.

Further Work Bearing upon the Canberra x Thew Cross

Further work was carried out to test the hypothesis that the resistance of Canberra and Thew to Forms 43 and 46 respectively was due in each case to one dominant factor. Each of these wheats was crossed with Federation, a variety very susceptible to all known rust forms, and the resultant material studied in the F_1 , F_2 , and F_3 generations.

FEDERATION x CANBERRA

The F_1 grain was in part sown in pots, tested in the seedling stage, and afterwards transplanted to open ground where it was grown to maturity. Other grains were sown at Cowra by Mr. J.T. Pridham to provide a large yield. In the seedling tests with Form 43, the F_1 plants gave a "1" reaction, whilst Federation and Canberra seedlings under the same conditions gave respectively "4" and "1" reactions. Clearly resistance was dominant.

F_2 tests were made by sowing grain in boxes and inoculating with Form 43. After note-taking one series of over 200 seedlings was sorted into groups on the basis of the reaction shown, and grown to maturity in open beds in order

that F₃ material of known phenotype might be available. In other cases F₂ grain was sown without any tests, and at maturity, complete single plants were harvested separately.

The results of the F₂ seedling tests were as follows:-

Reactions.	"0;"	"1"	"2"	"3"	"4"
Frequency.	213	153	111	141	17

Federation and Canberra seedlings tested at the same time gave respectively reactions of "3+" to "4-" and of "0;" to "2." Summarising the F₂ results it will be seen that there were 477 resistant and 158 susceptible plants, which is a close approximation to the expectancy of 476 resistant : 159 susceptible plants on a single factor hypothesis.

$$\frac{D}{P.E.} = \frac{1}{7.36} = 0.14.$$

F₃ tests were made with a small series of families taken at random from F₂ material which had been grown without test. Of 64 families tested, 11 were homozygous for resistance. They comprised 199 individuals thus averaging 18 plants per pot. There were 32 families heterozygous for resistance. These comprised 625 individuals, an average of 19 per pot. Of them, 446 were resistant and 179 susceptible. On a single factor hypothesis the expectancy would be 469 : 156.

$$\frac{D}{P.E.} = \frac{23}{7.3} = 3.145.$$

The remaining 21 families were homozygous for susceptibility. They comprised 342 plants, an average of 16 plants per pot.

The results of these tests can be taken as pointing to a single dominant factor determining resistance to Form 43 in this cross.

Inheritance of Morphological Characters

Federation and Canberra differ mainly in respect of the tip beard which is present in the latter variety. In chaff colour and grain colour, differences are not apparent.

Tip Beard

In the F_1 an intermediate condition occurs.

An F_2 generation was analysed, being sorted into classes similar to the two parents and to the intermediate hybrid. There were 44 beardless : 90 intermediate : 59 tip bearded. The expectancy is 48 : 97 : 48. Although the numbers are small, the result clearly indicates that a single factor determines the production of the tip beard in this cross.

The inheritance of tip beard was found to be independent of inheritance of resistance to form 45.

Grass Tufts

In no case did examination of an F_2 generation reveal the presence of any but normal plants.

FEDERATION x THEW.

An examination of this cross was also made for confirmatory evidence regarding the factorial basis for resistance in the Canberra x Thew cross.

F_1 seedlings were tested with Form 46 and afterwards grown to maturity, and in other cases the grain was grown at Cowra. The reaction to Form 46 was "2+" and under the same conditions Federation and Thew gave respectively "4" and "2." Resistance is dominant in the F_1 .

A series of F_2 tests was made with the following result :-

Reaction.	"0;"	"1" & "2"	"X"	"3"	"4"
Frequency.	145	132	191	179	7

Simultaneously pots of Federation and Thew were tested. Federation gave "3" and Thew gave "1", "2", and "X" reactions. It has already been pointed out that a number of varieties of wheat showed that whereas inoculations under winter conditions gave strongly resistant reactions, a repetition of the experiment in the summer gave susceptible reactions. In this instance the test was made in August under rather warm conditions.

Summarising the result it is seen that regarding the "X" reaction as indicating resistance, there were 468 resistant and 186 susceptible plants. The expectancy on a single factor hypothesis is 490 resistant : 164 susceptible plants.

$$\frac{D}{P.E.} = \frac{22}{7.47} = 2.9.$$

A series of F_3 families was tested. These were obtained from the plants which had been tested as F_2 seedlings, sorted on the basis of their F_2 reaction, and planted out into open beds. The occurrence of "grass-tufts" accounted for the loss of some plants, and in other cases the yield of grain was so low (fewer than 20 grains) that the results were rejected.

In all 101 F_3 families were tested with Form 46. There were 22 which were homozygous for resistance; they averaged 31 plants per pot in the test. Of families homozygous for susceptibility, there were 30, averaging 29 plants per pot. There were 50 families heterozygous for resistance, and they averaged 24 plants per pot. Summarising the results in the heterozygous families, there were 925 resistant : 304 susceptible plants. This gives a very close approach to a 3 : 1 ratio and confirms the opinion that the resistance of Thew is due to a single dominant factor.

RESISTANCE TO P. TRITICINA

It has already been pointed out that Thew is resistant to one Australian form of P. triticina. An examination of the mode of inheritance of this character was made in this cross.

In the F₁ a group of six seedlings was tested and found to give flecks, whilst at the same time Federation and Thew respectively gave "4" and "0;" reactions.

In the F₂, the same series of seedlings which had been tested with Form 46 was pruned and later tested with the form of P. triticina designated Aust. 1. The result was as follows:-

Reactions.	"0;"	"2"	"4"
Frequency	166	239	154

That is to say there were 405 resistant : 154 susceptible plants, on the basis of a single factor difference the expectancy is 419 resistant : 140 susceptible plants.

$$\frac{D}{P.E.} = \frac{14}{6.91} = 2.0$$

The relation between the resistance to Form 46 and to the form Aust. 1 of P. triticina on a phenotypic basis was examined. If "B" represents a factor for resistance to Form 46 and "b" its allelomorph which determines susceptibility, and "C" and "c" respectively the factors for resistance and susceptibility to the Form Aust. 1 of P. triticina, the results were as follows:-

	<u>BC</u>	<u>Bc</u>	<u>bC</u>	<u>bc</u>
Observed	249	81	94	21
Expected	245	82	82	27

It will be seen that there is a fairly close agreement between the frequency and the expectancy if free assortment took place. The two factors for resistance are inherited independently.

In the F_3 tests, the same series of 101 families used in the Form 46 tests was pruned and later tested with the leaf rust. The numbers of seedlings used has already been set out. Owing to damage by Helminthosporium sp. and accident, ten of the families were lost. Of the remaining 91, it was found that 23 were homozygous resistant, 17 homozygous susceptible, and 51 heterozygous. Of the latter, 1350 were resistant and 474 susceptible. The expectancy on a single factor hypothesis is 1368 resistant : 456 susceptible.

Here again the question of correlation between resistance to Form 46 and Aust. 1 of P. triticina was considered. The genotypes of the 91 families were summarised with the following result:-

	BBCC	BbCC	BBCc	BbCc	BBcc	Bbcc	bbCC	bbCc	bbcc
Observed	4	13	15	23	3	7	6	13	7
Expected	5.7	11.4	11.4	22.6	5.7	11.4	5.7	11.4	5.7

There is a fairly close agreement with the expectancy, confirming the result that the resistance to Form 46 is independent of resistance to P. triticina.

RESISTANCE TO ERYSIPIHE GRAMINIS

It has been found in the plant house work that at least two forms of the powdery mildew occur. Inoculation tests have shown that "Thew" is resistant to one form and susceptible to another. Federation is completely susceptible to both.

Using the first form, inoculations of six F_1 seedlings showed that they were resistant. In the F_2 , a series of 212 seedlings gave 161 resistant : 51 susceptible, a near approach to a 3 : 1 ratio. These F_1 and F_2 tests were made simultaneously with tests of Federation and Thew as controls.

A series of F_3 tests was made, using the same seedlings which had been tested first with Form 46 and later with P. triticina. After taking notes on the later results, the plants were pruned, and then atomised with an abundant suspension of conidia in water. Notes were taken on homozygosity for resistance and susceptibility and on heterozygosity. It was found that the results agreed with those for leaf rust. In the series tested, there was linkage between resistance to leaf rust and resistance to powdery mildew. Further extensive tests are needed for a determination of crossing-over if this occurs. It may be mentioned that 9 other varieties of wheat have been found which behave in this same way.

INHERITANCE OF MORPHOLOGICAL CHARACTERS

Federation differs from Thew in having bronze chaff and white grain whilst Thew has white (golden) chaff and reddish grain.

The F_1 is intermediate in character.

A small F_2 generation was analysed.

Chaff Colour

The material was sorted by direct comparison with the parental types, no attempt being made to sort the coloured types further. They were 72 coloured : 23 white chaffed plants, indicating a single factor difference.

Grain Colour

In this case again the F_2 grain was compared directly with the parental types and sorted into three classes. The result was 26 coloured : 47 intermediate : 22 white grained plants. This again points to a single factor difference.

Grass Tufts

Counts in F₂ generations showed a total ratio of 468 normals : 119 grass tufts. The expectancy on a 15 : 3 basis is 477 normals : 110 grass tufts, showing the presence of an inhibiting factor. That this is present in Thew is now evident.

These characters were inherited independently of each other and apparently were also inherited independently of resistance to Form 46 and the form of P. triticina called Aust. 1.

RIVERINA x THEW

An examination of the inheritance of resistance to P. triticina was carried out in this cross, which was made in the first instance because of the reciprocal reactions of the parents to Forms ⁴³ and 46 of P. graminis tritici.

In the F₁, six seedlings were tested and gave "0;" when Riverina and Thew gave respectively "4" and "0;"

In the F₂ the results were as follows:-

Reactions	"0;"	"2"	"4"
Observed	108	247	99
Expected	113.5	227	113.5

That is, there were 355 resistant : 99 susceptible plants.

$$\frac{D}{P.E.} = \frac{14.5}{6.22} = 2.33$$

The F₃ families were tested in the usual way, with the result that there were 34 homozygous resistant comprising an average of more than 21 plants each, 37 homozygous susceptible families each comprising more than 23 plants, and 91 heterozygous families which comprised 1756 resistant : 547 susceptible plants. This is in close agreement with the expectancy on a single factor hypothesis which is 1727 resistant : 576 susceptible plants.

In 1925 a back-cross was made. Pollen from an F_1 plant was used to pollinate emasculated Riverina flowers. A poor setting of grain took place, only 13 grains being obtained. These were sown and tested in the seedling stage prior to being planted out. Eight gave "4" reactions, the remaining five gave flecks. The progeny of each plant was saved separately and tested. The eight susceptible plants yielded a progeny of 433 plants, all of which gave "4" reactions. The five resistant were each heterozygous, producing a total of 183 resistant to 74 susceptible plants. The expectancy on a single factor hypothesis is 193 : 64

$$\frac{D}{P.E.} = \frac{10}{4.68} = 2.13$$

Here again is good evidence of a single factor determining the resistance of Thew to the Aust. 1 form of P. triticina.

GLUYAS x THEW

This cross was primarily made for resistance to Forms 43 and 46 of P. graminis tritici. Resistance to P. triticina also received some attention.

In the F_1 , four seedlings tested all gave "0;" reactions, whilst the parents gave respectively "4" and "0;".

An F_2 test gave 165 resistant : 60 susceptible plants. The expectancy on the basis of a single factor difference is 169 : 56.

A small number of F_3 families taken at random was tested. They gave 19 families which were homozygous resistant, 12 families homozygous susceptible, and 27 families heterozygous. The latter comprised 292 resistant : 87 susceptible. The expectancy on a single factor basis is 284 : 95.

In this cross also the evidence points to the resistance of Thew to leaf rust being dependent upon a single factor.

FEDERATION x JAPANESE BEARDED

The variety known as "Japanese Bearded", received from Mr. F. T. Brooks of Cambridge, was found to show strong resistance to P. triticina Aust. Form 1. In 1924 it was crossed with Federation for study of the inheritance of this character.

INHERITANCE OF RESISTANCE TO LEAF RUST.

In the F₁, ten seedlings showed a "2" reaction under conditions such that Federation and Japanese Bearded gave respectively "4" and "2" reactions.

In the F₂ a series of tests was made with the following result.

Reactions	"0;"	"2"	"4"
Observed	169	281	156
Expected	151.5	303	151.5

Summating the resistant types, it is seen that there were 450 resistant and 156 susceptible plants.

$$\frac{D}{P.E.} = \frac{5.5}{7.19} = 0.77$$

In the F₃ tests there were 45 families which were homozygous for resistance. These comprised 1249 individuals, or an average of more than 27 plants per pot. 52 families were homozygous for susceptibility, comprising 1627 plants or an average of more than 31 plants per pot. There were 98 heterozygous families comprising 2809 plants, an average of more than 28 plants per pot. Taking the number of families occurring in these three classes, the ratio is 45 homozygous resistant : 98 heterozygous resistant : 52 homozygous susceptible families, which is a close approximation to a 1 : 2 : 1 ratio. In the heterozygous families there were 2138 resistant : 671 susceptible plants. The expectancy on a 3 : 1 basis is 2107 : 702.

Further confirmation of this analysis was forthcoming. After taking notes on one of the batches of F_2 seedlings, these were sorted into the "0;" "2", and "4" classes, and transplanted into an open bed under this classification. The plants were harvested separately and kept in these groups to provide the grain actually used in the F_3 tests recorded above. The homozygous susceptible F_3 families came in all cases from plants which were susceptible in the F_2 . The homozygous resistant F_3 families did not show the same accurate result. Of the F_2 plants classified as "0;", all proved to be homozygous for resistance in the F_3 , with the exception of five which produced heterozygous families. Similarly those classed as "2" in the F_2 gave heterozygous F_3 families with the exception of ten which proved to be homozygous resistant. An error of this magnitude could easily be induced by improved and by worse environmental conditions for the rust development.

It is clear that as in "Thew", there is a single dominant factor in "Japanese Bearded" for resistance to the form Aust. 1 of *P. triticina*.

INHERITANCE OF MORPHOLOGICAL CHARACTERS

An attempt was made to determine association of resistance to leaf rust with certain morphological characters.

Beard

Federation is a bald wheat, Japanese Bearded fully bearded. The F_1 plants were intermediate in character. In the F_2 450 plants were examined. Of them 29 were fully bearded. This indicates a two factor difference, since the expectancy on this basis is 28 plants. Several attempts were made to devise a scheme for classifying the other groups. A satisfactory fit was not obtained, the nearest result being

	Bald	Awnletted	Tip bearded	Half bearded	Full bearded
Observed	66	161	146	48	29
Expected	28	112	168	112	28

Grain Colour.

Federation has white grain, Japanese Bearded has red. The F_1 plants produced an intermediate red grain. In the F_2 , 450 plants were sorted into two classes, one containing coloured grain and one containing white grain. In the former class marked differences were noted in the degree of colour, but they were not finally classified. The total was 442 coloured : 8 white grained, indicating a three factor difference for this character, since the expectancy on this basis is 7 white grained individuals.

Coleoptile Colour

In very young seedlings there are marked differences in the colour of the coleoptile in Federation and Japanese Bearded. In the former the colour is white, in the latter, brown. The F_1 showed an intermediate amount of colour. In the F_2 , 212 individuals were examined, giving a ratio of 50 white : 96 intermediate : 66 dark.

This is an approximation to a 1 : 2 : 1 ratio. In the F_3 , a group of 132 families was examined. There were 34 families homozygous for light coleoptile. They comprised 865 individuals, or an average of 25 plants in each family. Also there were 34 families homozygous for dark coleoptile. These comprised 921 individuals, or an average of 27 plants per family. The heterozygous families numbered 64, comprising 1855 individuals or 29 per family. Totalling the classes in these heterozygous families, it was found that there were 450 dark : 971 intermediate : 434 light, which again approximates to a 1 : 2 : 1 ratio. Assuming dominance of colour, the ratio becomes 1421 : 434, as compared with an expectancy of 1391 : 464.

Rust Resistance and Coleoptile Colour

It was considered that there might perhaps be some correlation between the pigmentation and resistance. The F₃ families which were classified according to colour production were allocated their genotype on a resistance basis.

Assuming that A represents the factor for resistance to Aust. Form.1 and a its allelomorph for susceptibility, whilst B represents the factor for coloured coleoptile and b the allelomorph factor for white coleoptile, then the constitution of Federation would be aabb and of Japanese Bearded AABB. In the F₂ the following are the results:-

Genotypes.	AABB	AABb	AaBB	AaBb	AAbb	Aabb	aaBB	aaBb	aabb
Observed	6	15	18	34	5	18	9	19	8
Expected	8	16.5	16.5	33	8	16.5	8	16.5	8

Although the number of families examined was small, it is clear that the two characters are inherited independently.

Grain Colour

It has already been stated that out of the families examined, only three had white grain colour. On the genotypic basis just postulated, these three families had respectively the genotypes Aabb, AaBB and AaBb. This indicates the probability of no association between these three characters.

FURTHER CROSSING WORK.

It is not intended at this juncture to describe in detail other breeding results, but simply to indicate in general terms what has been found. A number of crosses have been examined, following the same general procedure outlined in connection with the Canberra x Thew cross. As in that case, the crosses before 1926 were made for resistance to the two groups of forms which were then present in Australia. Many of the crosses were made between varieties which showed

reciprocal reactions to these two groups of forms with a view to combining this double resistance. Others had as one parent a wheat like Kanred or Kota which showed the double resistance, the other parents being commercial wheats like Federation, Hard Federation, Canberra, etc. In yet other cases the crosses involved Federation and one other of the varieties showing the single resistance, as had been done in the Canberra x Thew cross. A number of the actual parents are set out in Tables 51, 52, and 53 and will not be repeated here. Since 1926 the crosses made have involved resistance to Form 34. Webster and Hope have been mainly used to contribute this.

F₁ Results.

In the stem rust work, to date F₁ plants from 286 crosses have been tested with Form 43 to represent the first group of forms, and with Form 46 to represent the second group. In all cases there has been dominance of resistance. The tests have been made in the autumn in order that the seedlings after being tested might be transplanted to open beds and grown to maturity. Pots of the parental varieties have been tested under the same conditions for comparative purposes. Almost complete dominance of resistance to Forms 43 and 46 has been shown.

When the first of the F₁ tests was made with Form 34 in 1927, it was very surprising to find almost complete dominance of susceptibility to this form. To date F₁ seedlings from 76 crosses have given this result. There has been no departure from it. In a number of cases the F₁ grain of a cross has been divided into three groups. One batch has been inoculated with Forms 34, 43, and 46 in that order, Form 43 being used for inoculation after note taking and removal of the leaves which gave the Form 34 reaction, and Form 46 being used after removal of the leaves giving the

Form 43 reaction. The other two groups of seedlings have been inoculated with these same cultures used in different chronological order. The result has been the same. There is dominance of resistance to Forms 43 and 46, and dominance of susceptibility to Form 34.

In the leaf rust tests of F_1 seedlings from 17 crosses, there has been dominance of resistance similar to that described in the crosses of Them with Canberra and other susceptible wheats.

F₂ Results

In the stem rust work, a number of the crosses have been tested in the seedling stage with Forms 43 and 46, or with one or other of these two, depending upon the parents. The rule has been to find segregation on a single factor basis. One of the interesting cases was in crosses of Kota with Federation, Hard Federation, and Canberra, where there were on an average, 3 resistant : 1 susceptible plant. Clark (24) made an extensive study of a Kota x Hard Federation cross and found segregation on the basis of 15 susceptible : 1 resistant. The form or forms of stem rust present are not indicated. An exception to the general happening has been in a Federation x Webster cross. In some of the tests, Form 43 has been used to inoculate the first seedling leaf, and Form 46 to inoculate a later leaf after note taking and removal of the leaf infected with Form 43. In other tests the order in which the forms have been used has been reversed. The result has been the same. There has been a ratio of 15 resistant : 1 susceptible plant. Furthermore, the plants susceptible to Form 43 are also susceptible to Form 46. This indicates that in Webster there are two dominant factors for resistance to Form 43 and 46 which are cumulative in their effects. The same cross tested

with Form 34 gives a result in which the dominance of susceptibility is outstanding, with an approximation to a ratio of 3 susceptible : 1 resistant. This is merely a tentative suggestion since a series of F_3 tests that are in progress seems to point to a 13 : 3 ratio. In the F_2 work it was found that the segregates which were susceptible to Forms 43 and 46 were not necessarily susceptible to Form 34. The inheritance of resistance to Form 34 is independent of resistance to Forms 43 and 46. The same F_2 plants used in these tests were also inoculated with the form of P. triticina known as Aust. 1. Segregation on the basis of 3 resistant : 1 susceptible plant occurred.

This again was independent of the inheritance of resistance to the three forms of stem rust.

Some of the morphological characters which are exhibited have been studied in certain of the crosses, with the following general results:-

Table 65. Summary of results of counts in F_2 generations of certain wheat crosses, showing ratios found in the inheritance of some morphological characters.

Parents of Cross	Chaff Colour	Full Beard	Grain Colour
Canberra x Kanred	3 : 1	3 : 1	15 : 1
Federation x Kanred	3 : 1	15 : 1	63 : 1
Canberra x Kota	3 : 1	3 : 1	63 : 1
Hard Federation x Kota	3 : 1	3 : 1	15 : 1
Federation x Kota	3 : 1	15 : 1	15 : 1
Federation x Webster	-----	15 : 1	15 : 1
Gullen x Thew	3 : 1	15 : 1	3 : 1
Gluyas x Thew	3 : 1	15 : 1	3 : 1

Tests of F_3 families of several crosses for resistance to Forms 43 and 46 have been made. These include Canberra x Kanred, Canberra x Kota, Hard Federation x Kota, Federation x Kota, Gullen x Thew, Canberra x Bobs, Firbank x Wandilla, Canberra x Clarendon, and Bena x Clarendon. The results confirm the F_2 hypothesis that one main dominant factor for resistance operates in these crosses. Evidence was obtained of modifying factors operating in some cases.

It has already been stated that a series of F_3 families of the Federation x Webster cross are under study, mainly in regard to the inheritance of resistance to Form 34. It is early to attempt to draw final conclusions from this work. The dominance of susceptibility has been very clear. It has meant that an abundance of inoculum of this form has been available in the plant house in which the tests have been made. In all other cross-bred tests, a sufficiency of the inoculum has only been ensured by frequent inoculations of an appropriate susceptible host variety. Several families have been found which are homozygous in the seedling stage for resistance to Form 34, in addition to showing satisfactory agronomic characteristics as F_2 individuals. A result in the flag smut work which is in progress promises to have an important bearing on these F_3 family tests. In pot tests carried out in 1928 and 1929, the strain of Webster used in the crosses for rust resistance has remained quite free of flag smut, alongside fully susceptible plants of Federation and Waratah derived from grain which was heavily smutted with flag smut spores as was the Webster grain.

RESULTS IN LATER GENERATIONS

In some of the crosses, notably those enumerated under the heading "F₃ Results", selections have been made in each generation up to F₇, largely by Mr. J. T. Fridham at Cowra. Plant house tests have been made before deciding upon the progeny for the next sowing. Certain of these types give considerable promise agronomically. The occurrence of Form 34 has greatly complicated matters, but crosses between a number of the best of these types and Webster and Hope are now in the F₂ and are expected to give fully resistant types of agronomic value.

OCCURRENCE OF GRASS TUFTS IN WHEAT CROSSES.

Attention has already been called to the occurrence of grass tufts in the F_2 generations of the crosses Canberra x Thew and Federation x Thew. The same condition has been found in a number of other crosses that have been made. It has indeed been a limiting factor in some of the crosses which were made to give rust resistance, completely preventing any progress being made.

Grass tufts have occurred in the F_1 of a number of crosses. Notice was drawn to this happening by Mr. G.S. Gordon of Werribee in 1922. He had found that from crosses of "Indian F" with "Jonathan", only grass tufts resulted. He kindly made further crosses between the two varieties and forwarded the crossed grain for testing purposes. All the grains produced grass tufts, and although these were grown under the best garden conditions and watered when necessary, they could not be brought beyond this stage. They died without flowering.

The crosses which have been made and which show this same occurrence are as follows:-

Hard Federation x Cedar
Federation x Cedar
Canberra x Cedar
Gluyas x Cedar
Wandilla x Cedar
Yandilla King x Cedar
Indian F x Cedar
Cedar x Indian F.
Indian F x Jonathan
Federation x Jonathan
Jonathan x Canberra
Federation x Warren
Hard Federation x Warren
Canberra x Warren
"Sun x Dawson" x Federation
Yandilla x Bobin (Cross made by R.E. Dwyer.)
Persian Black x Federation
Alberta Red x Persian Black
Federation x Iumillo
T. sphaerococcum B.L. x Federation.

Grass tufts in the segregating generations have been noted by many workers. In the work under review, the occurrence of grass tufts in the F_2 generation has been observed in the crosses which are set out in the following table.

Table 66.

Occurrence of grass tufts in F₂ generations of certain wheat crosses, compared with the expectancy on a 13 : 3 basis.

Parents of Cross.	Observed.			Expected.		D.
	Totals	Normal	Grass Tufts	Normal	Grass Tufts	P.E.
Gluyas x Thew	560	463	97	455	105	$\frac{8}{5.23} = 1.3$
Thew x Wandilla	710	581	129	577	133	$\frac{4}{7.01} = 0.6$
Thew x Gullen	585	485	100	375	110	$\frac{10}{5.37} = 1.6$
Thew x Indian F.	217	170	47	176	41	$\frac{6}{3.88} = 1.5$
Thew x Riverina	101	78	23	82	19	$\frac{4}{2.65} = 1.5$
Thew x Federation	296	232	64	240	56	$\frac{8}{4.53} = 1.8$
Thew x Barooga	40	32	8	32.5	7.5	$\frac{0.5}{1.66} = 0.3$
Thew x Duri	105	81	24	85	20	$\frac{4}{2.70} = 1.5$
Bena x Thew	120	95	25	97	23	$\frac{2}{2.88} = 0.7$
Wandilla King x Thew	335	272	63	272	63	$\frac{0}{4.82} = 0$
Thew x Roseworthy	102	83	19	83	19	$\frac{0}{2.66} = 0$
Thew x Marshall's No.3	98	84	14	80	18	$\frac{4}{2.61} = 1.5$
Marshall's No.3 x Thew	150	116	34	122	28	$\frac{6}{3.22} = 1.9$
Hard Federation x Florence	386	320	66	314	72	$\frac{6}{5.17} = 1.2$
Florence x Hard Federation	199	158	41	162	37	$\frac{4}{3.71} = 1.1$
Federation x Florence	145	128	17	118	27	$\frac{10}{3.17} = 3.1$
Florence x Federation	193	153	40	157	36	$\frac{4}{3.66} = 1.1$
Canberra x Florence	351	293	58	285	66	$\frac{8}{4.93} = 1.6$
Florence x Gullen	366	292	74	297	69	$\frac{5}{5.04} = 1.0$
Gullen x Florence	505	410	95	410	95	$\frac{0}{5.92} = 0$
Florence x Wandilla	752	626	126	611	141	$\frac{15}{7.22} = 2.1$
Florence x Wandilla King	100	80	20	81	19	$\frac{1}{2.63} = 0.4$
Canberra x Clarendon	548	461	87	445	103	$\frac{16}{6.16} = 2.6$
Clarendon x Waratah	166	137	29	135	31	$\frac{2}{3.39} = 0.6$
Clarendon x Bena	172	142	30	140	32	$\frac{2}{3.45} = 0.6$
Bena x Clarendon	184	159	25	150	34	$\frac{9}{3.57} = 2.8$
Canberra x Bomen	469	387	82	371	88	$\frac{6}{5.70} = 1.1$
Gluyas x Bomen	501	407	94	407	94	$\frac{0}{5.89} = 0$
Wandilla King x Bomen	297	250	47	241	56	$\frac{9}{4.54} = 2.0$
Canberra x Rieti	488	407	81	397	91	$\frac{10}{5.82} = 1.7$
Ridet x Federation	196	170	26	159	37	$\frac{11}{3.69} = 3.0$
Chinese White x Canberra	176	142	34	143	33	$\frac{1}{3.49} = 0.3$
T. sphaerococcum S.B. x Fed.	286	242	44	232	54	$\frac{10}{4.45} = 2.2$

It will be seen that in some of the crosses the number of individuals available was small. The figures are given just as they were taken in the field. In these 33 crosses there is the clear indication of an inhibiting factor in each case. Examination of the results points to the inhibitor being carried by Thew, Florence, Clarendon, Bomen, Rieti, Redit, Chinese White, and T. sphaerococcum S.B.

A study of ~~the~~ F_3 families^{ies} of the Canberra x Thew cross further confirmed this result.

In addition to these cases in which there has been the 13 : 3 ratio typical of the action of an inhibitory factor, there have been three in which a 15 : 1 ratio occurs.

They are as follows:-

Table 67. Occurrence of grass tufts in F_2 generations of certain wheat crosses, compared with the expectancy on a 15 : 1 basis.

Parents of Cross.	Observed.			Expected.		D. P.E.
	Totals	Normal	Grass Tufts	Normal	Grass Tufts	
Exquisite x Thew	161	149	12	151	10	$\frac{2}{2.07} = 1.0$
Bomen x Warden	319	298	21	299	20	$\frac{1}{2.92} = 0.3$
Firbank x Wandilla	258	243	15	242	16	$\frac{1}{2.62} = 0.4$

RESISTANCE TO PUCCINIA GRAMINIS AVENAE

Unexpected difficulties have been met with in this work. There have been numerous attempts to make crosses between varieties of oats at the Sydney University. To date no crossed grain has been obtained from over 200 pollinations. Mr. J.T. Pridham of Cowra has kindly supplied some crossed grain from which a few results have been obtained. Losses from attack by grain moth have much further hampered work.

The crosses available have been -

1. Belar (susceptible) x Reid (resistant.)
2. Ruakura (susceptible) x Richland (resistant).
3. Algerian (susceptible) x White Tartar (resistant).
4. Algerian (susceptible) x Joannette (resistant).

The only rust used so far is P. graminis avenae 1.

The reactions indicated have to do with this physiologic form.

In the F_1 seedling tests, there was a close approach to complete dominance of resistance. Thus in the first cross, the F_1 reaction was "2+" while the resistant "Reid" gave "2". In the second cross, the F_1 reaction was "0"; similar to that given by Richland. In the third, the F_1 showed "2", similar to that given by White Tartar. In the fourth cross, the F_1 reaction was "2", whilst Joannette showed "1".

Only in the case of the first cross have F_2 results of value been obtained. In the other cases, loss of grain by attack of army worm in the field or by grain moth in the laboratory after harvest has reduced the numbers available too greatly for the figures to have a real significance.

But F_3 studies are planned which may give information regarding the genetics of resistance. In the cross Belar x Reid, the same culture of P. graminis avenae 1. which had been used in the F_1 tests was used to inoculate a group of F_2 seedlings.

There were 177 resistant :/susceptible plants, a close approach to a 3 : 1 ratio.

As regards resistance to P. graminis avenae 1, in the cross Belar x Reid there appears therefore to be a single dominant factor which determines resistance.

ACKNOWLEDGEMENTS.

Help has been received throughout the work from very many sources and is gratefully acknowledged. Professor R.D. Watt, Dean of the Faculty of Agriculture, in which the investigations have been carried out, has always given every possible assistance. Many members of the N.S.W. Department of Agriculture have given invaluable help. Thanks are due to the Under Secretary for Agriculture for official cognisance and furthering of the work. Principal E.A. Southes of H.A. College has done all in his power since the commencement of the work to render assistance, by personally helping in the rust plot, by providing hospitality and assistance, and by always expressing helpful interest in the investigations. Mr. T.H. Harrison also of H.A. College, has been indefatigable in giving invaluable help on all occasions. Mr. J.T. Pridham of the Cowra Experiment Farm has been at pains to help forward the work since commencement. Members of the Biology Branch have helped greatly, as have also the officers of the Plant Breeding Branch since its inception in 1927; particular mention of the services of Messrs. N.S. Shirlow, W.H. Darragh, and R.E. Dwyer should be made in this regard. Many of the field officers of the Department of Agriculture by collecting and forwarding material from various parts of the State have extended the rust survey. The same applies to workers in other States and N.Z., notably Messrs. W.M. Carne, of West Aust., G. Samuel of Sth. Aust., H. Pye and G.S. Gordon of

Victoria, C.E.W. Oldaker of Tasmania, J.R.A. McMillan of Queensland, and Dr. K. Curtis of the Cawthron Institute in N.Z., Mr. A.D. Cotton of Kew, and Miss K. Sampson of Aberystwyth have similarly helped. Mr. G. Wright of the University of Sydney has kindly loaned photographic apparatus. To Mr. J.A. Tunnicliffe of the Fisher Library are due thanks for help in connection with the historical records.

Messrs. Peters' Ltd. have made space available in their ice house for the storing of specimens. Without the help of Drs. E.C. Stakman and M.W. Levine of Minnesota, U.S.A., the stem rust work would not have been possible, and the same applies to Dr. E.B. Mains in connection with the leaf rust studies. Financial assistance has been thankfully received from the McCaughey Research Fund and the University of Sydney. By no means least, the members of the attendant staff have cheerfully given loyal help, often under circumstances of great difficulty, and their assistance is gratefully acknowledged.

SUMMARY AND CONCLUSIONS.

1. Studies of various graminaceous rusts have been in progress for several years. Stem rusts have received main attention, and of these, stem rust of wheat has had most time devoted to it.
2. Work with the aecidial stage of Puccinia graminis further disproved the old statement that stem rust of wheat in Australia has lost its power of attacking the barberry. Many successful infections were obtained from Australian teleutospores, as well as from material received from overseas. It appears that Form 43

of P. graminis tritici is capable of successfully infecting the barberry. Further indirect evidence was obtained that change in the specialisation of a rust may be brought about in the aecidial stage on the barberry. The teleutospore received from overseas have given rise to physiologic forms of P. graminis tritici and P. graminis avenae which are unlike those occurring in Australia.

3. Germination studies were made of teleutospores naturally occurring on straw which had been exposed throughout the winter to weather conditions. The most abundant germinations occur about October, but sparse germinations are found as early as March. Between March and October germinations are sporadic, and they cease altogether in November. There was no discernable difference between the germination capacity of teleutospores of different physiologic forms.
4. Uredospore studies have involved questions of specialisation. Tests of the grain of varieties used as differential hosts have shown that steps must be continuously taken to maintain the purity of these plants.
5. It was found that variations in the environmental conditions in the plant house may profoundly influence the reactions of differential hosts to various rusts.
6. In the case of P. graminis tritici there was change from complete susceptibility in the hotter months to complete resistance in the cooler part of the year. The reactions on several of the differential hosts showed this change. Some physiologic forms show much more violent alterations of this nature than do others.
7. Changes from summer to winter conditions alter the reactions shown by P. graminis avenae 1 on "Joanette" from complete susceptibility to complete resistance.

8. Tests with cultures of P. triticina showed that the reactions it gives on certain varieties of wheat are changed by altering the environmental conditions.
9. Essentially similar results are shown by P. simplex on certain varieties of barley.
10. Such major variations in the reactions given on differential hosts may completely alter determinations of the physiologic specialisation of the fungus concerned. In field practice such changes may also be of the utmost importance.
11. But under identical conditions of cultivation, physiologic forms may be sorted out upon the basis of their rust reactions on selected hosts.
12. In Australasia, seven naturally occurring forms of P. graminis tritici have been found by this means. They are Forms 34, 43, 44, 45, 46, 54 and 55. Form 11, a form tentatively designated Form 45A, and a hitherto unrecorded form have been obtained from the aecidial stages on barberries. Two other forms - 16 and 27 - have similarly been produced from overseas teleutospore material.
13. The distribution of these naturally occurring forms has been studied in regard to time and space.
14. Prior to 1926, Forms 43, 44, 45, 46, 54 and 55 were present, but in 1926, Form 34 was found in Western Australia. Next year it occurred in the Eastern states, and at the present time it appears to be the only form which occurs in Australasia.
15. P. graminis tritici is most abundant in wheat crops about November, but investigations have shown that it has been present in the viable uredospore stage on volunteer wheat and other hosts all the year round.

16. Barley serves as a host for various forms of P. graminis tritici, as does also rye. On the latter crop, there has been no indication of the presence of P. graminis secalis.
17. Several grasses have been found to be natural hosts for various forms of P. graminis tritici. Hordeum murinum and Agropyron scabrum are outstanding examples.
18. Specialisation studies of P. graminis avenae have shown the presence in Australasia of Forms 1, 2, 3, 6 and 7. Cultures of the organisms have also been obtained from the aecidial stage produced by rust sent from Ireland.
19. The distribution of P. graminis avenae has been studied, as has also its natural occurrence on grasses. Festuca bromoides, Phalaris minor, and Hordeum murinum are outstanding hosts of various forms of the rust.
20. A number of other grasses have been tested in the plant house, mainly in the seedling stage, with various cultures of the available rusts, and their reactions recorded.
21. Specialisation studies of P. triticina have shown that the differential hosts used by Mains and Jackson do not differentiate the Australian leaf rusts. But this is readily accomplished by "Thew". The two physiologic forms thus sorted out have been studied in their distribution and in their host range on grasses.
22. Biometrical studies of the spore forms of the available rusts have been made. Comparisons of some forms show that there are biometrical affinities between some forms which are in agreement with those determined by the reactions shown on the differential hosts. In other cases no parallelism exists.
23. Results obtained in breeding for rust resistance are given. The technique of crossing was tested, and the method adopted found to give 75% successful crossing in wheat.

24. Attempts to cross wheat and barley failed, and sterility was found in the one successful cross made between wheat and rye.
25. Numerous unsuccessful efforts were made to cross Khapli emmer with varieties of vulgare wheat. Attempts to incorporate the Khapli resistance in vulgare wheats are being made by indirect methods.
26. Crosses were made between Canberra x Thew to give resistance to the six forms of P. graminis tritici present in Australia prior to 1926. These parents behave reciprocally in their resistance. The results given in the F₁, F₂, and F₃ generations indicate in each parent the presence of a single dominant factor for resistance. The results are given of later generation tests and the development from the cross of the variety "Euston". The inheritance of resistance to Australian Form 1 of P. triticina was also traced in this cross, together with the inheritance of certain morphological characters.
27. To link up with the Canberra x Thew cross, studies were made of crosses between Canberra and Federation, and between Thew and Federation. Confirmatory evidence was obtained of the resistance in each case depending upon a single dominant factor. In the case of Thew, an additional independent dominant factor for resistance to Australian Form 1 of P. triticina is present, and is linked with resistance to one form of Erysiphe graminis.
28. The general results of studies of other wheat crosses are given, as well as a certain amount of work with oats.
29. Observations on the occurrence of grass tufts or dwarfing have been made in a number of the crosses, and its mode of inheritance studied.
30. Despite many complexities, there would seem to be no valid reason why success should not be attained in breeding fully resistant varieties of wheat which are also agronomically desirable.

A BIBLIOGRAPHY.

(1) Asmødt, O.S.

1922. "The Inheritance of Resistance to Several Biologic Forms of Puccinia graminis tritici in a Cross Between Kanred and Marquis." (Abstract).
Phytopathology, 12 : p.32.

(2) Asmødt, O.S.

1922. "Correlated Inheritance in Wheat of Winter-Spring Habit of Growth and Rust Resistance." (Abstract).
Phytopathology, 12 : p.32-33.

(3) Asmødt, O.S.

1923. "The Inheritance of Growth Habit and Resistance to Stem Rust in a Cross Between Two Varieties of Common Wheat."
Jour. Agr. Research, 24 : p.457-470.

(4) Allen R.F.

1923. "Cytological Studies of Infection of Baart, Kanred, and Mindum Wheats by Puccinia graminis tritici forms 3 and 19."
Jour. Agr. Research, 26 : 571-604.

(5) Armstrong, S.F.

1922. "The Mendelian Inheritance of Susceptibility and Resistance to Yellow Rust (Puccinia glumarum E.&H.) in Wheat.

Jour. Agr. Sci. 12 : 57-96.

(6) Arthur, J.C.

- 1905-1908. "Cultures of Uredineae in 1904-1907."
Jour. Mycol., 11 : p.50-67, 1905;
,, 12 : p.11-27, 1906;
,, 13 : p.189-205, 1907;
,, 14 : p.7-26, 1908.

(7) Arthur J.C.

- (7a) Atkinson, "Cultures of Uredineae in 1908-1909."
Mycologia, 1 : p.225-256, 1909;

1826. "An Account of the State of Agriculture and Grazing in N.S.W."

(8) Bailey, D.L.

London, J. Cross. pp.200.

1923. "Physiologic Specialisation in Puccinia graminis avenae E.&H.

Minn. Agr. Exp. Sta. Tech. Bull. 35.

(9) Barker, H.D. and Hayes, H.K.

1924. "Rust Resistance in Timothy."
Phytopathology, 14 : p.363-371, 1 fig.

(10) Beauverie, J.

1923. "On the Development of Wheat Rusts in Relation to Climatic Conditions." (Abstract).
Internat. Conf. Phytopath. Econ. Ent.
Holland Rept. 1923. p.202-203.

A BIBLIOGRAPHY.

(1) Aamodt, O.S.

1922. "The Inheritance of Resistance to Several Biologic Forms of Puccinia graminis tritici in a Cross Between Kanred and Marquis." (Abstract).
Phytopathology, 12 : p.32.

(2) Aamodt, O.S.

1922. "Correlated Inheritance in Wheat of Winter-Spring Habit of Growth and Rust Resistance." (Abstract).
Phytopathology, 12 : p.32-33.

(3) Aamodt, O.S.

1923. "The Inheritance of Growth Habit and Resistance to Stem Rust in a Cross Between Two Varieties of Common Wheat."
Jour. Agr. Research, 24 : p.457-470.

(4) Allen R.F.

1923. "Cytological Studies of Infection of Baart, Kanred, and Mindum Wheats by Puccinia graminis tritici forms 3 and 19."
Jour. Agr. Research, 26 : 571-604.

(5) Armstrong, S.F.

1922. "The Mendelian Inheritance of Susceptibility and Resistance to Yellow Rust (Puccinia glumarum E.&H.) in Wheat.

Jour. Agr. Sci. 12 : 57-96.

(6) Arthur, J.C.

1905-1908. "Cultures of Uredineae in 1904-1907."
Jour. Mycol., 11 : p.50-67, 1905;
" " 12 : p.11-27, 1906;
" " 13 : p.189-205, 1907;
" " 14 : p.7-26, 1908.

(7) Arthur J.C.

1909-1910. "Cultures of Uredineae in 1908-1909."
Mycologia, 1 : p.225-256, 1909;
" " 2 : p.213-240, 1910.

(8) Bailey, D.L.

1925. "Physiologic Specialisation in Puccinia graminis avenae E.&H.

Minn. Agr. Exp. Sta. Tech. Bull. 35.

(9) Barker, H.D. and Hayes, H.K.

1924. "Rust Resistance in Timothy."
Phytopathology, 14 : p.363-371, 1 fig.

(10) Beauverie, J.

1923. "On the Development of Wheat Rusts in Relation to Climatic Conditions." (Abstract).
Internat. Conf. Phytopath. Econ. Ent.
Holland Rept. 1923. p.202-203.

- (11) Biffen, R.H.
1905. "Mendel's Law of Inheritance and Wheat Breeding."
Jour. Agr. Sci. 1 : p.4-48. Pl. 1.
- (12) Biffen, R.H.
1907. "Studies in the Inheritance of Disease Resistance."
Jour. Agr. Sci. 2 : 109-128.
- (13) Biffen, R.H.
1912. "Studies in the Inheritance of Disease Resistance."
Jour. Agr. Sci. 4 : 421-429.
- (14) Biffen, R.H.
1916. "The Suppression of Characters on Crossing."
Jour. Genetics 5 : 225-228.
- (15) Biffen, R.H.
1917. "Systematised Plant Breeding."
Seward's "Science and the Nation." p.146-175.
University Press, Cambridge, England.
- (16) Bolley, H.L.
1891. "Wheat Rust: is the Infection Local or General
in Origin?."
Agr. Sci. 5 : 259-264.
- (17) Bolley, H.L.
1904. "Cause of Wheat Rust, its Method of Attack, Conditions
Favourable to Infection and Rust Development, and
What to do."
N.Dak. Agr. Exp. Sta. Press Bull. 20.
- (18) Bolley, H.L., and Pritchard, F.J.
1906. "Rust Problems. Facts, Observations, and Theories.
Possible Means of Control."
N.Dak. Agr. Exp. Sta. Bull. 68, p.607-672. 30 fig.
- (19) Butler, E.J., and Haymen, J.M.
1906. "Indian Wheat Rusts."
India Dep. Agr. Mem. Bot. Ser. 1 : 1-58.
- (20) Carleton, M.A.
1893. "Notes on the Occurrence and Distribution of Uredineae."
Science 22 : p.62-63.
- (21) Carleton, M.A.
1899. "Cereal Rusts of the United States."
U.S.D.A. Div. Veg. Phys. and Path.
Bull. 16, pp.74, illus.
- (22) Carleton, M.A.
1904. "Investigations of Rust."
U.S.D.A., B.P.I. Bull. 63, pp32. 2 pl.
- (23) Carleton, M.A.
1905. "Lessons from the Grain Rust Epidemic of 1904."
U.S.D.A. Farmers' Bull. 219.

- (24) Clark, J.A.
1925. "Segregation and Correlated Inheritance in Crosses Between Kota and Hard Federation wheats for Rust and Drought Resistance."
Jour. Agr. Res. 29 : 1-47., illus.
- (25) Clark, J.A., and Smith, R.W.
1928. "Inheritance in Nodak and Kahla Durum Wheat Crosses for Rust Resistance, Yield and Quality at Dickinson, North Dakota."
Jour. Amer. Soc. Agron. : 20: 12, p.1297-1304.
- (26) Cobb, N.A.
1890. "Contributions to an Economic Knowledge of Australian Rusts (Uredineae).
Agr. Gaz. N.S.W. Vol.1., p.186.
- (27) Cobb, N.A.
1892. "Contributions to an Economic Knowledge of Australian Rusts (Uredineae).
Agr. Gaz. N.S.W. Vol.3., p.44-68.
- (28) Cobb, N.A.
1893. "Contributions to an Economic Knowledge of Australian Rusts (Uredineae).
Agr. Gaz. N.S.W. Vol. 4., p.431-470.
- (29) Cobb, N.A.
1893. "Contributions to an Economic Knowledge of Australian Rusts." (Uredineae.)
Agr. Gaz. N.S.W. Vol.4., p.503-513.
- (30) Cobb, N.A.
1894. "Contributions to an Economic Knowledge of Australian Rusts." (Uredineae.)
Agr. Gaz. N.S.W. Vol.5., p.239-252.
- (31) Collins, D.
1798. "An Account of the English Colony in New South Wales. Vol.1."
London, T.Cadell Jun., and W.Davies.
- (32) Cunningham, G.H.
1923. "Relation of Biologic Specialisation in the Taxonomy of the Grass Rusts."
N.Z. Jour. Sci. Tech. 6 : 157-166.
- (33) Darnell-Smith, G.P.
1929. "The First Wheat in Australia."
Agr. Gaz. N.S.W. Vol. 40. p. 335-338.
- (34) Davies, D.W., and Jones, E.T.
1926. "Studies in the Inheritance of Resistance and Susceptibility to Crown Rust (Puccinia coronata Cda.) in a cross between selections of Red Rust Proof (Avena sterilis L.) and Scotch Potato (Avena sativa L.)
Welsh Jour. Agr. 2 : p.212-221. 4 pl.
- (35) Dietel, P.
1887. "Beiträge zur Morphologie und Biologie der Uridineen."
Bot. Centralb. 32 : 54.
- (36) Dietz, S.M.
1925. "The Inheritance of Resistance to Puccinia graminis avenae." (Abstract).
Phytopathology, 15 : 54.

- (37) Dietz, S.M.
1928. "Inheritance of Resistance in Oats to Puccinia graminis avenae."
Jour. Agr. Res. : 37, 1, p.1-23., 5 fig.
- (38) Eriksson, J.
1894. "Über die Specialisirung des Parasitismus bei den Getreide- Rostpilzen."
Ber. Deut. Bot. Gesell. : 12 :p292-331.
- (39) Eriksson, J.
1896. "Welche Grasarten Können die Berberitze mit Roste anstecken?."
Ztschr. Pflanzenkrank. Bd.6. Heft 4. p. 193-197.
- (40) Eriksson, J.
1897. "Weitere Beobachtungen über die Spezialesierung des Getreide- schwarzrostes."
Ztschr. Pflanzenkrank. Bd.7. Heft 4. p.198-202.
- (41) Eriksson, J.
1902. "Über die Spezialisierung des Getreideschwarzrostes in Schweden und in anderen Ländern."
Centbl. Bakt. Abt. 2. Bd.9., No.16.p.590-607;
No.17/18. p.654-658.
- (42) Eriksson, J., and Henning, E.
1894. "Die Hauptresultate einer neuen Untersuchung über die Getreideroste."
Ztschr. Pflanzenkrank. Bd.4. 66-73, 140-142, 197-203, 197-203, 257-262.
- (43) Eriksson, J., and Henning, E.
1896. "Die Getreideroste". p.463, 5 fig. 13 col. pl. Stockholm.
- (44) Evans, I.B.Pole
1911. "South African Cereal Rusts, with Observations on the Problem of Breeding Rust Resistant Wheats."
Jour. Agr. Sci. 4 : p. 95-104.
- (45) Ferrer, W.
1898. "The Making and Improvement of Wheats for Australian Conditions."
Agr. Gaz. N.S.W. 9 : 131-168, 241-250.
- (46) Florell, V.H.
1924. "Studies on the Inheritance of Earliness in Wheat."
Jour. Agr. Res. 19 : p.333-347.
- (47) Freeman, G.F.
1919. "Heredity of Quantitative Characters in Wheat."
Genetics 4 : p.1-93.
- (48) Freeman, E.A., and Johnson, E.C.
1911. "The Rusts of Grains in the United States".
U.S.D.A., B.P.I. Bull. 216, pp87. 2 fig. 1 pl.
- (49) Gaines, E.F.
1917. "Inheritance in Wheat, Barley and Oat Hybrids."
Wash Agr. Exp. Sta. Bull. 135. pp.61, 9 fig.
- (50) Garber, R.J.
1921. "A Preliminary Note on the Inheritance of Rust Resistance in Oats."
Jour. Amer. Soc. Agron. 13.: p.41-43. 1 fig.
- (51) Garber, R.J.
1922. "Inheritance and Yield with Particular Reference to Rust Resistance and Panicle Type in Oats."
Minn. Agr. Exp. Sta. Tech. Bull. 7. pp.62, illus.

- (52) Gordon, W.L., and Bailey, D.L.
1928. "Physiologic Forms of Oat Stem Rust in Canada."
Sci. Agric. 9. 1. p.30-38, 3 figs., 3 maps, 1 chart.
- (53) Goulden, C.H.
1926. "A Genetic and Cytological Study of Dwarfing in Wheat and Oats."
Minn. Agr. Exp. Sta. Tech. Bull. 33, pp37, illus.
- (54) Goulden, C.H., Neatby, K.W., and Welsh, J.N.
1928. "The Inheritance of Resistance to Puccinia graminis tritici in a cross between two varieties of Triticum vulgare.
Phytopathology, 18 : 631-658, 8 fig.
- (55) Griffee, F.
1922. "Breeding Oats Resistant to Stem Rust."
Jour. Heredity, 13 : 187-190. 3 fig.
- (56) Grove, W.B.
1913. "The British Rust Fungi."
Cambridge University Press.
- (57) Harrington, J.B., and Aamodt, O.S.
1923. "Mode of Inheritance of Resistance to Puccinia graminis with Relation to Seed Colour in Crosses Between Varieties of Durum Wheat."
Jour. Agr. Res. 24; p.979-996, 4 pl.
- (58) Harrington, J.B.
1925. "The Inheritance of Resistance to Puccinia graminis in crosses between varieties of Durum Wheat."
Sc. Agric., 5 : p.265-288.
- (59) Hayes, H.K.
1918. "Natural Cross-pollination in Wheat."
Jour. Amer. Soc. Agron., 10 : p.120-122.
- (60) Hayes, H.K., and Parker, J.H., and Kurtzweil, C.
1920. "Genetics of Rust Resistance in Crosses of Varieties of Triticum Vulgare with varieties of T.durum and T.dicoccum.
Jour. Agr. Res. 19 : p.523)542, pl. 97-102.
- (61) Hayes, H.K., and Stakman, E.C.
1922. "Wheat Stem Rust from the Standpoint of Plant Breeding."
Proc.Second Ann.Mtg.Canad.Soc.Agron.1921. 22-35. 4 fig.
- (62) Hayes, H.K., and Aamodt, O.S.
1923. "A Study of Rust Resistance in a Cross Between Marquis and Kota Wheats."
Jour. Agr. Res., 24 : p.997-1012, 3 pl.
- (63) Hayes, H.K., Stakman, E.C., and Aamodt, O.S.
1925. "Inheritance in Wheat of Resistance to Black Stem Rust."
Phytopathology, 15 : 371-387, 1 pl.
- (64) Hayes, H.K., Griffee, F., Stevenson, F.J., and Lunden, A.P.
1928. "Correlated Studies in Oats of the Inheritance of Reaction to Stem Rust and Smuts and of Other Differential Characters."
Jour Agr Res. 36, p.437-457.
- (65) Hayes, H.K.
1929. "Breeding Disease Resistant Varieties of Crop Plants."
Proc.Internat.Congr. of Pl. Sciences 1., p.137-148.
- (66) Heald, F.D.
1926. "Manual of Plant Diseases."
McGraw Hill Book Co. Inc.
- (67) Historical Records of Australia. 1, 1788 - 1796.
The Library Committee of the Commonwealth Parliament.

- (68) Historical Records of Australia, II, 1797 - 1800.
The Library Committee of the Commonwealth Parliament.
- (69) Hitchcock, A.S., and Carleton, M.A.
1893. "Preliminary Report on Rusts of Grain".
Kansas Agr. Exp. Sta. Bull. 38, pp. 14, 3 pl.
- (70) Hitchcock, A.S., and Carleton, M.A.
1894. "Second Report on Rusts of Grain".
Kansas Agr. Exp. Sta. Bull. 46.
- (71) Homma, (Yasu).
1919. "A Statistical Study on the Biological Forms of
Erysiphe graminis D.C.
Trans. Sapporo Nat. Hist. Soc., X, 2, p. 157-161.4 figs.
- (72) Howard, A, and Howard, G.L.C.
1913. "On the Inheritance of Some Characters in Wheat."
Mem. Dept. Agr. Ind. Bot. Ser. 5, p. 1-47, 3 pl.
- (73) Hungerford, C.W.
1914. "Wintering of Timothy Rust in Wisconsin."
Phytopathology, 4, p. 337-338.
- (74) Hursh, C.R.
1922. "The Relation of Temperature and Hydrogen-ion
Concentration to Urediniospore Germination of
Biologic Forms of Stem Rust of Wheat."
Phytopathology, 12 : p. 353-361.
- (75) Hursh, C.R.
1924. "Morphological and Physiological Studies on the
Resistance of Wheat to Puccinia graminis tritici E.&H."
Jour. Agr. Res. 27 : p. 381-412.
- (76) Hynes, H.J.
1926. "Studies of the Reaction to Stem Rust in a Cross
Between Federation Wheat and Khapli Emmer, with
Notes on the Fertility of the Hybrid Types."
Phytopathology, 16 : p. 809-827, 4 pl.
- (77) Hynes, H.J.
1928. "Stem Rust of Wheat. The Isolation of Resistant
Types from a Federation x Khapli cross."
Agr. Gaz. N.S.W., 39, p. 371-880.
- (78) Jaczewski, A.A.
1910. "Studien über das Verhalten des Schwarzrostes des
Getreides in Russland."
Ztschr. Pflanzenkrank. Bd. 20, Heft 6, p. 321-359, 8 figs.
- (79) Johnson, E.C.
1911. "Timothy Rust in the United States."
U.S.D.A., B.P.I., Bull. 224, pp. 20.
- (80) Johnston, C.O., and Melchers, L.E.
1929. "Greenhouse Studies on the Relation of Age of Wheat
Plants to Infection by Puccinia triticina."
Jour. Agr. Res. 38, p. 147-157, pl. 3.
- (81) Klebahn, H.
1896. "Kulturversuche mit heteröischen Rostpilzen."
Zeits. Pflanzenk. 6 : p. 261-270.
- (82) Klebahn, H.
1904. "Die Wirtwechselnden Rostpilze."
pp. 447, illus. 6 pl. Berlin.
- (83) Levine, M.N., and Stakman, E.C.
1918. "A Third Biologic Form of Puccinia graminis on Wheat."
Jour. Agr. Res. 13 : 651-654.

- (84) Levine, M.N.
1919. "The Epidemiology of Cereal Rusts in General and Black Stem Rust in Particular."
U.S.D.A., B.P.I., Off. Cereal Investig. (Mimeograph) pp. 78.
- (85) Levine, M.N.
1923. "A Statistical Study of the Comparative Morphology of Biologic Forms of Puccinia graminis."
Jour. Agr. Res. 24 : 539-568.
- (86) Levine, M.N., and Stakman, E.C.
1923. "Biologic Specialisation of Puccinia graminis secalis."
Phytopathology, 13 : p. 35 (Abstract).
- (87) Levine, M.N.
1928. "Biometrical Studies on the Variation of Physiologic Forms of Puccinia graminis tritici and the Effects of Ecological Factors on the Susceptibility of Wheat Varieties."
Phytopathology 18 : p. 7 - 125, 1 pl., 37 fig.
- (88) Lind, J.
1915. "Effect of Destruction of the Barberry (Berberis vulgaris) on the Common Rust of Wheat (Puccinia graminis) in Denmark."
Inter. Rev. Sc. Prac. Agr., Year VII, No. 3, p. 442.
- (89) Magnus, P.
1894. "Die Systematische Unterscheidung nächst Verwandter parasitischer Pilze."
Hedw. 33 : 362.
- (90) Magnus, P.
1895. "Eine Bemerkung zu E. Fischer's erfolgreichen Infectionen einiger Centaurea - Arten durch die Puccinia auf Carex Montana."
Bot. Centralb. 63 : 39.
- (91) Maiden, J.H.
1906. "History of Sydney Botanic Gardens."
Kew Bulletin, London. p. 205-218.
- (92) Mains, E.B., and Jackson, H.S.
1923. "Strains of the Leaf Rust of Wheat, Puccinia triticina, in the United States." (Abstract).
Phytopathology, 13 : 36.
- (93) Mains, E.B., and Leighty, C.E.
1923. "Resistance in Rye to Leaf Rust, Puccinia dispersa, Erikss."
Jour. Agr. Res. 25 : p. 243-252, illus.
- (94) Mains, E.B.
1924. "Notes on Greenhouse Culture Methods Used in Rust Investigations."
Ind. Acad. Sci. Proc. 33 : p. 241-257.
- (95) Mains, E.B., Leighty, C.E., and Johnston, C.O.
1926. "Inheritance of Resistance to Leaf Rust, Puccinia triticina, Erikss. in crosses of Common Wheat, Triticum vulgare Vill."
Jour. Agr. Res. 32 : p. 931-972, pl. 5.
- (96) Mains, E.B., and Jackson, H.S.
1926. "Physiologic Specialisation in the Leaf Rust of Wheat, Puccinia triticina, Erikss."
Phytopathology, 16 : p. 89-120 illus.
- (97) Marchal, E.
1903. "Wesentlichen Ergebnisse einer Aufrage über den Getreiderost in Belgien."
Ztschr. Pflanzenkrank. 13 : p. 145-147
- (98) Medley, R.N.
1929. "Field Experiments with Cereal Crops."
Agr. Gaz. N.S.W. 40., p. 346-352.

- 99) Melchers, L.E., and Parker, J.H.
1918. "Three Varieties of Hard Red Winter Wheat Resistant to Stem Rust."
Phytopathology, 8 : 79.
- 100) Melchers, L.E. and Parker, J.H.
1918. "Another Strain of Puccinia graminis."
Kansas Agr. Exp. Sta. Circ. 68, pp4.
- 101) Melchers, L.E., and Parker, J.H.
1922. "Inheritance of Resistance to Black Stem Rust in Crosses Between Varieties of Common Wheat." (Abstract).
Phytopathology, 12 : p. 31-32.
- 102) Melchers, L.E., and Parker, J.H.
1922. "Rust Resistance in Winter Wheat Varieties."
U.S.D.A., Bull. 1046, pp.32., illus.
- 103) Melhus, I.E., Durrell, L.W., and Kirby, R.S.
1920. "Relation of the Barberry to Stem Rust in Iowa."
Iowa Agr. Exp. Sta. Res. Bull. 57 : p. 283-325.
- 104) Mercer, W.H.
1914. "Investigations of Timothy Rust in North Dakota during 1913."
Phytopathology, 4., p. 20-22.
- 105) McAlpine, D.
1906. "The Rusts of Australia."
~~vii~~, pp. 349, pl. 55.
Melbourne, Literature, p. 213-221.
- 106) McFadden, E.S.
1888. "Rust Proof Bread wheats."
Dakota Farmer, 45 : p. 102.
- (106a) Montague Smith, J.
1818(?). "An Enquiry into the Nature, Cause and Prevention of Rust in Wheat."
1920. "Biologic Forms of Wheat Stem Rust in Western Canada."
H. Thomas and J. Moore. pp.15. p. 34-35.
- 108) Newton, M.
1922. "Studies on Wheat Stem Rust (Puccinia graminis tritici.)"
Roy. Soc. Canad. Proc. and Trans. (iii) 16 (Sect. v) : p. 153-210.
- 109) Newton, M., and Johnson, T.
1927. "Greenhouse Experiments on the Relative Susceptibility of Spring Wheat Varieties to Seven Physiologic Forms of Wheat Stem Rust."
Sci. Agr. 7 : p. 7-11.
- (110) Newton, M., and Johnson, T.
1927. "Colour Mutations in Puccinia graminis tritici (Pers) E. & H."
Phytopathology, 17 : No. 10.
- (111) Newton, M., Johnson, T., and Brown, A.M.
1928. "New Physiologic Forms of Puccinia graminis tritici."
Sc. Agric. 9 : No. 4, p. 209-215.
- (112) Nilsson-Ehle, H.
1908. "Einige Ergebnisse von Kreuzungen bei Hafer und Weizen."
Botan. Notisn. Lund's, p. 257-298.
- (113) Nilsson-Ehle, H.
1911. "Kreuzungsuntersuchungen an Hafer und Weizen II,"
Lund's Univ. Arsskr., n. F. Afd. 2. Bd. 7, No. 6, pp. 82.
- (114) Parker, J.H.
1918. "Greenhouse Experiments on the Rust Resistance of Oat Varieties."
U.S.D.A., Bull. 629, pp. 16.
- (115) Parker, J.H.
1920. "A Preliminary Study of the Inheritance of Rust Resistance in Oats."
Jour. Amer. Soc. Agron. 12 : p. 23-38, illus.

- 99) Melchers, L.E., and Parker, J.H.
1918. "Three Varieties of Hard Red Winter Wheat Resistant to Stem Rust."
Phytopathology, 8 : 79.
- 100) Melchers, L.E. and Parker, J.H.
1918. "Another Strain of Puccinia graminis."
Kansas Agr. Exp. Sta. Circ. 68, pp4.
- 101) Melchers, L.E., and Parker, J.H.
1922. "Inheritance of Resistance to Black Stem Rust in Crosses Between Varieties of Common Wheat." (Abstract).
Phytopathology, 12 : p. 31-32.
- 102) Melchers, L.E., and Parker, J.H.
1922. "Rust Resistance in Winter Wheat Varieties."
U.S.D.A., Bull. 1046, pp.32., illus.
- 103) Melhus, I.E., Durrell, L.W., and Kirby, R.S.
1920. "Relation of the Barberry to Stem Rust in Iowa."
Iowa Agr. Exp. Sta. Res. Bull. 57 : p. 283-325.
- 104) Mercer, W.H.
1914. "Investigations of Timothy Rust in North Dakota during 1913."
Phytopathology, 4., p. 20-22.
- 105) McAlpine, D.
1906. "The Rusts of Australia."
~~vii~~, pp. 349, pl. 55.
Melbourne, Literature, p. 213-221.
- 106) McFadden, E.S.
1925. "Synthetic" Rust Proof Bread Wheats."
Dakota Farmer, 45 : p. 102.
- 107) Newton, M.
1920. "Biologic Forms of Wheat Stem Rust in Western Canada."
Proc. West Canad. Soc. Agron. 1 : p. 34-35.
- 108) Newton, M.
1922. "Studies on Wheat Stem Rust (Puccinia graminis tritici.)"
Roy. Soc. Canad. Proc. and Trans. (iii) 16 (Sect. v) : p. 153-210.
- 109) Newton, M., and Johnson, T.
1927. "Greenhouse Experiments on the Relative Susceptibility of Spring Wheat Varieties to Seven Physiologic Forms of Wheat Stem Rust."
Sci. Agr. 7 : p. 7-11.
- 110) Newton, M., and Johnson, T.
1927. "Colour Mutations in Puccinia graminis tritici (Pers) E. & H."
Phytopathology, 17 : No. 10.
- 111) Newton, M., Johnson, T., and Brown, A.M.
1928. "New Physiologic Forms of Puccinia graminis tritici."
Sc. Agric. 9 : No. 4, p. 209-215.
- 112) Nilsson-Ehle, H.
1908. "Einige Ergebnisse von Kreuzungen bei Hafer und Weizen."
Botan. Notizn. Lund's, p. 257-298.
- 113) Nilsson-Ehle, H.
1911. "Kreuzungsuntersuchungen an Hafer und Weizen II,"
Lund's Univ. Arsskr., n. F. Afd. 2. Bd. 7, No. 6, pp. 82.
- 114) Parker, J.H.
1918. "Greenhouse Experiments on the Rust Resistance of Oat Varieties."
U.S.D.A., Bull. 629, pp. 16.
- 115) Parker, J.H.
1920. "A Preliminary Study of the Inheritance of Rust Resistance in Oats."
Jour. Amer. Soc. Agron. 12 : p. 23-38, illus.

- (116) Peltier, G.L.
1923. "A Study of the Environmental Conditions Influencing the Development of Stem Rust in the Absence of an Alternate Host : II. Infection Studies with Puccinia graminis tritici form 3 and form 9.
Nebr. Agr. Exp. Sta. Res. Bull. 25.
- (117) Piemeisel, F.J.
1917. "A New Strain of Puccinia graminis." (Abstract).
Phytopathology, 7 : p.73.
- (118) Potts, H.W.
1924. "Early Struggles of the Wheat Farmers of N.S.W."
Jour. Roy. Aust. Histor. Soc. 9, : p. 260-274.
- (119) Pritchard, F.J.
1911. "A Preliminary Report on the Yearly origin and Dissemination of Puccinia graminis."
Bot. Gaz. 52, : p. 169-192. pl. 4.
- (120) Pritchard, F.J.
1911. "The Wintering of Puccinia graminis tritici E.&H. and the Infection of Wheat through the Seed."
Phytopathology, : p. 150-154., 2 fig., pl. 22.
- (121) Puttick, G.F.
1921. "The Reaction of the F₂ Generation of a Cross Between a Common and a Durum Wheat to Two Biologic Forms of Puccinia graminis."
Phytopathology, 11 : 205-213.
- (122) Raines, M.A.
1922. "Vegetative Vigour of the Host as a Factor Influencing Susceptibility and Resistance to Certain Rust Diseases of Higher Plants. 1."
Amer. Jour. Bot. 9 : p. 183-203.
- (123) Reed, G.M.
1918. "Physiologic Specialisation of Parasitic Fungi."
Mem. Brooklyn Bot. Gard. 1, : p. 348-409.
- (124) Rostrup, E.
1894. "Mykologiske Meddelelser."
Bot. Tidssk. 19 : p. 36-51.
- (125) Ramsey, H.J.
1915. "Notes on the Early History of Dundas."
Jour. Roy. Aust. Geographical Soc. 3 : p. 25-31.
- (126) Rust in Wheat Conference.
1891. Agr. Gaz. N.S.W. 2 : p. 403-406.
- (127) Rust in Wheat.
1891. Agr. Gaz. N.S.W. 2 : 729-730.
- (128) Rust in Wheat Conference.
1892. Agr. Gaz. N.S.W. 3 : p.221.
- (129) Rust in Wheat ^{Investigations.} ~~Conference.~~
1892. Agr. Gaz. N.S.W. 3 : p. 632-633.
- (130) Rust in Wheat Conference.
1896. Agr. Gaz. N.S.W. 7 : p. 438-443.
- (129A) Rust in Wheat Conference.
1892. Report of the Proceedings of the Conference.
C.E. Bristow, Govt. Printer, Adelaide, 74 p.

- (131) Stakman, E.C.
1914. "A Preliminary Report on the Relation of Grass Rusts to the Cereal Rust Problem." (Abstract)
Phytopathology, : 4., p. 411.
- (132) Stakman, E.C.
1914. "A Study in Cereal Rusts : Physiological Races."
Minn.Agr.Exp.Sta.Bull. 138, 9 pl.
- (133) Stakman, E.C.
1915. "Relation Between Puccinia graminis and plants Highly Resistant to its Attack.
Jour.Agr.Res. 4, : p. 193-200., pl. 28.
- (134) Stakman, E.C., and Jensen, L.
1915. "Infection Experiments with Timothy Rust."
Jour.Agr.Res. 5, : p. 211-216.
- (135) Stakman, E.C., and Piemeisel, F.J.
1916. "Biologic Forms of Puccinia graminis on Wild Grasses and Cereals. A Preliminary Report." (Abstract).
Phytopathology, 6, : p. 99-100.
- (136) Stakman, E.C., and Piemeisel, F.J.
1916. "Infection of Timothy by Puccinia graminis."
Jour.Agr.Res. 6, : p. 813-816.
- (137) Stakman, E.C., and Piemeisel, F.J.
1917. "A New Strain of Puccinia graminis." (Abstract).
Phytopathology, 7, : p. 73.
- (138) Stakman, E.C., and Piemeisel, F.J. and
1917. "Biologic Forms of Puccinia graminis on Cereals and Grasses."
Jour.Agr.Res. 10, : p. 429-496, pl. 53-59.
- (139) Stakman, E.C., Parker, J.H., and Piemeisel, F.J.
1918. "Can Biologic Forms of Stem Rust in Wheat Change Rapidly Enough to Interfere With Breeding for Rust Resistance?"
Jour.Agr.Res. 14, : p. 111-124, pl. 13-17.
- (140) Stakman, E.C., and Levine, M.N.
1918. "Plasticity of Biologic Forms of Puccinia graminis."
Jour.Agr.Res. 15, : 221-250, pl. 17-18.
- (141) Stakman, E.C., Levine, M.N., and Leach, J.G.
1919. "New Biologic Forms of Puccinia graminis."
Jour.Agr.Res. 16, : p. 103-105.
- (142) Stakman, E.C.
1919. "The Black Stem Rust and the Barberry."
Yearbook U.S.D.A. 1918, P. 75-100, pl 1-10.
- (143) Stakman, E.C., and Aamodt, O.S.
1922. "The Effect of Fertilisers on the Development of Stem Rust of Wheat." (Abstract).
Phytopathology, 12, : p. 31.
- (144) Stakman, E.C., and Levine, M.N.
1922. "The Determination of Biologic Forms of Puccinia graminis on Triticum spp."
Minn.Agr.Exp.Sta.Tech.Bull. 8, pp.10, illus.
- (145) Stakman, E.C., Henry, A.W., Curran, G.C., and Christopher, W.N.
1923. "Spores in the Upper Air."
Jour.Agr.Res. 24 : p. 599-606.
- (146) Stakman, E.C., Levine, M.N., and Bailey, D.L.
1923. "Biologic Forms of Puccinia graminis on varieties of Avena spp."
Jour.Agr.Res. 24 : p. 1013-1018.

- (147) Stakman, E.C., and Aasmot, O.S.
1924. "The Effect of Fertilisers on the Development of Stem Rust in Wheat."
Jour.Agr.Res. 27 : p. 341-380.
- (148) Stakman, E.C. and Levine, M.N.
1924. "Puccinia graminis poae E.&H. in the United States."
Jour.Agr.Res. 28 : p. 541-548.
- (149) Stewart, G.
1928. "Origin of a Segregate Resistant to Black Stem Rust in a Cross Between Two Susceptible Parents."
Amer.Naturalist 62 : P. 188-192.
- (150) Tehon, L.R., and Young, P.A.
1924. "Notes on the Climatic Conditions Influencing the 1923 Epidemic of Stem Rust on Wheat in Illinois."
Phytopathology, 14 : p. 94-100.
- (151) Thiel, A.F., and Weiss, F.
1920. "The Effect of Citric Acid on the Germination of the teliospores of Puccinia graminis tritici."
Phytopathology, 10 : No.10, p. 448-453.
- (152) Thompson, W.P.
1918. "The Inheritance of the Length of the Flowering and Ripening Periods in Wheat."
Trans.Roy.Soc.Canada (Ser.3) 12 : p. 69-87.
- (153) Thompson, W.P.
1925. "Cytologic Conditions in Wheat in Relation to the Rust Problem."
A Sc. Agr. 8 : p. 237-239.
- (154) Vavilov, N.I.
1913. "Beiträge zur Frage über die Verschiedene Widerstandsfähigkeit der Getreide gegen parasitische Pilze."
Trudy Sel.Sta.Mosk.Selskokhoz Inst. 1 : p. 1-108.
- (155) Vavilov, N.I.
1914. "Immunity to Fungous Diseases as a Physiological Test in Genetics and Systematics, Exemplified in Cereals."
Jour.Genetics. 4 : 49-65.
- (156) Waldron, L.R., and Clark, J.A.
1919. "Kota, a Rust Resisting Variety of Common Spring Wheat."
Jour.Amer.Soc.Agron. 11 : p. 187-195.
- (157) Waldron, L.R.
1921. "Inheritance of Rust Resistance in a Family Derived From a Cross Between Durum and Common Wheat."
N.Dak.Agr.Exp.Sta.Bull. 147 : pp. 24, illus.
- (158) 1924. "A Study of Dwarfness in Wheat Accompanied by Unexpected Ratios."
Waldron, L.R. Genetics 9 : p. 212-46.
- (159) Ward, H. Marshall
1902. "On the Relations Between Host and Parasite in the Bromes and their Brown Rust, Puccinia dispersa Erikss."
Ann.Bot. 16 : p. 233-315.
- (158A) Ward, H. Marshall.
1901. "The Bromes and their rust fungus, Puccinia dispersa."
Ann. Bot. 15 ; p. 560-562.

- (160) Waterhouse, W.L.
1920. "A Note on the Over-summering of Wheat Rust in Australia."
Agr.Gaz.N.S.W. 31 : p. 165-166.
- (161) Waterhouse, W.L.
1921. "Studies in the Physiology of Parasitism, vii. Infection of Berberis vulgaris by Sporidia of Puccinia graminis."
Annals of Botany 35 : p. 557-564.
- (162) Waterhouse, W.L.
1921. "On the Production in Australia of Aecidial Stage of Puccinia graminis Pers."
Jour.and Proc.Roy.Soc.N.S.W. 55 : p. 278-288, 1 pl.
- (163) Waterhouse, W.L.
1923. "Notes on Rust Investigations in Progress." (Abstract.)
Proc.Pan/Pacific Sci.Cong.Aust.(1923) 1 : p. 142.
- (164) Waterhouse, W.L.
1927. "Studies in the Inheritance of Resistance to Leaf Rust Puccinia anomala Rostk. in Crosses of Barley I."
Jour. and Proc.Roy.Soc.N.S.W. 61 : p. 218-247, 2 pl.
- (165) Waterhouse, W.L.
1929. "A Preliminary Account of the Origin of Two New Australian Physiologic Forms of Puccinia graminis tritici"
Proc.Linn.Soc.N.S.W. 54 : 2 : p. 96-106, pl.1.
- (166) Weaver, J.E.
1916. "The Effects of Certain Rusts upon the Transpiration of Their Hosts."
Minn.Bot.Studies 4 : p. 379-406, Pl. 41-42.
- (167) Weiss, F.
1924. "The Effect of Rust Infection Upon the Water Requirements of Wheat."
Jour.Agr.Res. 27 : 107-118.

EXPLANATION OF PLATES.

- Plate I. (a) Aecidial stage of P. graminis tritici on a barberry which gave rise to the unusual physiologic form styled Form 45A.
- (b) Aecidial stage of P. graminis avenae on a barberry arising from inoculations with teleutospores on oaten straw from Ireland.

Plate II. Seedling leaves of wheat showing types of reaction produced by P. graminis tritici.

- (a) Leaves showing the flecks of the immune type designated "0;" of the resistant class. Two show the upper and two the lower surfaces of the leaves.
- (b) Leaves showing the very resistant or "1" type of reaction belonging to the resistant class.
- (c) Leaves showing the moderately resistant or "2" type of reaction belonging to the resistant class.
- (d) Leaves showing the relatively susceptible or "3" type of reaction belonging to the susceptible class.
- (e) The under surfaces of leaves showing the completely susceptible or "4" type of reaction belonging to the susceptible class.
- (f) The under surfaces of leaves showing the intermediately susceptible or "X" type of reaction belonging to the indeterminate class.

Plate III. Seedling leaves showing types of rust reaction.

- (a) (b) and (c) show infections of P. triticina.
- (a) Lower and upper surface views of leaves showing the flecks or "0;" reaction of the immune type belonging to the resistant class.
- (b) Lower and upper surfaces of leaves showing the moderately resistant or "2" type of the resistant class.
- (c) Lower and upper surfaces of leaves showing the completely susceptible or "4" type of reaction of the susceptible class.
- (d) (e) (f) and (g) show infections of P. graminis avenae.
- (d) Lower and upper surfaces of leaves showing the very resistant or "1" type of reaction belonging to the resistant class.
- (e) Upper and lower surfaces of leaves showing the ordinary occurrence of the moderately resistant or "2" type of reaction of the resistant class.
- (f) Lower surfaces of leaves showing the unusual occurrence of the teleutosori in the moderately resistant or "2" type of reaction exhibited by a physiologic form obtained from Irish straw.
- (g) Upper and lower surfaces of leaves showing the completely susceptible or "4" type of reaction belonging to the susceptible class.

Plate IV. Natural crossing in a variety of Triticum sphaerococcum. In the top row at the left is shown an ear of the normal wheat. In the top row at the right is the ear of a wheat derived from the grain grown at Cambridge, England. In the bottom row are typical ears of some of the wheats derived as segregates from the last-mentioned wheat illustrated in the right of the top row.

Plate V. Variations in the reactions shown by P.graminis tritici on wheat seedlings.

- (a) Upper and lower surfaces of leaves of "Khapli" showing the very resistant reaction normally given by Form 27.
- (b) Similar leaves of the same host infected by the same physiologic form but showing the susceptible reaction found in the spring and autumn.
- (c) and (d) Under surfaces of leaves of "Mindum" infected with Form 34. In (c) the flecks which occur in the winter months are shown, and in (d) the completely susceptible "4" reaction which is exhibited in the summer.
- (e) Upper and lower surfaces of leaves of "Federation" showing the "2" reaction produced by Form 46 in the winter. These should be compared with the leaves illustrated in Plate II (e), which represent the fully susceptible "4" reaction exhibited in the summer by this same form on the same host.

Plate VI. Variations in the reactions shown by the same culture of P. graminis avenae 1 on "Joanette" oats, brought about by change in the environmental conditions.

- (a) The completely susceptible or "4" type of reaction found in the summer.
- (b) The indeterminate or "X" type of reaction exhibited in the late spring and autumn.
- (c) The very resistant or "1" type of reaction exhibited in the winter.

Plate VII. (a) (b) (c) and (d) illustrate types of reactions shown by P. simplex on barley.

- (a) represents the immune or "0;" type of reaction in which a black discolouration takes the place of the ordinary whitish flecks.
- (b) shows the very resistant or "1" type of reaction.
- (c) illustrates the moderately resistant or "2" type of reaction.
- (d) shows the completely susceptible or "4" type of reaction.
- (e) and (f) illustrate leaves of wheat from H.A. College naturally infected with P. triticina. In (e) are shown the mixtures of susceptible "4" pustules and flecks on leaves of "Thew", and in (f) are shown leaves of "Federation" on which pustules of both physiologic forms are present.

Plate VIII. (a) (b) (c) and (d) illustrate the types of Federation seedlings derived from grain of different ages, sown the same day and photographed the same day.

- (a) Seedlings from grain 6 days old, "very spindly".
- (b) Seedlings from grain 10 days old, "spindly".
- (c) Seedlings from grain 17 days old, "almost normal".
- (d) Seedlings from grain 20 days old, "normal".
- (e) and (f) The side and face views of ears of the F_1 plant of the cross made between "Federation" and "Rosen Rye".

Plate IX. Crosses between vulgare wheats and "Khapli".

- (a) Grain of "Federation".
- (b) Grain of "Khapli".
- (c) Grain produced as a result of crossing Federation and Khapli.
- (d) Some of the F_1 seedlings of the Waratah x Khapli cross after inoculation and removal from the incubation chamber.
- (e) F_1 leaves showing the "X" reaction produced by Form 34.

Plate X. (a) The F_1 plants of the crosses of vulgare wheats with Khapli made in 1927, growing in the open after having been tested and transplanted. The normal growth and heading of wheat plants of other crosses which had been treated in exactly the same fashion are seen to the left.

(b) The same batch of F_1 plants of vulgare x Khapli crosses, showing the differences in the degree of growth made depending upon the particular vulgare variety used as a parent.

Plate XI. (a) An F_2 batch of wheat seedlings showing the stage at which inoculations were made.

(b) A similar box of seedlings showing the stage of development at which note-taking was done.

(c) A pot of wheat seedlings showing the stage at which inoculations were made in the tests.


(d) A pot of similar seedlings showing the stage of development at note-taking time.

(e) Part of an F_2 generation of Canberra x Thew, showing the occurrence of "grass tufts" amongst the normal plants.

Plate XII. Photomicrographs of uredospores of physiologic forms of P. graminis tritici x 250.

- (a) Form 43. (b) Form 44. (c) Form 54 (d) Form 34.
(e) Form 11. (f) Form 33. (g) Form 46. (h) Form 45
(i) Form 45A. (j) Form 55. (k) Form 27. (l) Form 16
(m) "New Form".

Plate XIII. Photomicrographs of spore forms of Puccinia spp. x 250.

- (a) Aecidiospores of P. graminis tritici 46.
(b) Aecidiospores of P. graminis tritici 11.
(c) Aecidiospores of Irish form of P. graminis avenae 1.
(d) to (h) Uredospores of P. graminis avenae.
(d) Form 1 from Australian material.
(e) Form 1 from Irish material.
(f) Form 2.
(g) Form 3.
(h) Form 7.
(i) Teleutospores of P. graminis avenae 1 from Ireland.
(j) Teleutospores of P. graminis avenae 1 from Australia.
(k) (l) and (m) Teleutospores of P. graminis tritici.
(k) Form 34.
(l) Form 43.
(m) Form 46.
(n) and (o) Uredospores of P. triticina.
(n) Australian Form 1.
(o) Australian Form 2.
- 



(a.)

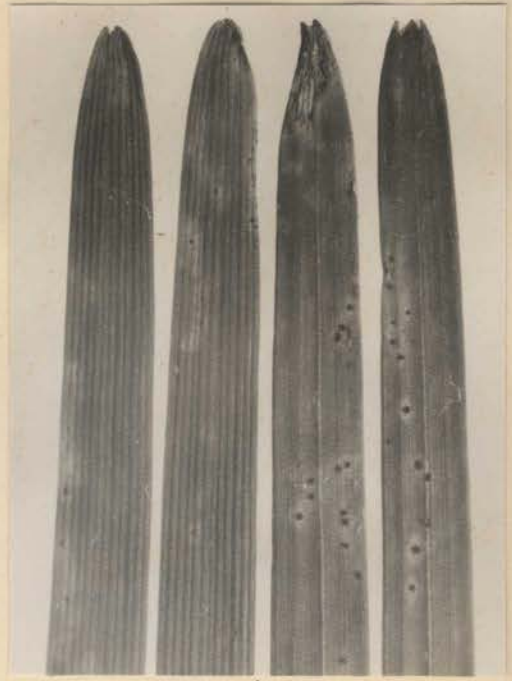


(b.)





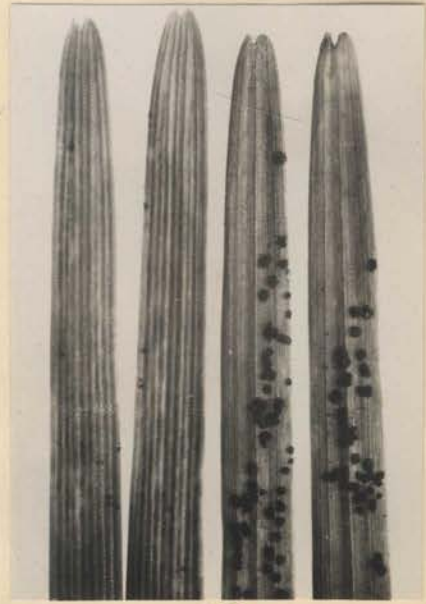
(a.)



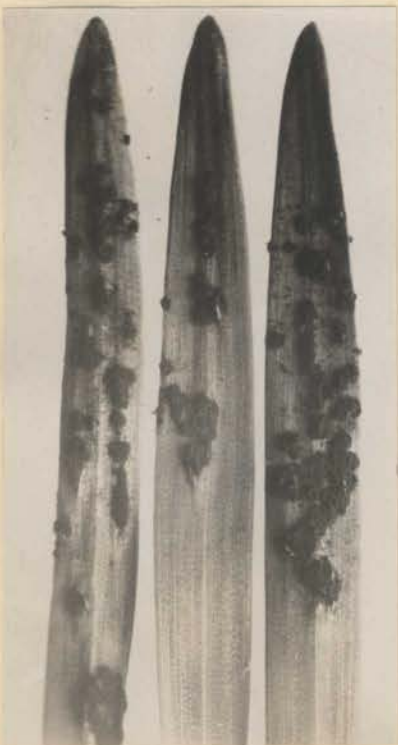
(b.)



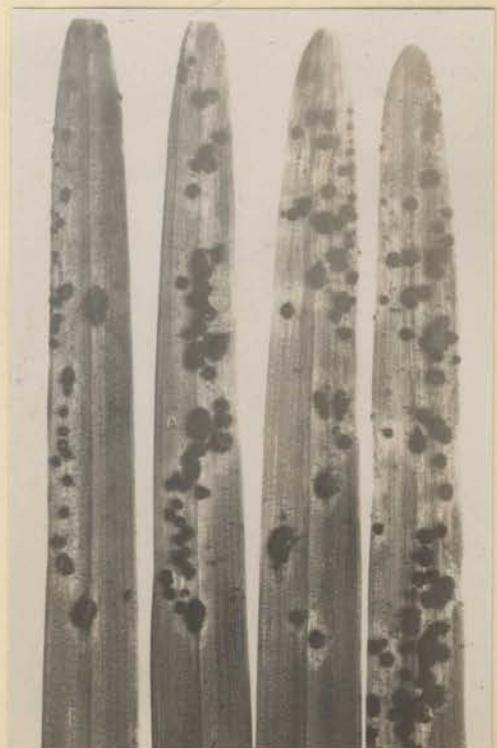
(c.)



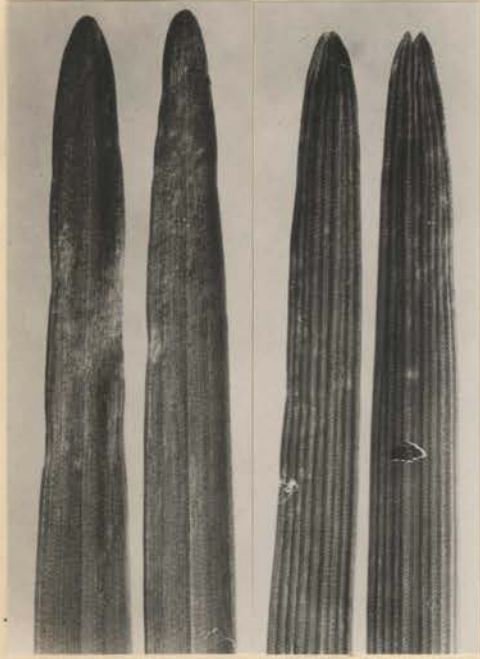
(d.)



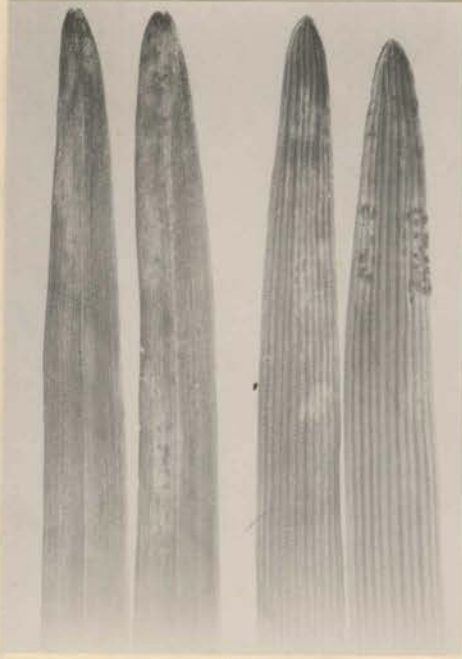
(e.)



(f.)



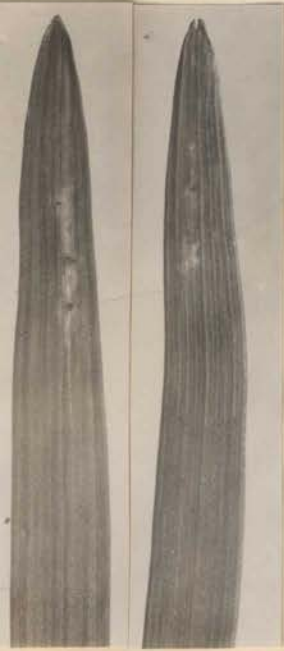
(a.)



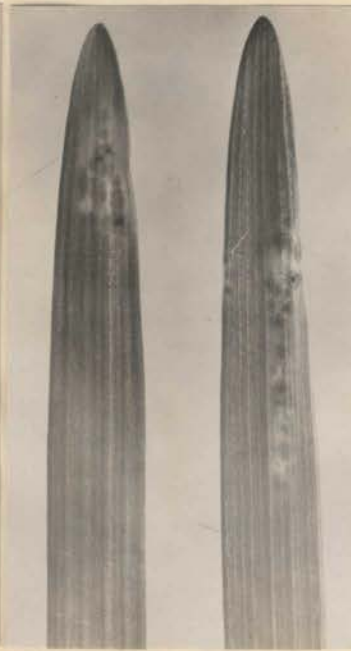
(b.)



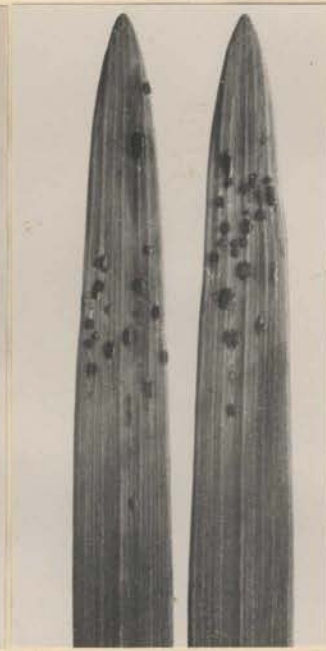
(c.)



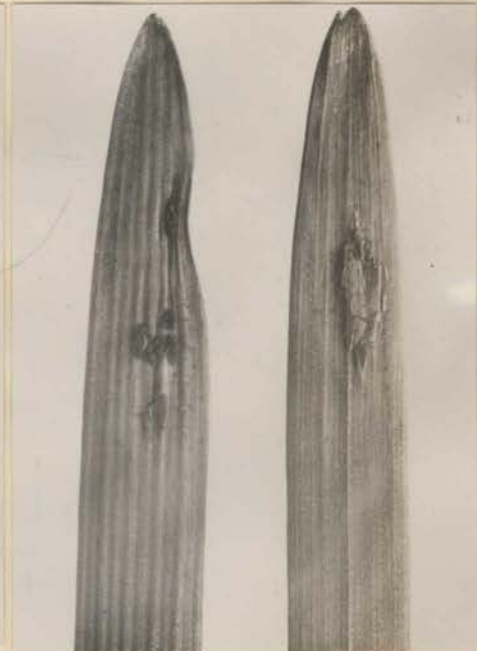
(d.)



(e.)



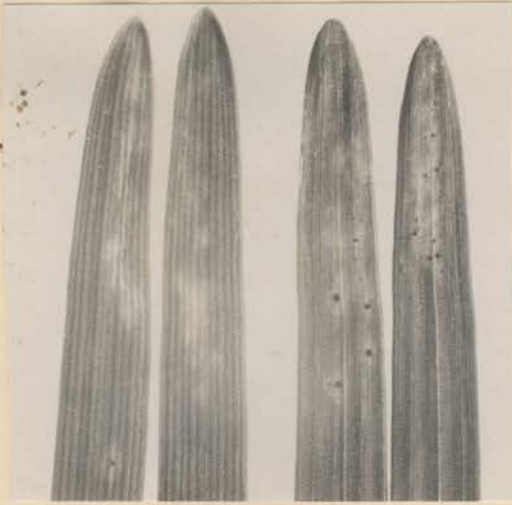
(f.)



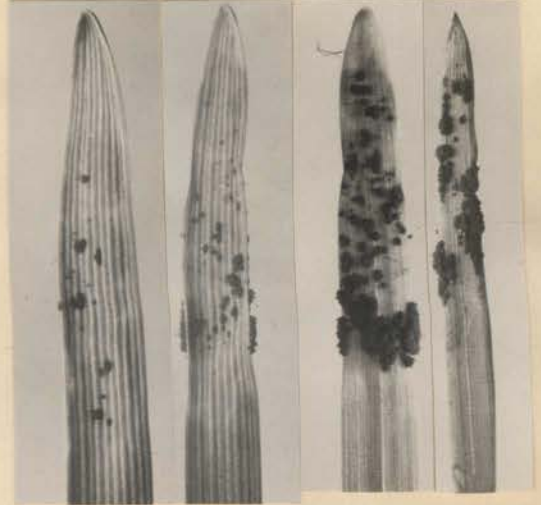
(g.)







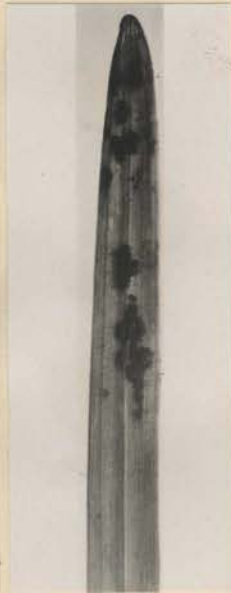
(a.)



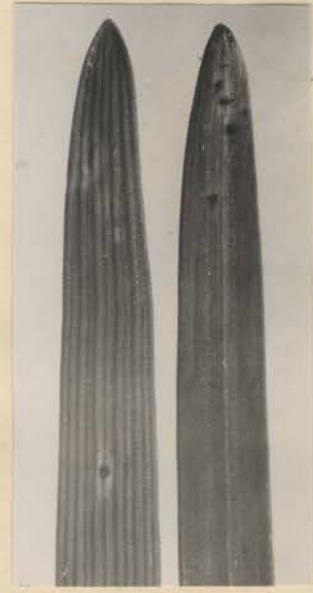
(b.)



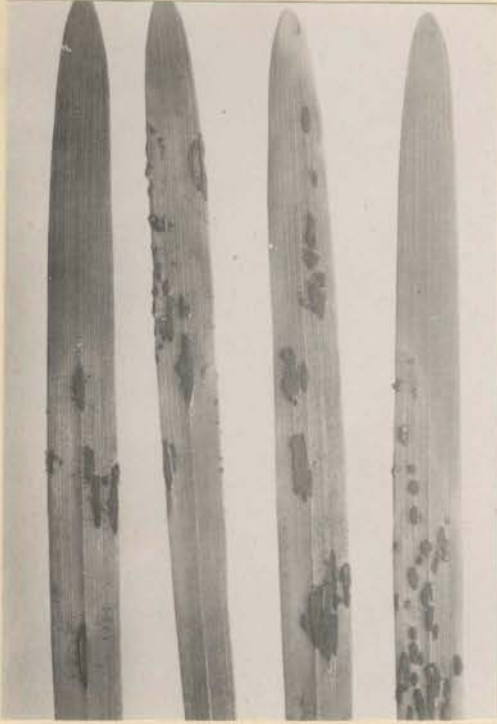
(c.)



(d.)



(e.)



(a.)



(b.)



(c.)



(a.)



(b.)



(c.)



(d.)

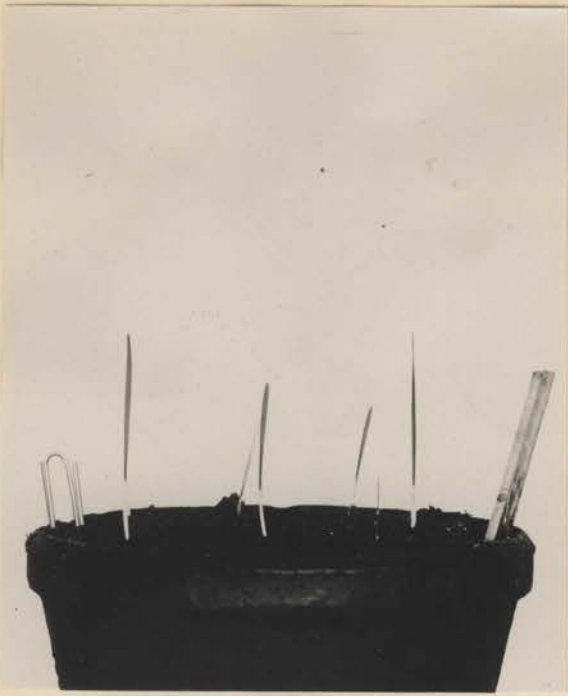


(e.)



(f.)





(a.)



(b.)



(c.)



(d.)



(e.)



(f.)





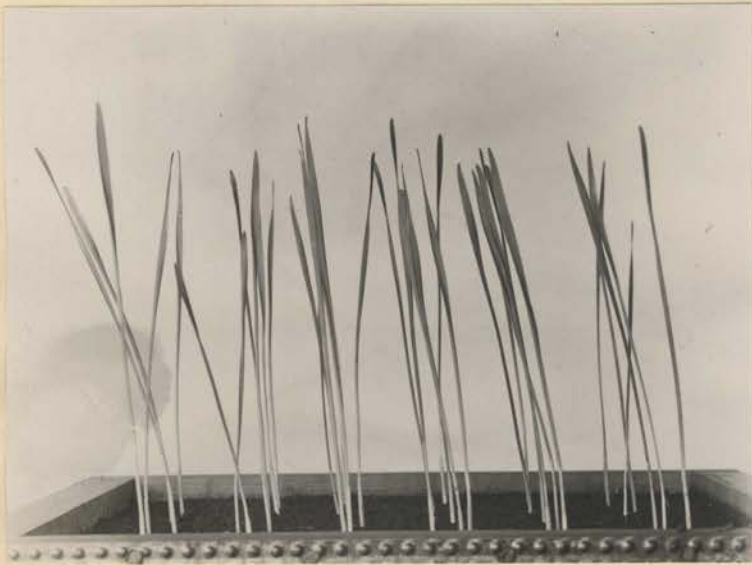
(a.)



(b.)



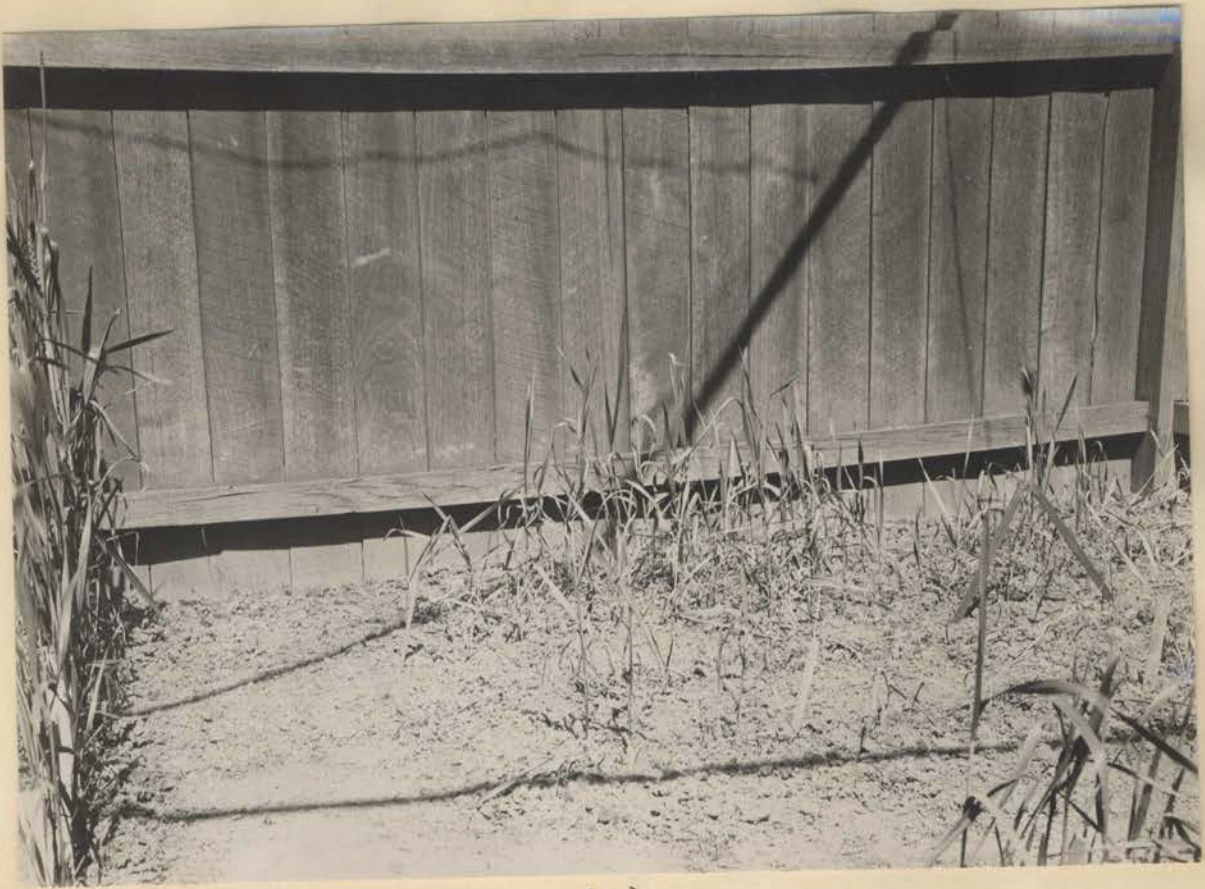
(c.)



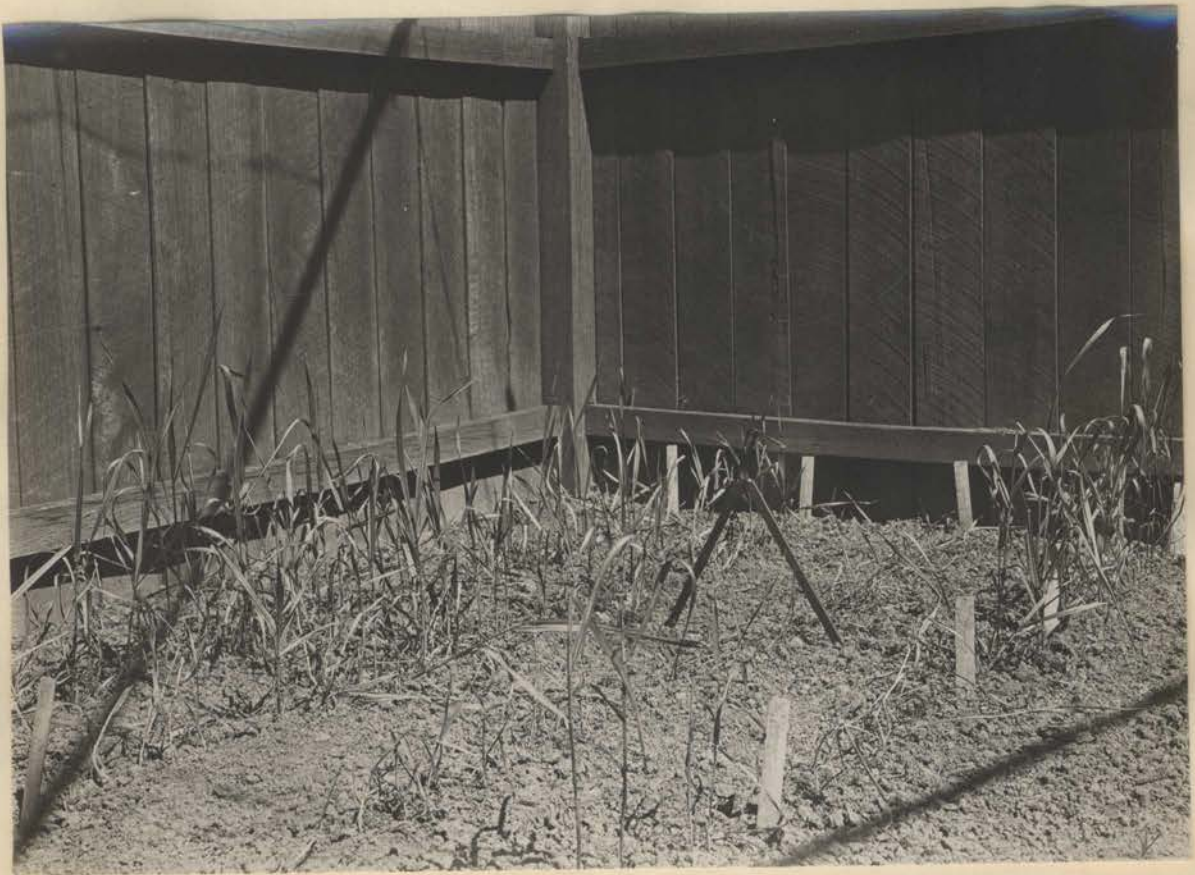
(d.)



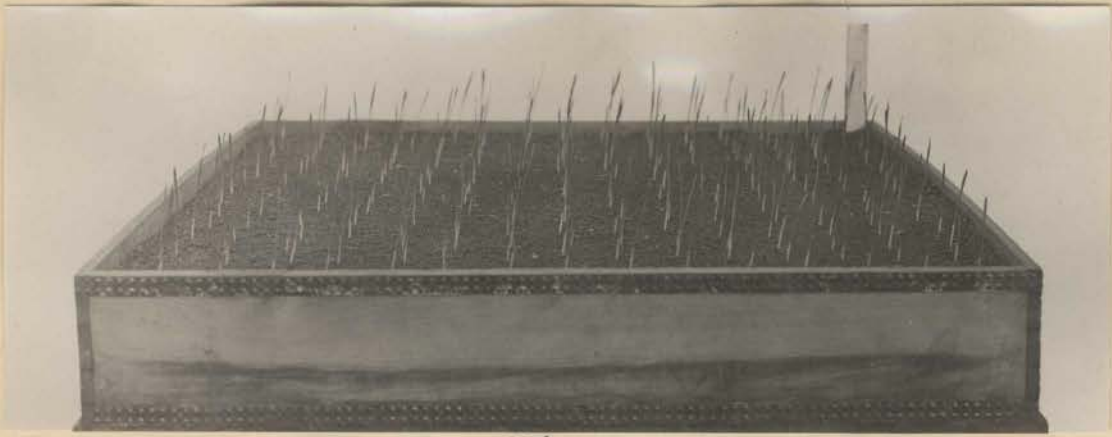
(e.)



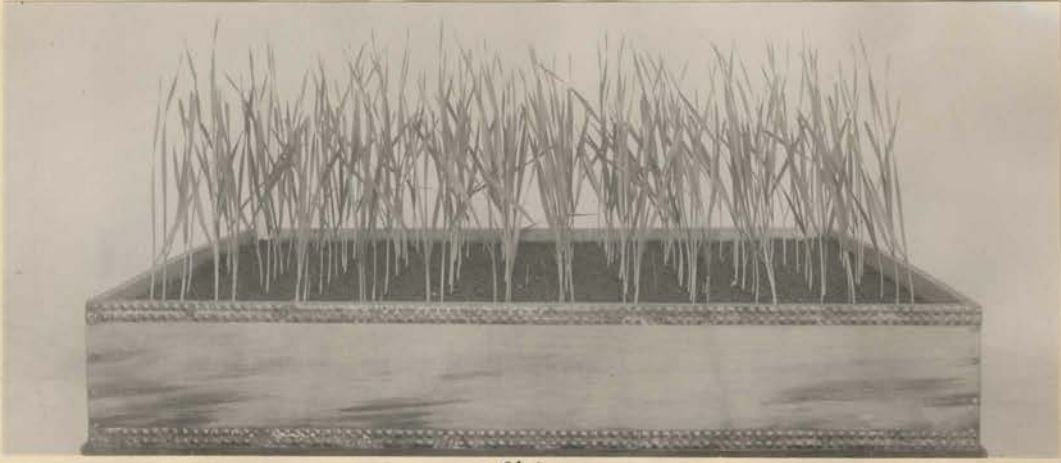
(a.)



(b.)



(a.)



(b.)



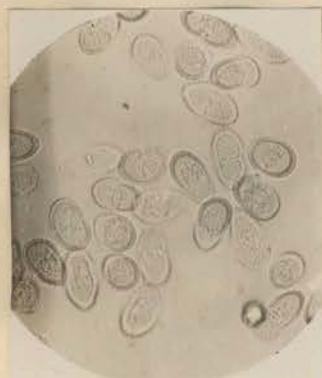
(c.)

(d.)

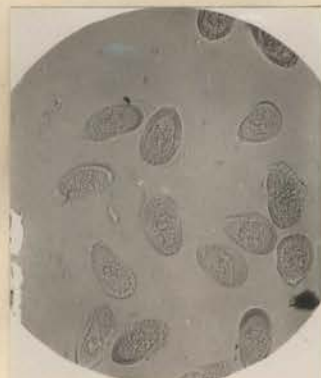


(e.)





(a.)



(b.)



(c.)



(d.)



(e.)



(f.)



(g.)



(h.)



(i.)



(j.)



(k.)

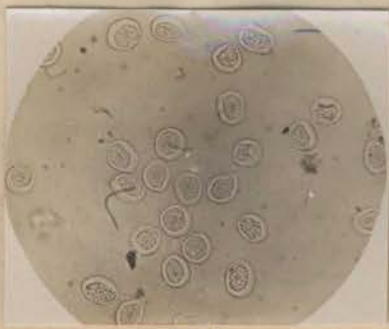


(l.)



(m.)

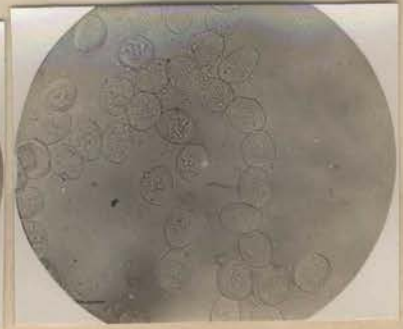




(a.)



(b.)



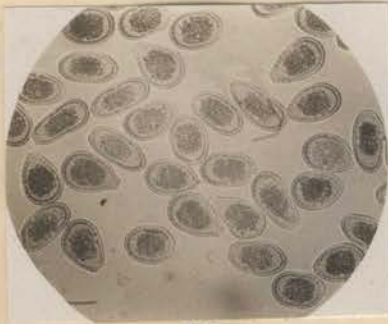
(c.)



(d.)



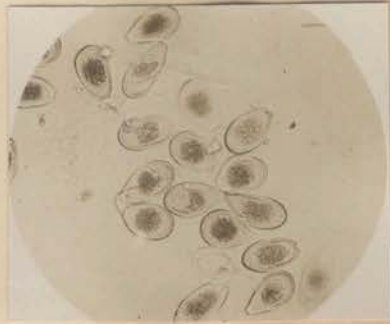
(e.)



(f.)



(g.)



(h.)



(i.)



(j.)



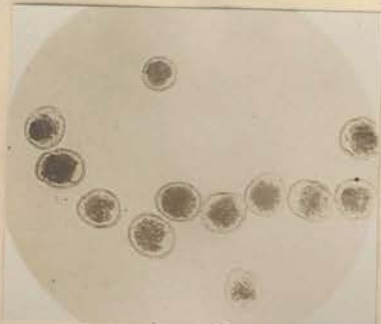
(k.)



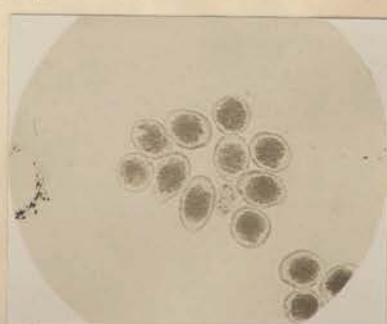
(l.)



(m.)



(n.)



(o.)



(Reprinted from the Journal and Proceedings of the Royal
Society of New South Wales, Vol. LV.)

ON THE PRODUCTION IN AUSTRALIA OF THE
AECIDIAL STAGE OF *Puccinia graminis* Pers.

By W. L. WATERHOUSE, M.C., B.Sc., Agr. (Syd.), D.I.C. (Lond.)

With Plate XIX.

[Read before the Royal Society of N.S. Wales, December 7, 1921.]

Issued March 9th, 1922.

AECIDIAL STAGE OF PUCCINIA GRAMINIS.

ON THE PRODUCTION IN AUSTRALIA OF THE
AECIDIAL STAGE OF *Puccinia graminis* Pers.

By W. L. WATERHOUSE, M.C., B.Sc., Agr. (Syd.), D.I.C. (Lond.)

With Plate XIX.

[Read before the Royal Society of N. S. Wales, December 7, 1921.]

Introduction.

There are several kinds of rust which attack the cereals and grasses, but in Australia the one which does most damage is the black stem rust, *Puccinia graminis* Pers. In some seasons the losses it causes to wheat-growers are enormous. In 1916, the losses in the United States and Canada were estimated at 280,000,000 bushels of wheat, or one-third of the entire crop.⁽¹⁾ Indeed the ravages of the rust have become so great in these countries as to constitute, in parts, the limiting factor in wheat production. The damage done in Australia is not so great. McAlpine⁽²⁾ states that the loss to Australia in 1889 was estimated at between £2,000,000 and £3,000,000. More recent figures concerning New South Wales are available. Mr. A. H. E. McDonald, Chief Inspector of Agriculture, estimates that in 1903 the losses in the North-Western and Northern Tableland Districts reached 3,000,000 bushels valued at over £400,000. In 1916 he estimates the losses in New South Wales at £2,000,000. All these are losses occasioned in "epidemic years" when the weather conditions are particularly favourable to the parasite. But in ordinary seasons the rust is to be found in most districts, and is probably responsible for a depleted grain yield of perhaps five to ten per cent. of the crop. Such immense losses have led to a very careful investigation of the fungus by numerous workers.

Life History of the Rust.

In the 18th century farmers in Europe associated the presence of the common barberry (*Berberis vulgaris* L.) with the spread of rust on wheat. But it remained for De Bary⁽³⁾ to prove in classic fashion that the relation between the two was causal. He worked out the complete life history of the rust. Teleutospores on wheat straw that has been exposed to winter conditions germinate in the following spring and produce sporidia. If these sporidia fall upon the young growth of the barberry or several allied plants, they bring about infection and the production of the spermagonial and the aecidial or "cluster-cup" stage of the parasite. Aecidiospores from the barberry will infect wheat and lead to the production of the uredospore or "red-rust" stage. Later in the summer the same uredo mycelium in wheat may give rise to the teleutospore stage, thus completing the life history.

The proof of this connection of the barberry with cereal rust led to the eradication of the plant in parts of Europe. In U.S.A., a vigorous Barberry Eradication Campaign has been in progress since 1918. A sum of \$200,000 annually is being spent on this work. In these countries the absence of the barberry has been found to decrease the amount of rust on wheat. For example, in Denmark⁽⁴⁾ a gradual disappearance of the stem rust has been contemporaneous with the destruction of the barberry. But it does not always follow that the elimination of the barberry will necessarily get rid of the rust. The uredo-spore stage is capable, under some conditions, of keeping the fungus alive on stray wheat plants and certain grasses until the next season's wheat crop is grown.

Previous Work in Australia.

In Australia no native species of *Berberis* are known. But several introduced species are present in the cooler

parts where they have been planted for ornamental purposes. Up to the present the aecidial stage of the rust has not been found in this country, although repeated attempts have been made to produce it.

Cobb⁽⁵⁾ states that the aecidial stage is unknown in Australia.

McAlpine⁽²⁾ made numerous attempts to infect barberries from rusted wheat straw. In 1892 and succeeding years various means were adopted. In some cases, rusted straw was scattered round the barberry plants or tied on to them. In others, susceptible wheat was sown round barberries, and after it had become severely rusted, was allowed to die down on the spot. In yet other instances, "germinating spores were applied directly to the leaves." McAlpine states that "some plants were kept under bell-jars, others were exposed, and all were attended to and watered freely. Not the slightest trace of any fungus appeared on any of the barberry leaves." He also forwarded rusted wheat straw to Dr. C. B. Plowright in England to be there tested on barberries. But in all cases the teleutospores failed to germinate upon arrival in England.

McAlpine concludes that as far as Australia is concerned, "the rust apparently has no intermediate stage." This has led most workers to consider that the teleutospore stage is merely vestigial. For example Butler⁽⁶⁾ states that the aecidial stage has been completely lost in Australia. Similarly Levine⁽⁷⁾ says that the Australian rust has lost the power of infecting the barberry.

In South Africa and in Ecuador, it is reported that the aecidial stage has similarly been lost. But in the light of the results herein reported, further experimental work in these countries would appear desirable.

The form of stem rust which occurs in Australia has been determined by Eriksson,⁽²⁾ and other authorities as

Puccinia graminis Pers. But if the aecidial stage on the barberry cannot be produced, and if this feature is a requisite⁽⁸⁾ in the determination, then as McAlpine suggests its identity with *Puccinia graminis* Pers. is not proved.

Inoculation Experiments.

(a) *British Material.*

It was planned to attempt the barberry infections in 1919. In July of that year a number of species of *Berberis* were kindly supplied by Mr. J. H. Maiden, F.R.S., Director of the Sydney Botanic Gardens, and planted as a hedge at the Sydney University; a few were also planted at certain of the Government Experiment Farms. It was not until the present year, however, that the work became possible. In the meantime the plants had been attended to and had become well established.

In 1920, while the writer was at the Imperial College of Science and Technology at South Kensington, attempts were made to get Australian teleutospore material to germinate in order to test it on barberries there. Rusted straw was kept in a cool cabin during the voyage and other material was sent through the post in the usual way. But in all cases the spores failed to germinate upon arrival in England. These results are in accord with those reported by Plowright.⁽⁹⁾

In March 1921, rusted wheat straw from Pembrokeshire, Wales, was collected and forwarded by Mr. A. D. Cotton of the Institute of Plant Pathology at Harpenden. It showed abundant teleutosori of *Puccinia graminis* on the sheaths and stems, and was in a viable condition upon arrival in Sydney at the end of April. At this time two plants of *Berberis vulgaris*, which had been cut back a short time before, were putting out fresh young growth. Small bundles of the rusted straw were suspended over one

of the plants. The straw and both plants were sprinkled with water daily for a week.

No case of rust infection occurred. This negative result was expected, because the same method had been tried and had failed in England where better conditions for infection—a higher humidity—prevailed.

A successful method which had been worked out at South Kensington was then tried. It was there noted that more abundant teleutospore germinations appeared to be obtained by leaving the spores intact in the sori than by separating the teleutospores by scraping them off the straw.

Fragments of the straw on which teleutosori were present were soaked in tap-water for five minutes. The barberry plants were sprinkled with water and the soaked fragments of straw placed on young leaves. The whole shoot was then enclosed in a glass cylinder (a lamp chimney) which was then lightly packed with cotton wool at the top and bottom, and then supported by a stake. (Plate XIX, fig. 1.) A very humid atmosphere was thus maintained within the cylinder. The shoot was kept enclosed for 24 hours in some cases, and for 36 hours in others; there was no discernible difference in the results.

In each case, after four to five days, discoloured spots appeared on the upper surfaces of the inoculated leaves. In addition, in some cases, dead areas occurred, mainly at the edges of the leaves. The causal agents were not determined, but similar necrotic areas were also present on control shoots which had not been enclosed in the cylinders. It appears, therefore, that the teleutospore inoculation was not the cause of the necrosis.

After 8 to 10 days yellowish spermatogonia became visible on the upper surfaces of the leaves. Aecidia were produced on the lower surfaces 4 to 5 days later. Aecidiospores

were taken from them to inoculate wheat plants and produced typical uredosori. By means of uredospores the British rust is being kept in culture for further study and comparison with Australian forms.

It was thus shown that the two particular plants of *Berberis vulgaris* were susceptible, under the set conditions, to sporidia of *Puccinia graminis* obtained from England.

(b) *Australian Material.*

Numerous unsuccessful attempts were made to obtain viable teleutospores from straw of the 1920-1921 wheat crop; some was kept at the Sydney University and some at the Hawkesbury Agricultural College and forwarded at intervals by Mr. W. M. Carne. In September 1921, Mr. L. G. Little, Experimentalist of the Glen Innes Experiment Farm, sent specimens of rusted wheat straw of the 1920-1921 crop which had been harvested in January 1921. Some of the varieties were almost destroyed by the stem rust in this season. Straw of these had been allowed to remain undisturbed on the ground during the winter months, when heavy frosts were frequent. Teleutospores were abundant on the stems and leaf-sheaths. The teleutospores germinated in hanging-drops of tap-water at room temperature (about 17-20° C.). An abundance of sporidia was produced overnight from each sorus.

On the 29th September and on several occasions during October, inoculations with soaked teleutospores were made on soft young leaves of the same two plants of *Berberis vulgaris* which had been tested with the British rust. The method of enclosing inoculated shoots in glass cylinders as already described was adopted. The method of merely suspending the rusted straw over the plants was not again tried.

The shoots were kept in the glass cylinders for 24 and 36 hours, again with no appreciable difference in the result obtained. After the removal of the glass cylinders, the plants were watered from time to time when the weather was dry.

After four to five days dark spots became visible on the upper surface of the leaves. As had happened when working with the British rust, sharply defined necrotic areas occurred on some of the inoculated as well as on control shoots. Similar diseased areas were present on leaves of other species of *Berberis* growing close by.

Spermagonia appeared on the upper surface of the inoculated leaves after 8 to 10 days (Plate XIX, fig. 2). Four to five days later, aecidia were produced on the under surfaces (Plate XIX, fig. 3). Aecidiospores were used to inoculate wheat seedlings. Uredo pustules were produced on the seedling leaves of Hard Federation wheat in 12 days. By means of uredospores the rust is being kept in culture for study.

Aecidiospore measurements were made of 50 spores shaken from an infected barberry leaf. The arithmetic mean was $19.1 \mu \times 16.7 \mu$ and the range from $24.2 \mu \times 18.6 \mu$ to $14.9 \mu \times 14.9 \mu$. Grove⁽¹⁰⁾ gives the measurement as varying from 14 to 26 μ in diameter. There is therefore no notable difference in the size of the Australian aecidiospores.

Sections were cut through infected leaves (Plate XIX, figs 4 and 5). A comparison of these slides with others of similar material prepared in England again fails to reveal any differences of note.

General Discussion.

The results obtained show that it is not true that *Puccinia graminis* as it occurs on wheat in Australia has lost

the power of producing the aecidial stage on the barberry. This may be true for teleutospores kept in hot dry districts where they lose their power to germinate. But in some cases at least the complete life-history of the fungus can be gone through under Australian conditions. Further work is planned to try and ascertain the conditions which are requisite.

The infections here recorded were obtained under extremely artificial conditions. But careful observations of barberry bushes growing in the cooler areas of the State should be made to determine whether infection may not also take place under natural conditions.

European and American observations have shown that the aecidial stage is of great importance in starting the early rust attack of cereals. Further, recent work of Dr. E. C. Stakman and his co-workers at Minnesota, U.S.A., has led him to believe that the aecidial stage on the barberry may be very important in multiplying the number of biologic forms of the rust. His theory is that infection at a spot of a barberry leaf may be brought about by sporidia of two different biological forms. Each gives rise to a uninucleate mycelium. It is possible that the two mycelia may intermingle. In the derivation of the binucleate aecidiospores at the base of an aecidium, one nucleus may be contributed from each mycelium. Such an aecidiospore, having a different nuclear constitution to that of aecidiospores of either of the two biologic forms, may be expected to behave differently, and probably to constitute a new biologic form.

It has already been stated that no species of *Berberis* are native to Australia but that barberry plants have been distributed throughout the country. In view of the proof that infection may be brought about, this distribution should cease. It is true that the positive results were

obtained under artificial conditions, but it is by no means impossible for the requisite conditions for infection to occur in Nature.

A further reason for urging that the barberry should be restricted is that American fodder is sometimes imported into Australia. If teleutospores of *Puccinia graminis* should be present on it and the sporidia find their way on to a barberry, the resultant infection might lead to the spread in Australia of a virulent American biologic form of the rust.

The greatest danger is present in the cool districts. It is difficult to understand why the place of the barberry should not be filled by one or other of our many harmless native shrubs. If this be not desired, then at least the distribution of barberries should be restricted to *Berberis Thunbergii*, which is immune to the rust.

Summary.

1. Under set conditions for inoculation, two plants of *Berberis vulgaris* were proved to be susceptible to sporidia of *Puccinia graminis* obtained from rusty British wheat.

2. Inoculations of the same plants under similar conditions with viable teleutospores obtained from Glen Innes, New South Wales, gave numerous infections.

3. Spermagonia and aecidia were produced, and from the latter aecidiospores were used to reinfect wheat.

4. The aecidial stage produced by the Australian rust shows no marked differences to the British rust.

5. The distribution of barberry plants should be discontinued.

Literature Cited.

- (1) 1918—STAKMAN, E. C. "The Black Stem Rust and the Barberry." Yearbook of U. S. Department of Agriculture, p. 4.

- (2) 1906—McALPINE, D. "Rusts of Australia," p. 64 *et seq.*
- (3) 1865—DEBARY, A. Neue Untersuchungen ueber die Uredineen, ins besondere die Entwicklung der *Puccinia graminis* und den Zusammenhang derselben mit *Aecidium berberidis*. Monatsber. K. Acad. d. Wiss., p. 25.
- (4) 1915—LIND, J. "Effect of Destruction of the Barberry (*Berberis vulgaris*) on the Common Rust of Wheat (*Puccinia graminis*) in Denmark. Inter. Rev. Sci. Prac. Agr. Year VII, No. 3, p. 442.
- (5) 1892—COBB, N. A. "Contributions to a Knowledge of Australian Rusts." Agr. Gaz. N.S.W., Vol. III, p. 57.
- (6) 1918—BUTLER, E. J. "Fungi and Disease in Plants," pp. 154 and 159.
- (7) 1919—LEVINE, M. N. "The Epidemiology of Cereal Rusts." U.S. Dept. Agr. Bureau of Plant Industry (Mimeog) p. 10.
- (8) 1904—FISCHER, Ed. "Die Uredineen der Schweiz." Beitrage zur Kryptogamenflora der Schweiz. (Heft II.)
- (9) 1898—PLOWRIGHT, C. B. "Barberry and Wheat Mildew." Gardeners' Chronicle, Vol. XXIII, p. 45.
- (10) 1913—GROVE, W. B. "British Rust Fungi," p. 250.

Explanation of Plate.

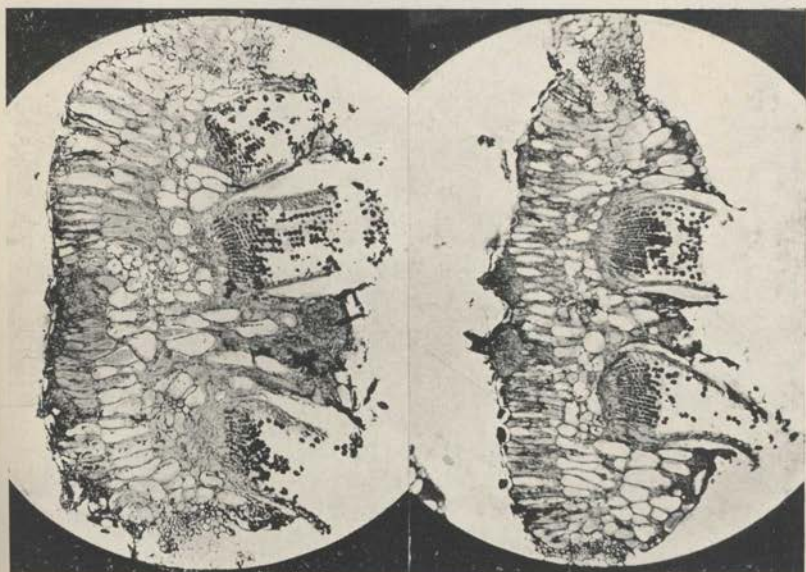
Photographs 2 and 3 and photomicrographs 4 and 5 by H. Gordon Gooch.

1. A plant of *Berberis vulgaris* with a young shoot enclosed in a glass cylinder during inoculation.

2. Showing spermagonia on the upper surface of a barberry leaf infected with sporidia of an Australian form of *Puccinia graminis* on wheat. $\times 2.5$.

3. Aecidia on lower surface of a barberry leaf infected with Australian rust. $\times 1.4$.

4 and 5. Sections through infected leaves showing spermagonia and aecidia. Fixed in Flemming's Solution and stained with Orange G. and Gentian Violet. $\times 60$.



4.

5.



1.



3.

2.

*(Reprinted from the Journal and Proceedings of the Royal
Society of New South Wales, Vol. LXI.)*

STUDIES IN THE INHERITANCE OF RESISTANCE
TO LEAF RUST, *Puccinia anomala* Rostr.,
IN CROSSES OF BARLEY. I.

W. L. WATERHOUSE, M.C., B.Sc.

The University of Sydney.

(With Plates IV.-V.)

(Read before the Royal Society of New South Wales, Aug. 3, 1927.)

Issued November 28th, 1927.

RESISTANCE TO LEAF RUST.

STUDIES IN THE INHERITANCE OF RESISTANCE
TO LEAF RUST, *PUCCINIA ANOMALA* ROSTR.,
IN CROSSES OF BARLEY. I.

W. L. WATERHOUSE. M.C., B.Sc.

The University of Sydney.

(With Plates IV.-V.)

(Read before the Royal Society of New South Wales, Aug. 3, 1927.)

INTRODUCTION.

Cereal rusts cause very heavy crop losses, and from the earliest days have received considerable attention. The most promising method of reducing losses is by breeding rust-resistant varieties. An understanding of the inheritance of resistance to rust thus becomes important.

The first recorded genetical study of the inheritance of resistance to disease was made by Biffen (6, 7, 8 & 9). He found that the susceptibility of certain wheats to yellow stripe rust (*Puccinia glumarum* Erikss) behaved as a simple Mendelian dominant character; the results showed a ratio of 3 susceptible plants to 1 resistant in the F₂ generation. Nilsson-Ehle (29, 30) working with other wheat varieties infected by this rust, obtained results which were not so simple, but explainable on the basis of multiple factors. Armstrong (4) carried out investigations with this same rust and confirmed Biffen's results.

The most extensive studies of rust resistance deal with stem rust of cereals and grasses caused by *P. graminis* Pers. Results have been reported by Hayes, Parker and Kurtzweil (19), Waldron (36), Puttick (33), Aamodt (1, 2, 3), Melchers and Parker (27, 28), Hayes and Stakman (20), Garber (13, 14), Griffie (15), Hayes and

Aamodt (21), Harrington and Aamodt (17), Barker and Hayes (5), Harrington (18), Hayes, Stakman and Aamodt (23), Clark (10), Dietz (12) and Hynes (24). The results are very varied. This is to be expected where such an extremely specialised pathogen and such a wide group of host plants are concerned. A general discussion is withheld until later in the paper.

The inheritance of resistance to leaf rust of wheat, *P. triticina* Erikss., has been studied by Mains, Leighty and Johnston (26).

Parker (31, 32) and Davies and Jones (11) have studied the inheritance of resistance in oats to crown rust, *P. coronata* Cda.

The results of studies on the inheritance of resistance to leaf rust of rye, *P. dispersa* Erikss. and Henn. have been reported by Mains and Leighty (25).

Apparently no study has yet been reported of the inheritance of resistance to the leaf rust of barley, *P. anomala* Rostr. Frequently this fungus heavily attacks the commercial varieties of barley which are grown for green feed in the coastal regions of New South Wales (Plate IV.). An investigation was therefore undertaken, of which only the results obtained in the glass house are herein reported. Barley is also susceptible to stem rust, *P. graminis tritici* E. and H. Work dealing with this rust will be reported later in another paper. The studies herein described are divided into two sections (1) varietal tests and (2) cross-breeding results.

MATERIALS AND METHODS.

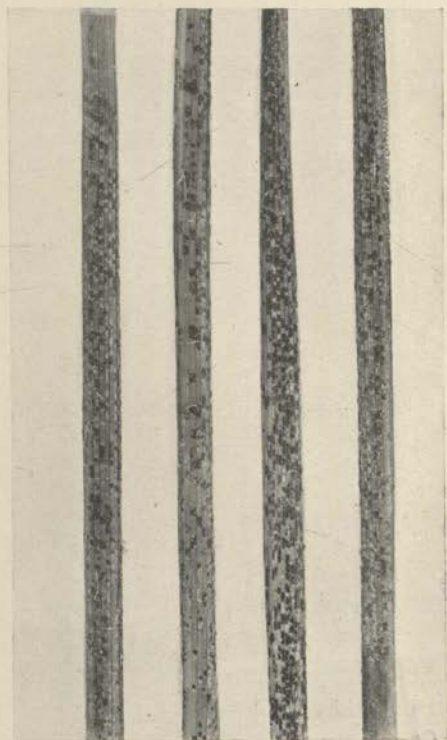
Several collections of *P. anomala* occurring on barley crops were made. Cultural tests in the glass house using some of the commercial varieties of barley which are usually grown gave no indication of physiological specialisation of

the fungus. But it is not improbable that further tests with a wider range of host varieties may reveal the presence of more than one physiologic form of the rust. The glass house studies herein reported deal with only one physiologic form. The culture used was obtained from material collected by Mr. G. S. Gordon at the Werribee Research Station, Victoria. No other culture of the rust was grown in the glass house throughout the course of these investigations.

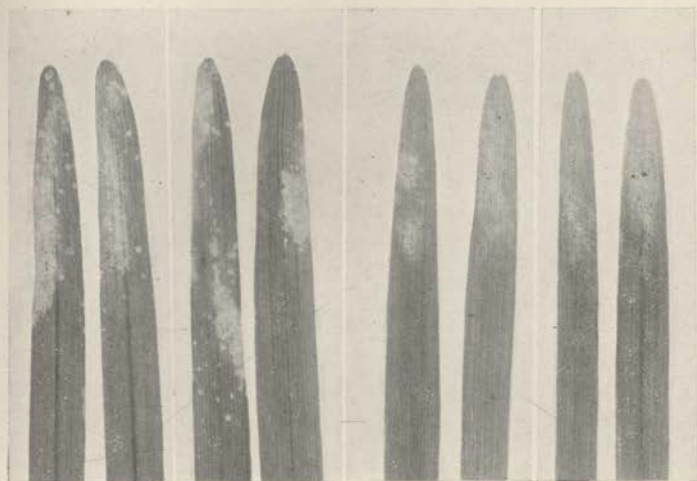
Barley crops in the coastal areas of New South Wales have always, when examined, proved to be more or less heavily infected by the leaf rust as they approach maturity. Although the attack in some cases has been severe and the loss considerable, there is no recorded case of the crop having been completely ruined. But if similar varieties to "Cape" and "Skinless", resistant to the leaf rust could be obtained, a good work would be accomplished. A search was therefore commenced for a variety or varieties resistant to the rust.

Grain of as many varieties as possible was obtained from different sources. About a dozen grains of each were sown in pots and the first leaf of the seedlings used in the tests. Where any doubt existed about the result, sowings were replicated until definiteness was reached.

The tests were made in the usual way by moistening the leaf with distilled water and then introducing to the moistened area a quantity of the inoculum upon a sterile flat needle. When first used, the strain of rust had been subcultured in the glasshouse for at least 50 uredospore generations on a susceptible variety of barley. After inoculation the pots were incubated in a saturated atmosphere at ordinary glasshouse temperatures for 48 hours. Preliminary tests showed that incubation for 24 hours gave results essentially similar to those obtained

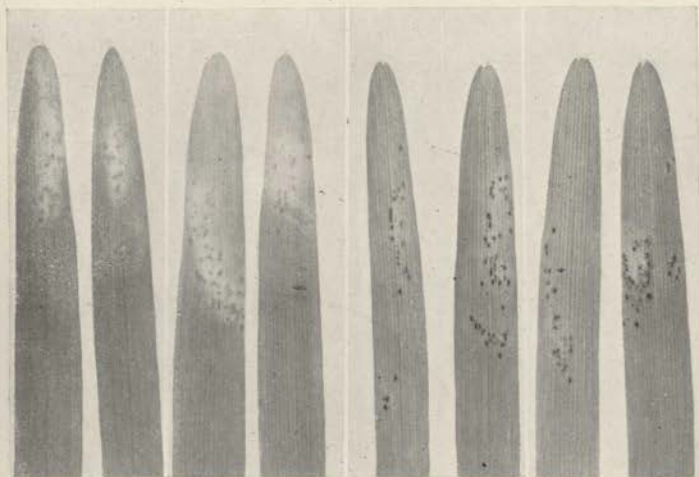


Four stems of "Cape" barley from a naturally infected crop growing at Hawkesbury Agricultural College, N.S.W., showing the teleutospore stage of *Puccinia anomala* Rostr. attacking the leaf sheath.



A

B



C

D

Representative barley seedling leaves illustrating 4 classes of reaction to the leaf rust. In each case two leaves show the upper and two show the lower surfaces. All were photographed 12 days after inoculation.

A, Highly resistant, styled the "0" reaction.

B, Very resistant, styled the "1" reaction.

C, Moderately resistant, styled the "2" reaction.

D, Very susceptible, styled the "4" reaction.

from the longer incubation. The longer period of 48 hours was adopted in order to bring this barley leaf rust work into conformity with the usual glasshouse routine, which deals mainly with stem rust; the best result with this latter rust is given by 48 hours incubation. After removal from the incubation chamber, the pots were placed in well lighted positions on the glass house benches to allow the rust to develop. Light and temperature variations markedly affected the rate of development. Tests using the same rust on the same varieties in cool dull winter weather and in bright hot summer showed that normal development of pustules took place in 14 days under the former, as compared with 8 days under the latter conditions.

Notes on the reactions given by the plants were taken when infection was best developed on the susceptible control plants. As already stated, this period varied from 8 to 14 days. The following classes of rust reaction were recognised, depending upon the type of infection produced on the leaves:—

Table 1.—Leaf rust reactions used in the determination of resistance or susceptibility.

Notation.	Class of Reaction.	Type of Reaction.
0.	Highly resistant	No uredosori produced; flecks of a necrotic nature present.
1.	Very resistant	Uredosori few in number, small, and always produced in the centre of necrotic spots; frequently many of the hypersensitive areas show no sori.
2.	Moderately resistant..	Uredosori fairly abundant, of fair size and always produced in necrotic or very chlorotic areas.
3.	Moderately susceptible.	Uredosori fairly abundant, of moderate size and produced in areas which show no necrosis, although sometimes slight chlorosis occurs.

4. Very susceptible Uredosori abundant, large and produced without necrosis or chlorosis. Occasionally a chlorotic ring occurs round the pustule.

Classes 0, 1, 2 and 4 are illustrated in Plate V.

The strict differentiation between certain of these classes was difficult, e.g., between "1" and "2", and especially between "3" and "4". But the distinction between the resistant and the susceptible classes could always be made. On account of this difficulty, and because results in the first of the crosses studied (A. Cape x Manchuria) indicated that a change from "0" to a "1" reaction was not of genetic significance, in a good deal of the work the notes were taken to record either resistant or susceptible reactions, without specifying the particular class to which the resistant individuals belonged. It appears that a change from a "0" to a "1", from a "1" to a "2", or from a "3" to a "4" class of reaction may be brought about in some cases by simply giving better environmental conditions of light and temperature for development of the rust. But in other instances, under extremely varied conditions, certain barley types showed striking necrosis (i.e., a "0" reaction) and only this reaction. Throughout the work the class of reaction referred to by Stakman and Levine (34) as the "heterozygous or X class" was not met.

EXPERIMENTAL RESULTS.

1. VARIETIES.

The varieties subjected to the glasshouse tests are here grouped according to the classification of Harlan (16). The origin of the seed is stated in each case. Those indicated by a Cereal Investigation Number (C.I. No.) were mostly supplied by Principal E. A. Southee of Hawkesbury Agricultural College, who obtained them from Dr. H. V. Harlan of U.S.A. Drs. H. K. Hayes and J. J. Christensen supplied seed of a number from

Minnesota. Mr. G. S. Gordon of the Werrabee Research Station, Victoria, contributed a number. The greatest number, however, came from Mr. J. T. Pridham of the Cowra Experiment Farm, who has always been most generous with his material.

As already stated, in these tests about a dozen plants of each variety were used. Where any doubt existed as to the class of reaction, further sowings were made and the tests repeated until definiteness was reached.

The results are set out in the following tables:—

Table 2.—Results with varieties of *Hordeum vulgare* L.
(a) Susceptible. Reaction = "4".

	Variety.	Source.
C.I.2202	<i>H.v. nigrum typica</i> (Black Russian)	U.S.A.
C.I.2204	<i>H.v. atrum typica</i>	"
C.I.2206	<i>H.v. duplinigrum typica</i>	"
C.I.2207	<i>H.v. trifurcatum typica</i> (Nepaul)	"
C.I. 908	<i>H.v. pallidum</i> (Luth)	"
C.I. 991	<i>H.v. coeleste</i>	"
C.I.2238	<i>H.v. nigrum leiorrhynchum</i> (Lion)	"
C.I.2256	<i>H.v. pallidum rikotense</i>	"
C.I.2269	<i>H.v. pallidum eurylepis</i>	"
Sel. C 81	Manchuria	Minnesota
Sel. C225	Manchuria	"
	Trabut	Cowra, N.S.W.
	Cape	"
	Skinless	"
	Reka	"
	Shorthead	"
	Chilian	"
	Albert	"
	Mariout	California
	Four Thousand	"
	No. 78	Cowra, N.S.W.
	Gold	"
	Hero	"
	White Hulless	"
	Meloy	Cowra, N.S.W.
	Pearl	"
	Chedret	"
	Coutsopodi	"
	Janina	"
	Larissa	"
	Kaylaria	"
	Senes	"
	Salonika	"

	Variety.	Source.
	Zea	Cowra, N.S.W.
	Sahara 3763	"
	" 3764	"
	" 3765	"
	" 3766	"
	" 3767	"
	" 3768	"
	" 3769	"
	" 3771	"
	Tennessee Winter	Werribee, Victoria.
	Himalaya	"
	Orzo nuda putignans	"
	Orzo maraina	"
	Gatami	"
	Tunis	"
	Sea of Azov	"
	White Hulless	"
	Roseworthy Oregon	"
	Wisconsin Pedigree	"
	Squarehead	"
	Odessa	"
(b) Moderately resistant. Reaction = "2".		
	Californian Feed	Cowra, N.S.W.
	Psaknon	"
	Locride	"
	Sahara 3770	"
	Coast	"
	Marionet	Werribee, Victoria.
	Orge Fourragère	"
	Minn. I 16.13 Lion	Minnesota, U.S.A.
	Minn. II 20.10 B	"
	Minn. II 21.14	"
(c) Resistant. Reaction = "0" or "1".		
C.I.2208	<i>H.v. aethiops typica</i>	U.S.A.
C.I.2290	<i>H.v. horsfordianum typica</i> (Virginia Hooded)	"
	Minn. II 21.15	Minnesota
	Smooth Awn x Manchuria	
	Minn. II 21.17	"
	Smooth Awn x Manchuria	
	Minn. II 21.18	"
	Smooth Awn x Manchuria	
	Sel. C163 Manchuria	"
	Sel. C168 Manchuria	"
	Minn. 184 Manchuria	"
	Manchuria	"
	O.A.C. 21	Cowra, N.S.W.
	No. 22	"
	No. 305	"
	Colsess	"
	Orge 4th	Werribee, Victoria
	Orge 14J	"

Table 3.—Results with varieties of *Hordeum intermedium* Kcke. Susceptible. Reaction = "4".

	Variety.	Source.
C.I.2209	<i>H.i. haxtoni typica</i>	U.S.A.
C.I.2210	<i>H.i. mortoni typica</i>	"
C.I.2213	<i>H.i. nudihaxtoni typica</i>	"
C.I.2214	<i>H.i. nudimortoni</i>	"
C.I.2215	<i>H.i. cornutum typica</i>	"
C.I.2237	<i>H.i. haxtoni tonsum</i>	"
C.I.2254	<i>H.i. haxtoni tonsum</i>	"
C.I.2280	<i>H.i. haxtoni tonsum</i> (Arlington Awnless)	"
	"Intermediate"	Cowra, N.S.W.

Table 4.—Results with varieties of *Hordeum distichon* L. (a) Susceptible. Reaction = "4".

	Variety.	Source.
C.I.2219	<i>H.dis. angustispicatum typica</i>	U.S.A.
C.I.2221	<i>H.dis. nudum typica</i> (McEwan's)	"
C.I.2222	<i>H.dis. nigrinudum</i>	"
C.I.2223	<i>H.dis. laxum typica</i>	"
C.I.2224	<i>H.dis. nigrilaxum</i>	"
C.I.2250	<i>H.dis. nudum ianthinum</i>	"
	Princess	Cowra, N.S.W.
	Goldthorpe	"
	Primus	"
	Standwell	"
	Kinver	"
	Pryor	"
	Cowra 27	"
	Volga	"
	Golden Grain	"
	Gisborne	Werribee, Victoria
	<i>H. distichon nutans</i>	"
	<i>H. distichon erectum</i>	"
	Hanchen	"
	Duckbill	"
	Burton's Malting	"
	Binder	"
	Garton's Regenerated Malster	"
	Archer	"

(b) Resistant. Reaction = "0".

C.I.2220	<i>H.dis. rimpaui typica</i>	U.S.A.
----------	---	--------

Table 5.—Results with varieties of *Hordeum deficiens* Steud. Susceptible. Reaction = "4".

	Variety.	Source.
C.I.2225	<i>H.def. deficiens typica</i>	U.S.A.
C.I.2226	<i>H.def. steudelii typica</i>	"
C.I.2228	<i>H.def. tridax typica</i>	"
C.I.2229	<i>H.def. nudificiens typica</i>	"

In addition to these tests of varieties of the cultivated barleys, tests were made of two other species of *Hordeum* which were available. One of these was "Wild Barley", *Hordeum spontaneum* Koch., obtained from Dr. H. V. Harlan in the U.S.A. It proved to be completely susceptible to the rust, giving the typical "4" reaction. The other was "Barley Grass", *Hordeum murinum* L., which was resistant, giving a sharp "0" reaction.

An examination of these results shows that of the cultivated varieties tested, no one belonging to *H. intermedium* or *H. deficiens* proved resistant, and only 1 of *H. distichon*. Of *H. vulgare*, 15 varieties were found to be strongly resistant, and 10 varieties were moderately resistant.

Comparison of varietal resistance to Leaf Rust and Spot Blotch.

An interesting point is that some of the strains of "Manchuria" are susceptible to leaf rust, whilst others are resistant. This is in agreement with the results of the study of the resistance of these barleys to attack of *Helminthosporium sativum* Pammel, King, and Bakke, as reported by Hayes et al, (22). This organism causes Spot Blotch of barley. A direct comparison of the two results is possible in some cases, thanks to Drs. Hayes and Christensen who forwarded grain of some of their pure lines. In the following table are shown the reactions of these barleys to the two pathogens. The "H. No." is that showing the reaction to *Helminthosporium* found by Hayes et al. (22). A high figure, of which 30 is the maximum, denotes very high resistance, whilst a low figure, of which 3 is the minimum, denotes complete susceptibility.

Table 6.—Comparison of the resistance of certain Barleys to *Puccinia anomala* and *Helminthosporium sativum*.

Variety.	Reaction to <i>P. anomala</i> .	Reaction to <i>H. sativum</i> .
Manchuria C81... ..	"4"=Susceptible	H. No. = 16.0
Manchuria C225... ..	"4"= do.	H. No. = 7.0
Minn. I. 16.13 Lion.	"2"=Moderately resistant	H. No. = 10.0
Minn. II. 20.10 B ..	"2"= do. do.	H. No. = 21.5
Minn. II. 21.14... ..	"2"= do. do.	H. No. = 23.0
Minn. II. 21.15... ..	"0"=Very resistant	H. No. = 20.0
Minn. II. 21.17... ..	"0"= do.	H. No. = 20.0
Minn. II. 21.18... ..	"0"= do.	H. No. = 20.5
Manchuria C163 ..	"0" do.	H. No. = 21.0
Manchuria C168 ..	"0" do.	H. No. = 19.5
Manchuria Minn. 184	"0" do.	H. No. = 21.0

It will be noted that in general, varieties resistant to leaf rust are also resistant to *Helminthosporium sativum*, and those which are susceptible to rust are also susceptible to *Helminthosporium sativum*. An exception is Manchuria C81. The correlation is slight also in the case of Minn. I. 16.13 Lion. It is difficult to suggest any common morphological basis for the resistance to these two pathogens, and no work has been undertaken relative to physiological resistance.

The varietal tests show that there are available several barley varieties which are resistant to the leaf rust. But as so often happens, these resistant varieties in themselves have certain failings in N.S.W. from an agronomic point of view, as for example, late maturity, low yield and poor stooling capacity. Hence cross-breeding work was undertaken involving these resistant varieties and good agronomic sorts which are susceptible to the rust.

2. CROSS-BREEDING RESULTS.

In 1923 and in each succeeding year a number of crosses were made at the University of Sydney between susceptible commercial varieties like Cape, Skinless and Kinver, and certain of the varieties which have been shown to be resistant, e.g., Manchuria, *H. distichon rimpau* *typica* and

Virginia Hooded. These crosses were tested in the F_1 , F_2 , and F_3 generations. During all the work, frequent sowings were made of each of the parents concerned, and these seedlings tested for resistance as controls alongside the cross-bred seedlings. The F_1 grains were sown in pots, and after being tested the seedlings were transplanted to open ground and grown to maturity. In the F_2 generation the same procedure was followed. In many cases additional F_2 grain was sown directly in open ground, and these plants harvested at maturity without having been tested as F_2 individuals.

At harvest time it was found convenient to pluck heads of individual plants into paper bags which were then labelled. These were later strung in order on wire. Loss of grain from shattering of the heads was thus avoided and thorough drying-out facilitated.

A. *Cape* x *Manchuria*.

F_1 Results.

From the cross, 17 grains were secured. Sixteen of these F_1 seedlings gave a "0" reaction, and one of them gave a "4" reaction. Control inoculations of the parents gave a "4" reaction on "Cape" and a "0" reaction on "Manchuria". It thus seemed that 16 were true crosses and that the one susceptible plant was the result of accidental self-pollination. Each F_1 plant at maturity was harvested separately. The susceptible plant did not show any marked agronomic differences from the others. Ninety seedlings were grown from it. All proved susceptible, thus confirming the idea that accidental pollination had occurred in this instance.

Rejecting this result, therefore, it is clear that resistance is dominant. Moreover the resistance of the F_1 plant is similar to the resistance of the resistant parent. Intermediacy of reaction is not shown.

F₂ Results.

Grain from two of the F₁ plants was sown in boxes and each resultant F₂ plant inoculated with the rust. After notes had been taken, the seedlings were transplanted to open beds, each under its proper number which was retained throughout the F₃ tests. Grain from the other F₁ individuals was sown directly in open ground and the individual F₂ plants harvested at maturity. These plants, therefore, were not tested in the F₂ generation.

The results of the F₂ tests are shown in the following table:—

Table 7.—Reactions of plants tested in the F₂ generation.

Plant Breeding Number.	Number of Resistant Plants.		Number of Susceptible Plants.
	Reaction = "O".	Reaction = "1".	Reaction = "4".
II. 23.1.1	79	21	33
II. 23.1.2	55	18	21
Totals	134	39	54

From this table it will be seen that the resistant plants were divided into two classes, viz., "0" and "1", on the basis of the rust reaction exhibited. As already pointed out in connection with the varietal tests, a change from one class to the other may sometimes be brought about by an alteration of the environmental conditions. The control pots of the resistant parent (Manchuria) gave a "0" reaction usually, but occasionally the reaction was "1". It was thought that the "1" reaction of the F₂ segregates might be an index to the heterozygosity of these individuals. In other words, these plants might be the intermediates. But in the F₃ tests, some of the families derived from F₂ plants which gave a "0" reaction, proved to be homozygous for resistance, whilst others were heterozygous for this character. Exactly the same thing occurred with families derived from F₂ plants showing a "1" reaction. A close

scrutiny of the figures showing the number of individuals producing the "0" and "1" reactions in a number of the families, failed to indicate any genetic significance. Clearly then, intermediacy of reaction is not shown in this cross.

The results of these F_2 tests may therefore be summarised as 173 resistant and 54 susceptible seedlings. This is an approximation to a 3:1 ratio. On the basis of one genetic factor difference between resistance and susceptibility, the expectancy would be 170:57. The deviation is 3 and the probable error on the basis of 227 individuals is 4.4.*

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{3}{4.4} = 0.68.$$

At maturity the F_2 plants showed a certain amount of diversity in regard to time of maturing and stooling capacity, but otherwise morphological segregation was not marked.

F_3 Results.

The series of 227 tested F_2 seedlings was transplanted into open beds and grown to maturity. Attack by larvae of the "army worm" shortly before harvest time seriously reduced the yield and completely destroyed some of the plants. For this reason there are included in these results some of the F_3 families which had not been previously tested as F_2 individuals. Such families were purely random selections. Individual F_2 plants were harvested separately. For the F_3 tests a number of grains of each was sown. Of some families only a few grains were available, but results are included only where at least 20 grains were sown. In some cases more than 50 grains were sown. Throughout the tests, control pots of each parent were sown and tested, giving the typical "4" reaction for "Cape", and "0" for "Manchuria."

* Probable errors of the Mendelian ratios were obtained from the tables of probable errors from the Department of Plant Breeding, Cornell University.

The families tested fell into three groups. One group was homozygous for resistance, the second heterozygous for resistance, and the third homozygous for susceptibility. There were 66 homozygous resistant families, 117 heterozygous resistant families and 52 homozygous susceptible families. The average number of tested individuals in each family was slightly more than 25. In all, 5986 plants came under test. The 66 homozygous resistant families comprised 1688 individuals, the 117 heterozygous resistant families comprised 2218 resistant and 752 susceptible individuals, and the 52 homozygous susceptible families comprised 1328 individuals. In every case where an F_2 test had been made, the last-named class came from susceptible F_2 parents. The homozygous and heterozygous resistant classes were derived from resistant F_2 parents.

These classes approximate to a 1:2:1 ratio, which is to be expected on a one-factor hypothesis. This is supported by an examination of the totals given by the heterozygous resistant families. The observed ratio is 2218 resistant: 752 susceptible plants. The expectancy on the basis of 2970 individuals is 2226 : 744. The deviation on the basis of a single factor difference is 8 individuals. The probable error is 18.14 individuals.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{8}{18.14} = 0.44.$$

B. Manchuria x *Skinless*.

F_1 Result.

From this cross 8 grains were obtained. The F_1 seedlings all gave a "0" reaction, whilst the parents gave respectively "0" and "4". The F_1 plants were somewhat bearded and produced hulled grain.

F_2 Result.

A series of 210 F_2 seedlings was tested, and afterwards these were transplanted to open ground and grown to

maturity. The F_2 test gave 159 resistant and 51 susceptible plants. The particular class of resistant reaction was not noted in individual cases. This result closely approximates to a 3 : 1 ratio. The expectancy on the basis of a single factor difference is 157 : 53. The deviation is 2 and the probable error for 210 individuals is 4.23 individuals.

$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2}{4.23} = 0.47$. Test pots of the parent varieties gave the usual reactions. In the open beds the F_2 generation was somewhat attacked by the army worm and its numbers depleted at harvest time.

F_3 Results.

For the F_3 tests, 140 families were used. Prior to sowing, notes were taken on head characters of the F_2 plants, dealing with beardedness or hoodedness, and with hulled or naked grain. "Manchuria" is bearded with hulled grain, and "Skinless" is hooded with naked grain. In recording notes on the F_2 plants, the true naked-grained and the true bearded classes were carefully determined. In the "hulled grain" class were included intermediate types, i.e., those showing a tendency to shell, but not completely naked. Similarly there are included in the "hooded" class intermediate types showing slight beard. The results showed 100 hooded : 40 bearded, which approximates to a 3 : 1 ratio. There were 104 hulled : 36 naked-grained plants, a still closer approximation to a 3 : 1 ratio. Unfortunately it has not been possible to grow these families on to maturity to obtain confirmation of this F_2 analysis for morphological characters.

The F_3 tests showed that there was a group of 33 families, each homozygous for resistance. There was a second group of 60 families, each heterozygous for resistance. A third group of 46 families was homozygous for susceptibility. On an average there were about 23

tested individuals in each of the families. The total number of plants tested was 3359. The 33 homozygous resistant families comprised 753 individuals, the 60 heterozygous resistant families comprised 1126 resistant and 376 susceptible plants, and the 46 homozygous susceptible families comprised 1104 individuals. It is interesting to compare the observed number of the individuals in the heterozygous families with the expectancy. Assuming a single genetic factor difference between resistance and susceptibility, the expectancy on the basis of 1502 individuals is 1126.5 resistant to 375.5 susceptible plants. This is an extraordinarily close fit.

Combining these rust results with those for the morphological characters, the following is obtained:—

Table 8.—Inheritance of three Mendelian characters which are seemingly inherited independently.

Phenotypes.	Expected.	Observed.
Resistant, hooded, hulled	59.1	51
Susceptible, hooded, hulled	19.7	27
Resistant, bearded, hulled	19.7	18
Resistant, hooded, naked	19.7	13
Susceptible, bearded, hulled	6.6	9
Resistant, bearded, naked	6.6	11
Susceptible, hooded, naked	6.6	9
Susceptible, bearded, naked	2.2	2
	<hr/> 140.2	<hr/> 140
	<hr/>	<hr/>

It is realised that the number of individuals here dealt with is small, but the results point to the characters being independently inherited.

C. Manchuria x *Kinver*.

F₁ Result.

From the cross 16 grains were obtained. The F₁ seedlings were tested alongside the parent varieties, with the result that all showed a "0" reaction. The F₁ plants at maturity were bearded two-rowed plants having hulled grain.

F₂ Result.

In the F₂ generation a series of 194 seedlings was tested. The result was that 147 were resistant and 47 susceptible. This is an approximation to a 3 : 1 ratio. On the basis of a single factor difference the expectancy is 145 : 49. The deviation is 2 and the probable error 4.07.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2}{4.07} = 0.49.$$

After being tested, these F₂ seedlings were transplanted to open beds and grown to maturity. At harvest it was found that the yield in many cases was very poor, so that only a small quantity of grain from some families was available for the F₃ work.

F₃ Result.

In the F₃ tests, 97 families were used. There proved to be a group of 29 homozygous resistant families, a group of 48 heterozygous resistant families, and a group of 20 homozygous susceptible families. The average number of individuals in each of the tested families was rather more than 16. The total number of plants tested was 1605. The 29 homozygous resistant families comprised 465 individuals, the 48 heterozygous resistant families comprised 611 resistant and 208 susceptible plants, and the 20 homozygous susceptible families comprised 321 plants. Again there is the approximation to a 1 : 2 : 1 ratio which is to be expected on a single genetic factor basis. Examination of the heterozygous families confirms this. They comprise 611 resistant : 208 susceptible plants. The expectancy is 614 resistant : 205 susceptible. The deviation is 3 and the probable error 8.36 individuals.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{3}{8.36} = 0.36.$$

D. Virginia Hooded x Cape.

Four grains were obtained from the cross. The F_1 reaction was "0". In the F_2 test, 171 seedlings were tested and gave a ratio of 121 resistant : 50 susceptible plants. On the basis of a single genetic factor difference the expectancy is 128 : 43. The deviation is 7 and the probable error is 3.82 individuals.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{7}{3.82} = 1.83.$$

For the F_3 tests there were available 99 families. These tests showed that there were three groups. There was a group of 25 homozygous resistant families, a group of 50 heterozygous resistant families, and a group of 24 homozygous susceptible families. The average number of individuals in the families tested was about 22. There were 2188 plants in these tests. The 25 homozygous resistant families comprised 518 plants, the 50 heterozygous resistant families comprised 858 resistant and 284 susceptible plants, and the 24 homozygous susceptible families comprised 528 plants. The close approximation to a 1 : 2 : 1 ratio is evident. The heterozygous families show a ratio of 858 resistant and 284 susceptible plants. The expectancy on a single factor difference is 856 : 286. The deviation is 2 and the probable error is 11.59 individuals.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2}{11.59} = 0.17.$$

E. Cape x Hordeum distichon rimpauvica typica.

Three grains were obtained from the cross. The F_1 reaction was "0". In the F_2 test 165 individuals were tested, giving a ratio of 123 resistant : 42 susceptible plants. The expectancy on the basis of a single genetic factor difference is 124 : 41. The deviation is 1 and the probable error for 165 individuals is 3.75.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{1}{3.75} = 0.267.$$

For the F_3 tests there were available 145 families. They fell into three groups. There was a group of 42 homozygous resistant families, a group of 69 heterozygous resistant families and a group of 34 homozygous susceptible families. The average number of individuals in the tested families was about 20. The total number of plants tested was 2860. The 42 homozygous resistant families comprised 828 individuals, the 69 heterozygous families comprised 1046 resistant and 357 susceptible plants, and the 34 homozygous susceptible families comprised 629 individuals all of which were susceptible. The heterozygous families show a ratio of 1046 resistant : 357 susceptible individuals. On the basis of a single genetic factor difference the expectancy is 1052 : 351. The deviation is 6 individuals and the probable error is 11 individuals.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{6}{11} = 0.55.$$

SUMMATION OF RESULTS OF THE CROSSES.

If it is assumed that the resistance in each of the cases reported is due to the one factor, it becomes interesting to add the results of the several crosses.

In the F_2 tests of the 5 crosses, 975 plants were used. The results are set out in the following table:—

Table 9.—Summation of the F_2 results in all 5 crosses.

Cross Number.	Parents.	Number of Individuals in Tested F_2 Generations.	
		Resistant.	Susceptible.
II. 23.1	Cape x Manchuria	181	54
II. 23.6	Manchuria x Skinless	159	51
II. 23.7	Manchuria x Kinver	147	47
II. 23.8	Virginia Hooded x Cape . . .	121	50
II. 23.9	Cape x <i>H. dist. rimpaui typica</i>	123	42
	Totals	731	244

On the basis of a single genetic factor difference, the result shows a deviation of less than 1 individual from the expectancy where 975 individuals are concerned.

In the F_3 tests of the 5 crosses, 715 families were dealt with. These comprised 15998 plants. Thus there was an average of rather more than 22 tested individuals in each of the families. The results are shown in the following table:—

Table 10.—Summation of the results of the tests of the F_3 families in all 5 crosses.

Cross Number.	Parents.	Number of F_3 Families Tested.		
		Homozygous Resistant.	Heterozygous Resistant.	Homozygous Susceptible.
II. 23.1	Cape x Manchuria ..	66	117	52
II. 23.6	Manchuria x Skinless	33	60	46
II. 23.7	Manchuria x Kinver	29	48	20
II. 23.8	Virginia Hooded x Cape	25	50	24
II. 23.9	Cape x <i>H. dist. rimpai typica</i>	42	69	34
	Totals	195	344	176

On the basis of a single factor difference, the expectancy would be 178.75 : 357.5 : 178.75.

Table 11.—Summation of individuals in the F_3 heterozygous families of all 5 crosses.

Cross Number.	Parents.	Resistant.	Susceptible.
II. 23.1	Cape x Manchuria	2231	760
II. 23.6	Manchuria x Skinless	1126	376
II. 23.7	Manchuria x Kinver	611	208
II. 23.8	Virginia Hooded x Cape	858	284
II. 23.9	Cape x <i>H. dist. rimpai typica</i> ..	1046	357
	Totals	5872	1985

On the basis of a single factor difference, the expectancy for 7857 individuals is 5893 : 1964. The deviation is 21 and the probable error is 28 individuals.

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{21}{28} = 0.75.$$

DISCUSSION.

The results herein reported show that resistance to leaf rust of barley (*Puccinia anomala* Rostr.) is inherited in certain varietal crosses in a very definite manner. It is Mendelian in character. Moreover it is dependent upon a single genetic factor which is dominant to the allelomorphic factor for susceptibility. The observed results upon which this generalisation is based show a striking approach to the expectancy.

It is instructive to review briefly the results which have been reported dealing with the genetics of inheritance of resistance to cereal rusts.

In the investigations of inheritance of resistance to Yellow Stripe Rust of wheat, *P. glumarum* E. and H., Biffen (6, 7, 8, & 9) found in the crosses of certain varieties, a simple ratio of 3 susceptible plants : 1 resistant. Resistance in these crosses was due to a single genetic factor which was recessive. It was inherited independently of other observed morphological characters. Armstrong (4) obtained substantially similar results. Nilsson-Ehle (29 & 30) used different parental varieties and did not obtain such clear-cut results. In the F_2 generation he found transgressive segregation and various types intermediate in resistance between the parents. He considered that his results were only to be explained upon a multiple factor hypothesis.

The inheritance of resistance of wheats to Leaf Rust, *P. triticina* Erikss., was studied by Mains, Leighty and Johnston (26). They found some cases in which resistance was dependent upon a single dominant genetic factor. In other cases it was dependent for its expression upon a single main factor difference. In yet others it was demonstrated that more than one genetic factor was concerned.

Many workers have investigated the inheritance of resistance to stem-rust in wheat, *P. graminis tritici* E. and H. Hayes, Parker and Kurtzweil (19) found that resistance to a physiologic form of the rust was dominant in crosses between varieties of *Triticum vulgare* and *T. dicoccum*, whilst it was recessive in crosses between varieties of *T. vulgare* and *T. durum*. In the latter there were strong linkages between rust resistance and "durum" characters, but some resistant plants of the "vulgare" type were obtained. That is to say, crossing-over took place occasionally. Waldron (36) examined a cross between a variety of *Triticum vulgare* and *T. durum*, and was unable to explain his results on the basis of a single factor for resistance. Puttick (33) reported results of the study of the reactions in glasshouse tests of the F₂ generation of a cross between varieties of *T. vulgare* and *T. durum*, using two physiologic forms of the rust to which the parents reacted reciprocally. He found all gradations between complete susceptibility to and immunity from the two forms of the rust. Melchers and Parker (27 & 28) found that a single genetic factor was responsible for resistance to one physiologic form of the rust in crosses between Kanred and Marquis. Aamodt (1, 2 & 3) working with a cross between these same parents, reported that a single genetic factor apparently determined the reaction to several physiologic forms of the rust. Hayes and Aamodt (21) studied a cross between Kota and Marquis, using two physiologic forms of the rust. The results were explainable on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents respectively, each factor being allelomorphic and dominant to the factor for susceptibility. Clark (10) crossed Hard Federation and Kota, and showed that resistance to the rust was recessive. He obtained a

15 : 1 ratio in the F_2 generation, indicating the presence of 2 Mendelian factors for resistance in this cross. In the F_3 generation not one of about 300 resistant F_2 families bred true for resistance, although a number of individual plants did. It was considered that homozygous strains could be obtained in the F_4 generation by selection within the F_3 families. Harrington (18) studied crosses between certain varieties of *T. durum* dealing with resistance to several physiologic forms. He found that several factors were involved and that environmental factors modified the expression of the reaction. Thompson (35) demonstrated that in crosses of Iumillo (resistant) and Marquis (susceptible), a ratio of 13 susceptible : 1 resistant was obtained in the F_2 generation, and that there was very close correlation between "durum" characters and rust resistance. Hayes, Stakman and Aamodt (23) state that the resistance of the "durum" parent can be combined with the characters of the common wheat. In a double cross of (Marquis x Kanred) x (Marquis x Iumillo), the inheritance of 2 types of resistance was studied. A single factor was found to explain the Marquis x Kanred type of immunity, while at least 2 factors were required to explain the Marquis x Iumillo resistance. Hynes (24) studied a cross between Federation (a common wheat) and Khapli (an emmer), obtaining results explainable on a multiple factor hypothesis.

It is not surprising that these reported results dealing with stem rust of wheat should be so varied. The pathogen is very highly specialised. In the U.S.A. more than 40 physiologic forms have been determined, and in Australia the writer has determined several others. In addition to the specialisation of the rust fungus, it must be remembered that the wheats used in the crosses belong in some cases to widely different species of *Triticum*.

The inheritance of resistance to stem rust of oats, *P. graminis avenae* E. and H., was investigated by Garber (13 & 14). He found in crosses of the two susceptible varieties Minota and Victory with White Russian, a resistant parent, that the F_1 plants were as resistant as the resistant parent and that the F_2 generation showed a simple ratio of 3 resistant plants : 1 susceptible. The F_3 generation results confirmed this. There appeared to be no linkage between rust reaction and panicle type. Griffee (15) in similar work obtained results which gave a close approximation to the expectancy on the hypothesis of a single dominant genetic factor for resistance being present. Dietz (12) used 2 physiologic forms of the rust, and found that resistance was dominant and based on a single factor difference in National x White Tartar and in Lincoln x White Tartar. In crosses between 3 genetically different strains of Burt, at least 2 factors,—one of them an inhibitor—were involved. In the crosses Iowa 105 x Green Russian and Ruakura x White Russian, the result in the F_2 was a segregation into 300 resistant plants to 1 susceptible.

Here again there is evidence that resistance does not behave in the same manner in every instance. Clearly there are more factors than one underlying resistance to stem rust of oats.

In a study of rust of Timothy Grass, *P. graminis phleipratensis* E. and H. Stak. and Piem., Barker and Hayes (5) demonstrated that the inheritance of resistance was dependent upon a single dominant genetic factor.

Parker (31 & 32) studied the inheritance of resistance in oats to crown rust, *P. lolii-avenae* Mc.A. He concluded that resistance could hardly be considered as a simple character or as being determined by a single factor difference, but was explainable upon a multiple factor

hypothesis. Davies and Jones (11) studied the inheritance of resistance to this rust in a cross between a selection of Red Rust Proof (resistant) and Scotch Potato (susceptible). They obtained a ratio of 777 resistant and 258 susceptible plants in the F_2 generation. Of the resistant segregates, 117 showed a weakening of resistance as compared with the others, which closely resembled the resistant parent. But the results suggested a single factor basis for inheritance.

The inheritance of resistance of leaf rust of rye, *P. dispersa* E. and H., has received some attention from Mains and Leighty (25). The data were insufficient to justify conclusions as to the type of inheritance, but resistance is believed to be dominant.

From a consideration of the foregoing results, it is clear that the inheritance of resistance to various rusts varies in different crosses. In some cases the resistance is dependent upon a single genetic factor. This may behave as a Mendelian dominant in some crosses, as a recessive in others. In other cases the basis of inheritance is far more complex. This applies to yellow stripe rust, leaf rust, and stem rust of wheat, and to crown (leaf) rust and stem rust of oats.

The results herein reported show that in the crosses investigated, inheritance of resistance to leaf rust of barley is dependent upon a single dominant genetic factor. It appears that complete dominance was shown in the F_1 individuals. In the F_2 generation there was segregation not only into the 2 grandparental types, but some individuals appeared which showed an intermediate type of resistance. In the cases investigated, this intermediate type of reaction was not directly correlated with the heterozygosity of the F_2 segregates concerned, as indicated by the F_3 generation results.

Further work is in progress involving crosses which have been made in each year since 1923, from which it is hoped to get additional light on this point. Another question concerns the type of inheritance in crosses where one parent, as for example, "Californian Feed", shows the "2" type of reaction. These results will be reported on a future occasion.

It is worth while calling attention to the value of barley crosses of this nature for teaching work to illustrate fundamental Mendelian principles. The extremely sharp reactions which are given make it particularly useful for the purpose, and no technical difficulties are encountered in any of the operations.

Acknowledgments.—Thanks are due and gratefully tendered to Drs. H. K. Hayes and J. J. Christensen of Minnesota, U.S.A., Principal E. A. Southee of Hawkesbury Agricultural College, N.S.W., J. T. Pridham, Esq., of Cowra, N.S.W., and G. S. Gordon, Esq., of Werribee, Victoria, for generously supplying grain of varieties; to T. H. Harrison, Esq., of Hawkesbury Agricultural College, N.S.W., and Mr. Gordon for forwarding specimens of rust, and to the McCaughey Research Fund of the University of Sydney for financial assistance in connection with the investigation.

SUMMARY.

Glasshouse tests have been made with 119 varieties of barley belonging to 6 species to determine their reaction to one Australian physiologic form of *Puccinia anomala* Rostr.

Strong resistance was shown by 16 of them. These varieties belong to *Hordeum vulgare* and *H. distichon*.

None of these varieties is entirely suitable agronomically for N.S.W. conditions.

Crosses of certain of these resistant varieties were made with commercial N.S.W. varieties, and the inheritance of resistance studied throughout the F_1 , F_2 , and F_3 generations.

In the F_1 generation resistance was completely dominant.

The F_2 result showed segregation in a ratio of 3 resistant plants to 1 susceptible.

The F_3 generation studies confirm the hypothesis that a single dominant genetic factor underlies resistance.

From a few results, evidence of linkage between rust resistance and morphological characters was not found.

Tests of certain varieties seem to indicate correlation between resistance to *Helminthosporium sativum* and leaf rust.

LITERATURE CITED.

- (1) Aamodt, O. S.
1922. "The Inheritance of Resistance to Several Biologic Forms of *Puccinia graminis tritici* in a Cross Between Kanred and Marquis." (Abstract).
Phytopathology, 12 : p.32.
- (2) Aamodt, O. S.
1922. "Correlated Inheritance in Wheat of Winter-Spring Habit of Growth and Rust Resistance." (Abstract).
Phytopathology, 12 : p.32-33.
- (3) Aamodt, O. S.
1923. "The Inheritance of Growth Habit and Resistance to Stem Rust in a Cross Between Two Varieties of Common Wheat."
Jour. Agr. Research, 24 : p.457-470.
- (4) Armstrong, S. F.
1922. "The Mendelian Inheritance of Susceptibility and Resistance to Yellow Rust (*Puccinia glumarum* E. and H.) in Wheat."
Jour. Agr. Sci., 12 : 57-96.
- (5) Barker, H. D., and Hayes, H. K.
1924. "Rust Resistance in Timothy."
Phytopathology, 14 : p.363-371, 1 fig.

- (6) Biffen, R. H.
1905. "Mendel's Law of Inheritance and Wheat Breeding."
Jour. Agr. Sci., 1 : p.4-48.
- (7) Biffen, R. H.
1907. "Studies in the Inheritance of Disease Resistance."
Jour. Agr. Sci., 2 : p.109-128.
- (8) Biffen, R. H.
1912. "Studies in the Inheritance of Disease Resistance II."
Jour. Agr. Sci., 4 : p.421-429.
- (9) Biffen, R. H.
1917. "Systematised Plant Breeding."
Seward's "Science and the Nation," p.146-175.
University Press, Cambridge, England.
- (10) Clark, J. A.
1925. "Segregation and Correlated Inheritance in Crosses
Between Kota and Hard Federation Wheats for
Rust and Drought Resistance."
Jour. Agr. Research, 29 : p.1-47.
- (11) Davies, D. W., and Jones, E. T.
1926. "Studies in the Inheritance of Resistance and Sus-
ceptibility to Crown Rust (*Puccinia coronata* Cda.)
in a Cross Between Selections of Red Rust Proof
(*Avena sterilis* L.) and Scotch Potato (*Avena sativa*
L.)."
Welsh Jour. Agr., 2 : p.212-221, 4 pl.
- (12) Dietz, S. M.
1925. "The Inheritance of Resistance to *Puccinia graminis*
avenae." (Abstract).
Phytopathology, 15 : p.54.
- (13) Garber, R. T.
1921. "A Preliminary Note on the Inheritance of Rust
Resistance in Oats."
Jour. Amer. Socy. Agron. 13 : p.41-43.
- (14) Garber, R. T.
1922. "Inheritance and Yield with Particular Reference
to Rust Resistance and Panicle Type in Oats."
Minn. Agr. Expt. Sta. Tech. Bull. 7 : 40 pages,
6 pl.
- (15) Griffee, F.
1922. "Breeding Oats Resistant to Stem Rust."
Jour. of Heredity, 13 : p.187-190, 3 fig.
- (16) Harlan, H. V.
1918. "Identification of Varieties of Barleys."
U.S.D.A. Bull. 622 : 30 p., 4 pl.

- (17) Harrington, J. B., and Aamodt, O. S.
1923. "The Mode of Inheritance of Resistance to *Puccinia graminis* with Relation to Seed Colour in Crosses Between Varieties of Durum Wheat."
Jour. Agr. Research, 24 : p.979-996.
- (18) Harrington, J. B.
1925. "The Inheritance of Resistance to *Puccinia graminis* in Crosses Between Varieties of Durum Wheat."
Scientific Agriculture, 5 : p.265-288.
- (19) Hayes, H. K., Parker, J. H., and Kurtzweil, C.
1920. "Genetics of Rust Resistance in Crosses of Varieties of *Triticum vulgare* with Varieties of *T. durum* and *T. dicoccum*."
Jour. Agr. Research, 19 : p.523-542.
- (20) Hayes, H. K., and Stakman, E. C.
1922. "Wheat Stem Rust from the Standpoint of Plant Breeding."
Proc. Second Annual Mtg. West Canad. Soc. Agron. p.22-35.
- (21) Hayes, H. K., and Aamodt, O. S.
1923. "A Study of Rust Resistance in a Cross Between Marquis and Kota Wheats."
Jour. Agr. Research, 24 : p.997-1012.
- (22) Hayes, H. K., Stakman, E. C., Griffee, F., and Christensen, J. J.
1923. "Reaction of Barley Varieties to *Helminthosporium sativum*."
Minn. Agr. Expt. Sta. Tech. Bull. 21 : p.47.
- (23) Hayes, H. K., Stakman, E. C., and Aamodt, O. S.
1925. "Inheritance in Wheat of Resistance to Black Stem Rust."
Phytopathology, 15 : p.371-387, 1 pl.
- (24) Hynes, H. J.
1926. "Studies of the Reaction to Stem Rust in a Cross Between Federation Wheat and Khapli Emmer, with Notes on the Fertility of the Hybrid Types."
Phytopathology, 16 : p.809-827, 4 pl.
- (25) Mains, E. B., and Leighty, C. E.
1923. "Resistance in Rye to Leaf Rust *Puccinia dispersa* Erikss."
Jour. Agr. Research, 25 : 243-252.

- (26) Mains, E. B., Leighty, C. E., and Johnston, C. O.
1926. "Inheritance of Resistance to Leaf Rust *Puccinia triticina* Erikss., in Crosses of Common Wheat, *Triticum vulgare* Vill."
Jour. Agr. Research, 32 : 931-972.
- (27) Melchers, L. E., and Parker, J. H.
1922. "Inheritance of Resistance to Black Stem Rust in Crosses Between Varieties of Common Wheat, *Triticum vulgare*." (Abstract).
Phytopathology, 12 : 31-32.
- (28) Melchers, L. E., and Parker, J. H.
1922. "Rust Resistance in Winter Wheat Varieties."
U.S.D.A. Bull. 1046 : 32p.
- (29) Nilsson-Ehle, H.
1908. "Einige Ergebnisse von Kreuzungen bei Hafer und Weizen."
Botan. Notisn. Lund's. 257-298.
- (30) Nilsson-Ehle, H.
1911. "Kreuzungsuntersuchungen an Hafer und Weizen II."
Lunds Univs. Arsskr. N.F. Afd. 2, Bd. 7, No. 6, 82p.
- (31) Parker, J. H.
1918. "Greenhouse Experiments on the Rust Resistance of Oat Varieties."
U.S.D.A. Bull. 629 : 16p.
- (32) Parker, J. H.
1920. "A Preliminary Study of the Inheritance of Rust Resistance in Oats."
Jour. Amer. Socy. Agron. 12 : 23-38.
- (33) Puttick, G. F.
1921. "The Reaction of the F₂ Generation of a Cross Between a Common and Durum Wheat to Two Biologic Forms of *Puccinia graminis*."
Phytopathology, 11 : 205-213.
- (34) Stakman, E. C., and Levine, M. N.
1922. "The Determination of Biologic Forms of *Puccinia graminis* on *Triticum spp.*"
Minn. Agr. Expt. Sta. Tech. Bull. 8 : 10p., 1 fig.
- (35) Thompson, W. P.
1925. "Cytological Conditions in Wheat in Relation to the Rust Problem."
Scientific Agric. 8 : p.237-239.
- (36) Waldron, L. R.
1921. "Inheritance of Rust Resistance in a Family Derived from a Cross Between Durum and Common Wheat."
Nth. Dakota Agr. Expt. Sta. Bull. 147 : 24p.

A PRELIMINARY ACCOUNT OF THE ORIGIN OF TWO NEW
AUSTRALIAN PHYSIOLOGIC FORMS OF
Puccinia graminis tritici.

By W. L. WATERHOUSE, The University of Sydney.

(Plate iv.)

[Read 24th April, 1929.]

[Issued 15th May, 1929.]

• From the Proceedings of the Linnean Society of New South Wales,
Vol. liv, Part 2, 1929.

A PRELIMINARY ACCOUNT OF THE ORIGIN OF TWO NEW
AUSTRALIAN PHYSIOLOGIC FORMS OF
Puccinia graminis tritici.

By W. L. WATERHOUSE, The University of Sydney.

(Plate iv.)

[Read 24th April, 1929.]

[Issued 15th May, 1929.]

A PRELIMINARY ACCOUNT OF THE ORIGIN OF TWO NEW AUSTRALIAN
PHYSIOLOGIC FORMS OF *Puccinia graminis tritici*.

By W. L. WATERHOUSE, The University of Sydney.

(Plate iv.)

[Read 24th April, 1929.]

Introduction.

The occurrence of physiologic forms within well-defined morphological species of fungi has long been known and is widespread throughout the group.

Physiologic specialization in *Puccinia graminis tritici* E. & H., the fungus which causes stem rust in wheat, has received particular attention. Extensive studies, mainly carried out in North America, have revealed in it an extreme state of specialization. The most recent statement issued by Stakman and Levine (1928) sets out fifty-five physiologic forms which have been identified.

This extreme specialization of the pathogen seriously hinders efforts made to combat rust attack by the breeding of resistant varieties of wheat. There are instances in which certain varieties have been produced which were resistant to the forms of rust known to be present in that locality. But with the passage of time, these varieties have become liable to attack. Investigation has shown that this is due to the advent of new physiologic forms. It thus becomes a matter of importance to know how physiologic forms originate.

The writer is indebted to Dr. E. C. Stakman and Dr. M. N. Levine of the University of Minnesota for supplying grain of the differential hosts used in the investigation, to Mr. G. Wright of the University of Sydney for the loan of photographic apparatus, and to Messrs. G. S. Gordon, T. H. Harrison, W. H. Darragh and R. E. Dwyer for collecting and forwarding material, and gratefully acknowledges the help given.

HYPOTHESES ADVANCED TO ACCOUNT FOR SPECIALIZATION.

Several suggestions have been made to explain the origin of specialization in this fungus.

1. *Nuclear interchange in uredo mycelia.*

In the uredospore stage, it is known that multiple infection of a wheat plant frequently takes place. Numerous cases have occurred in the writer's investigations in which two quite distinct physiologic forms have been identified from the one plant. Levine (1928) records the occurrence of three forms on the one collection of material.

It has been suggested that in a plant infected by two forms of rust, commingling of the two mycelia may take place. In the anastomoses, a nuclear interchange is possible between hyphae belonging to the two forms. This would lead to the production of a hypha and later a mycelium having a hybrid origin. Uredospores from this might be expected to exhibit physiologic capabilities different from those of its components, to be, in fact, a new physiologic form.

In the investigations which are in progress, some time has been spent in testing this hypothesis, but with negative results.

2. *Nuclear fusions in aecidial stage.*

A more attractive hypothesis has been put forward dealing with the aecidial stage of the fungus. The uredospore stage is binucleate. It gives place to the

teleutospore stage. The teleutospore cells are uninucleate, as are also the sporidia they produce. These sporidia infect young growth of the barberry, in which they give rise to a uninucleate mycelium. The binucleate condition is restored at the base of the aecidia. Here a cellular fusion of adjoining uninucleate hyphae takes place and leads to the production of binucleate aecidiospores. These produce the binucleate mycelium of the uredospore stage in wheat.

The suggestion is that the barberry may be infected at the same spot by sporidia of two diverse physiologic forms. Two genetically different mycelia may thus be present in the young barberry growth, and may commingle. At the base of an aecidium, the migrating nucleus of one form may become associated in the newly-created binucleate hypha with a nucleus of the second form. This would give rise to aecidiospores of mixed or hybrid ancestry, and these might be expected to show new physiological capabilities.

Repeated efforts have been made in past years to have viable teleutospores of several well established forms available at the same time for a series of such tests, but without success until this season. The possibility that such nuclear fusions may explain the cases herein recorded is discussed later in the paper.

3. Mutations.

Mutation has been put forward to explain the origin of specialization. To date no case has been recorded. Mutations are known in regard to colour of the uredospore. Newton and Johnson (1927) describe such a case, and in the writer's studies one similar case has occurred.

Despite the suggestions which have been put forward, the position has been obscure in regard to the origin of specialization.

METHOD OF DETERMINING PHYSIOLOGIC FORMS.

In order to make clear the happenings herein described, it is well to indicate the method of determining physiologic forms of wheat stem rust. The procedure followed has been developed by Stakman and Levine (1922), also Levine (1928), and is briefly as follows. Twelve standard varieties of wheat have been selected as a result of much careful work. They are shown in the following table.

Table 1. The set of differential hosts selected by Stakman and Levine for the determination of physiologic forms of *Puccinia graminis tritici* E. & H.

Species of <i>Triticum</i> .	Varietal Name.	Cereal Investigation Number.
<i>T. compactum</i>	Little Club	C.I. 4066
<i>T. vulgare</i>	Marquis	C.I. 3641
"	Kanred	C.I. 5146
"	Kota	C.I. 5878
<i>T. durum</i>	Arnautka	C.I. 4072
"	Mindum	C.I. 5296
"	Arnautka (Spelmars)	C.I. 6236
"	Kubanka	C.I. 2094
"	Acme	C.I. 5284
"	Einkorn	C.I. 2433
<i>T. monococcum</i>	Vernal Emmer	C.I. 3686
<i>T. dicoccum</i>	Khapli	C.I. 4013
"		

Grain of these standard differential hosts was kindly supplied in 1921 by Dr. E. C. Stakman and Dr. M. N. Levine, and has since been grown in pure lines for use in the determinative work.

Seedlings to the number of about fifteen are grown in small pots. The first seedling leaf is moistened with water by means of a flat sterile needle, and the uredospores of the rust under examination are placed in this water adherent to the leaf. The pots are then incubated in a saturated atmosphere for 48 hours. They are afterwards placed on well-lighted benches in the plant house to allow the rust to develop. Notes are taken on the rust development twelve to sixteen days later, depending upon the prevailing weather conditions.

The types of infection shown by each host are recorded by a simple notation. Arabic numerals from 0 to 4 indicate the order of severity of infection. 0 indicates immunity, and 4 complete susceptibility. Fluctuations within these types are indicated by plus and minus signs. A sixth type is represented by x. This indicates that a heterogeneous reaction occurs on the one plant. A single leaf may show all classes of reaction ranging from 1 to 4, and further simplification of these reactions is impossible.

The explanation of these symbols is set out hereunder.

Resistant Class.

Type 0.—Host immune.

No uredosori are developed, but sharply defined hypersensitive flecks may be present, indicated then as "0;".

Type 1.—Host very resistant.

Infection is very light; uredosori are minute and scattered, and surrounded by sharp, continuous, necrotic areas.

Type 2.—Host moderately resistant.

Infection is light; uredosori are small to medium in size. Hypersensitive areas in the form of necrotic halos are present. The pustules occur in green, but slightly chlorotic islands.

Susceptible Class.

Type 3.—Host relatively susceptible.

Infection is moderate; uredosori are medium in size and show a tendency to coalesce. True hypersensitiveness is absent excepting when cultural conditions are unfavourable.

Type 4.—Host completely susceptible.

Infection is normal and heavy. Uredosori are large and generally confluent. Hypersensitiveness is absent excepting when cultural conditions are unfavourable.

Indeterminate Class.

Type x.—Host intermediately susceptible.

Infection is of a heterogeneous nature. Uredosori are very variable and apparently include all types and degrees of infection on the one leaf; no mechanical separation is possible since spores from small and from large uredosori alike produce the same heterogeneous infection.

When the class of reaction on the standard differential hosts has been determined, the results are tabulated. From these tabulated results a dichotomous key has been prepared, and by using this it is easy to determine the physiologic form of the rust under examination. A reference to Table 2 will show four of the forms set out in the conventional way.

EXPERIMENTAL MATERIAL AND METHODS.

In the investigations at the University of Sydney, the problem of how physiologic forms originate has long been under consideration. Numerous tests have been made, but not until the season which has just closed has a positive result been obtained.

It has several times been found in studies dealing with the aecidial stage that inoculation of young growths of the barberry with viable teleutospores has led to the production of spermagonia only. Craigie (1927) has recently published results of outstanding importance which throw much light on this occurrence. He has shown that *Puccinia graminis* Pers. is heterothallic. Further, the spermatia are not functionless male gametes as was formerly supposed, but are + and - haploid spores, which, by mingling, give rise to the diploid phase and lead to the development of aecidia. This work has led to attention being concentrated on the spermagonial stage in the life history.

During the summer of 1927-1928, numerous identifications of physiologic forms were made from uredospore material collected at widely separated localities in Australia. From certain of the collections, teleutospore material was also obtained. Three only of these need be considered.

1. *Werribee Material.*

One collection came from the State Research Farm, Werribee, Victoria. In November, 1927, a comprehensive series of samples of the rusted wheat crop was forwarded by Mr. G. S. Gordon, Cerealist. Investigation showed that the form present was Form 34. In July and August, 1928, two further batches of rusted wheat straw from the same area were submitted by Mr. Gordon. This material comprised stubble which had been cut at harvest and which showed abundant teleutosori, together with "second growth" shoots of wheat carrying abundant uredo- and teleuto-sori. The unusually copious autumn rains had been responsible for the development of this "second growth" and for its heavy rust infection. From both batches, uredospores were at once used to infect the standard set of differential hosts. The form present was Form 34. The straw was then placed in light wire frames to keep it intact, and exposed on the ground to the weather conditions prevailing at the University.

It is of interest to record that a further series of determinations made during the summer of 1928-1929—i.e., following upon the barberry work described herein—has shown the presence of only Form 34 at Werribee.

Early in September, 1928, germination tests proved that the teleutospores were viable. Later a young plant of *Berberis vulgaris* growing in a pot was inoculated with fragments of the straw carrying the teleutosori. The method used was to atomize the plant with sterile water. A certain amount of this remained on the young shoots. These were very gently rubbed with the fingers cleansed in alcohol and washed in running water. The rubbing was found to aid greatly in wetting the cuticle. On the wet shoots were placed wetted fragments of straw carrying teleutosori. The plant was incubated in this condition for 48 hours in an inoculation chamber containing a saturated atmosphere. Afterwards the pot was placed on the plant-house bench and watered when necessary.

After twelve days there were abundant spermagonia on some of the inoculated shoots. Aecidia developed in some cases, but other infections gave rise only to spermagonia. Over these were smeared spermatia from the Grafton rust, to be described presently as the third culture. In the same way, spermatia from the

Werribee rust were smeared over the spermatogonia on the barberry infected by the Grafton rust. The operation was carried out with the aid of a fine camel-hair brush sterilized in alcohol and then in distilled water. One of the infected petioles is illustrated in Figure 2 of Plate iv.

After about six days aecidia were developed on the shoots where mixing of spermatia had been carried out. Aecidiospores were used to inoculate seedlings of "Federation" wheat growing in pots. Two methods were used. In the first, the spores were collected on a small sterile camel-hair brush moistened with distilled water and lightly brushed over the aecidia. From the tip of the brush the aecidiospores were transferred to the moistened leaves of the "Federation" seedlings. In the second case, the aecidiospores were shaken from the shoot into a sterile petri dish and then transferred to the moistened wheat seedling leaves. In each case the pots of inoculated seedlings were incubated in a saturated atmosphere for 48 hours and then placed in isolated positions on the plant-house benches. The aecidiospores from the normally-produced aecidia were not kept separate from those borne in aecidia resulting from the spermatial intermixing.

Four cultures were thus obtained with intervals of three to four days between each. The aecidiospores were not nearly as abundant as in the case of the Bathurst rust, with the result that the uredosori produced on the "Federation" seedlings were scanty. All four cultures had, therefore, to be transferred from "Federation" to new pots of "Federation" seedlings in order to obtain a quantity of inoculum sufficient to inoculate the regular differential hosts.

Three out of the four cultures gave an identical result. The form present was not Form 34. It was the form which has been identified by Stakman and Levine (1922) as Form 11. It differs from Form 34 in the reaction shown by Einkorn. This host is susceptible to Form 11 but resistant to Form 34. The contrast in the reactions is as sharp as possible. This is illustrated in Fig. 3 of Plate iv.

This unexpected result was checked in many ways. Inoculum from each of the ten susceptible varieties of the standard differential hosts was used to inoculate further sets of differentials. So far thirty-six of these tests have been made. In all cases the result has been the same. The form is Form 11.

It was a coincidence that on the day the first of these inoculations of the differential set was made, a similar set was inoculated with a new uredospore culture which had just come to hand from Werribee. This gave Form 34. A mixing of seed or mistake in labelling was clearly not responsible for the unexpected identification of Form 11.

The fourth culture of "Federation" seedlings derived from the aecidiospores gave a different result. The form present proved to be Form 34. In this case the sojourn on the barberry had exerted no influence upon the form.

It is unfortunate that a differentiation was not made between the shoots which produced spermatogonia and aecidia without outside interference, and those which produced aecidia only as a result of admixture of spermatia with those of the Grafton rust. But both things happened, and it is believed that this is the explanation of Forms 11 and 34 appearing from these cultures.

2. Bathurst Material.

An extensive series of collections was made at the Bathurst Experiment Farm by Mr. R. E. Dwyer, B.Sc.Agr. From this area, rusted straw was obtained

at intervals of approximately a fortnight, commencing in November, 1927, and ending late in April, 1928. Thereafter it was obtained at monthly intervals until July, 1928, when no uredosori were found and only the teleutospore stage of the rust was present on the straw. This straw was allowed to remain out in the open at Bathurst, exposed to the winter conditions which prevailed there, and was used in the spring for the barberry inoculations.

This series of determinations of the uredospore stage involved sixteen distinct tests on the standard set of differential hosts. The first, made in November, 1927, showed that a mixture of Forms 34 and 43 was present. Thereafter no sign of Form 43 was found. Form 34 alone was identified. The collections were bulky and random samples from them were used in the determinations, so that it is unlikely that Form 43 was present and not detected. It may further be remarked that a later series of identifications made during the 1928-1929 season has shown only Form 34 at Bathurst.

Early in September, 1928, some of the teleutospore material was sent from Bathurst to the University and was found to be in a viable condition. The straw was used to inoculate a young barberry plant growing in a pot. This inoculation led to the production of a few spermagonia only.

In October, a further supply of the rusted straw was obtained from Bathurst and used for further barberry inoculations. Spermagonia were very abundant in nine days, and four days later numerous aecidia were present on many of the young growths. On certain shoots the infection was very heavy and aecidiospore production was abundant. An illustration of this is given in Figure 1 of Plate iv. On other shoots aecidial production was very scanty, and on a few leaves only a few spermagonia were found. Spermata from these last-named were mixed with those on the barberry which was infected by the Grafton rust, and spermata of this Grafton rust were in turn mixed with those on the plant under consideration.

At short intervals commencing early in November, seedlings of "Federation" wheat in pots were inoculated with aecidiospores. Both methods of transference already described were used. The result was that four pots of seedlings producing abundant uredosori were obtained at intervals of about four days. The uredospores were used for inoculating the standard set of wheat differentials in order to determine the physiologic form. Three out of the four pots gave the normal reactions for Form 34. That is to say, the completing of the life cycle on the barberry had not affected the parasitic capabilities of the rust as determined by the standard test. In the case of the fourth culture, there was a departure from the normal reaction shown by Form 34. The pot of Einkorn, instead of giving the characteristic flecking of the resistant reaction, showed eleven of the thirteen plants with this normal reaction, whilst the remaining two seedlings showed this reaction intermixed with pustules of the "3" type belonging to the susceptible class. Uredospores from these pustules were transferred to a pot of "Federation" and a pot of Einkorn, producing on each the normal susceptible reactions. This culture was used to inoculate the standard set of differentials. It proved to be Form 11, the form which also occurred in the aecidial cultures obtained from the Werribee rust.

3. *Grafton Material.*

A third collection of material was made at the Grafton Experiment Farm by Mr. W. H. Darragh, B.Sc.Agr. During the months of November and December,

1927, six different comprehensive sets of uredospore material were obtained from numerous wheat varieties. On some of the straw, teleutosori were also abundant. Tests with five of the batches showed the presence of only Form 34. In the sixth, two forms were present. One was Form 34, the other Form 43.

Again, it is of interest to know that a further series of tests made later with uredospore material collected in October, November and December, 1928, has shown only Form 34 to be present.

In January, 1928, Mr. Darragh forwarded a collection of wheat stubble showing heavy teleutospore infection. This, together with the straw infected by the uredo- and teleutospore stages which had been used in the form determinations, was placed in a light wire frame and exposed at the Hawkesbury Agricultural College to the prevailing winter conditions. At intervals a few straws were withdrawn from the frame by Mr. T. H. Harrison, B.Sc.Agr., and submitted for teleutospore germination tests. Early in September, 1928, the teleutospores proved to be viable. Later a barberry plant was inoculated with fragments of the straw, of which only a small amount was then available. Within twelve days, two of the shoots produced spermagonia. These were kept under observation for aecidial production, but none developed. Spermata from them were taken for admixture with the Bathurst and Werribee rusts, and spermata from these latter were in turn mixed with those produced by the Grafton rust. In eight days there were a few aecidia on the under surfaces of the two leaves bearing the spermagonia. The latter persisted for an unusually long period.

Aecidiospore production was necessarily scanty, and a number of attempts were made to obtain the uredospore stage on "Federation" seedlings. An inoculation made at the end of October gave one infected seedling. This inoculum was used to infect a fresh pot of "Federation" seedlings. From these, the standard set of differentials was inoculated in order to determine what form was present. It proved to be a form which has so far not been recorded. It resembles Form 18, but differs markedly in its reaction on Einkorn. Instead of giving on this host the susceptible "3" type of reaction which is characteristic of Form 18, the new form gives the sharp flecks of the strongly resistant reaction. These results have been repeatedly checked by numerous cross-inoculations of the standard set of differential hosts. To date, twenty of these tests have been made. In Figure 4 of Plate iv is illustrated the flecking of a leaf of "Mindum" by this form, in comparison with the susceptible reaction on the same host given by Form 34. In colour the new form differs markedly from any other form that is available. Judged by the Ridgway (1912) colour standards, it is "Raw Sienna" of Plate iii, whilst the Forms 11 and 34 are "Sanford's Brown" of Plate ii, and Form 43 is "Burnt Sienna" of Plate ii.

DISCUSSION.

Investigations with the uredospore stage of *Puccinia graminis tritici* E. & H. which have been in progress for a number of years, have revealed the presence in Australia of certain definite physiologic forms. Several hundred of these determinations have been made.

One of these forms is Form 34. Of late years this has been predominant and still more recently has been the only form found. It is one of the very virulent forms and has been recorded by Stakman and Levine from several continents.

Another form which used to be found very frequently is Form 43. Since December, 1927, this has not been found on the mainland of Australia, although one determination made in January, 1928, with material from Tasmania, revealed

its presence there. Form 43 has some characteristics which are unusual. One is its apparent inability to infect the barberry, although it produces viable teleutospores. So far it has not been found outside Australia.

Tests with the uredospore stage on wheat collected at Werribee, Bathurst and Grafton have shown that Form 43 was last found at the first-named station in 1925. The only Werribee rust found in 1927 and since then was Form 34; there has been no indication of the presence of any other form in that locality. At Bathurst, Form 43 occurred in November, 1927, intermixed with Form 34, but since then an extended search has revealed the presence of only this latter form. It is believed that the teleutospore material used from Werribee and Bathurst was Form 34. Part of the actual Grafton straw used in the teleutospore stage was shown in its uredospore stage to comprise Forms 34 and 43. These two forms may reasonably be considered to have been present in the teleutosori used to inoculate the barberry.

Previous work (Waterhouse, 1921) has shown that contrary to the old belief, stem rust of wheat in Australia has not lost its capacity to infect the barberry. In the 1927-1928 season, teleutospore material of Form 34 was on hand from many localities. The only available material known to have any other form present was the straw from Grafton comprising Forms 34 and 43. One of the reasons for using it was to extend further the attempts to produce the aecidial stage of Form 43. The straw carrying Form 34 was used partly in order to see whether the aecidial stage on the barberry differed in its physiologic capabilities. Although Craigie's work was known, the pressure of other duties at the time made it impossible to use monosporidial cultures on the barberry. By the time the results herein recorded were obtained, the teleutospores had lost viability and it was impossible to use this necessary refinement.

The fact remains that from the aecidia on the barberries, three distinct physiologic forms were obtained. One of these was Form 34 which was well known to have been present in the teleutospores used to produce the infections. Numerous tests have shown no difference between this aecidial culture and the stock uredospore cultures of Form 34, as judged by the reactions on the standard differentials. The other two forms which were obtained had never before been grown in culture, nor have they turned up since in the work that has been done. It is hardly possible that either of them could have been present and not detected in the teleutospore material that was used. Of the two, one is established as Form 11. The other is, as far as can be learned, new to Science and will have to be given its proper designation.

A comparison of the four forms which are herein referred to is instructive, and is set out in Table 2.

It will be seen that of the twelve differential hosts, the first two, viz., Little Club and Marquis, are susceptible throughout, and the last two, viz., Vernal Emmer and Khapli, are resistant throughout. The remaining eight differentials are therefore the ones to be considered.

The Forms 34 and 43 which were known to be present in the teleutospore stage are remarkably different. Form 34 is very virulent. Form 43 attacks but few of the differentials. Form 11, which is one of the two which have originated on the barberry, resembles Form 34, but is more virulent, since it attacks Einkorn. Susceptibility of Einkorn is one of the characteristics of Form 43. For this reason, in the large series of cross-inoculations that was made, an especial watch was kept on the results obtained when inoculum from Einkorn was used. Form 11

Table 2. Means of the reactions produced by certain physiologic forms of *Puccinia graminis tritici* E. & H. on differential varieties of *Triticum* spp.

Physiologic Forms.	Infection Means on											
	Lc.	Ma.	Krd.	Ko.	Arn.	Mnd.	SpM.	Kub.	Ac.	Enk.	Em.	Kpl.
	Little Club, C.I. 4066.	Marquis, C.I. 3641.	Kared, C.I. 5146.	Kofa, C.I. 5878.	Arnautka, C.I. 4072.	Mindum, C.I. 5296.	Arnautka (Spelmars), C.I. 6236.	Kubanka, C.I. 2094.	Acme, C.I. 5284.	Einkorn, C.I. 2433.	Vernal Emmer, C.I. 3686.	Khapl, C.I. 4013.
11	4	4=	3++	3+	4=	4=	4=	3++	3++	3	1=	1=
34	4+	4-	4-	4=	4	4=	4=	4=	3++	1=	0;	1=
43	4	3++	0	0;	0;	0;	0;	×	1	3	1	0;
New form	4	3++	3++	3	0;	0;	0;	3	3	0;	0;	0;

was obtained in every instance. Parallel sets were inoculated with a standard culture of Form 34 to serve as checks. The susceptibility of Einkorn, which is characteristic of Form 43, has replaced its resistance, characteristic of Form 34, thus leading to the production of Form 11. The fact that no one of the aecidial cultures gave the reactions of Form 43 goes further to confirm the conclusion previously arrived at that Form 43 does not infect the barberry.

The advent of such a form as Form 11 may have serious consequences if it should become widespread in Australia. As a result of the studies that have been made, an American variety of wheat named "Hope"—obtained from Stakman and Levine—has been found to be resistant to all known forms of Australian rust. It has, therefore, been used rather extensively in the work of breeding resistant varieties suitable for Australian conditions. "Hope", as a commercial variety, is valueless for our wheat-growing conditions. Now that cultures of Form 11 have become available, "Hope" has been tested with it in the plant house. It proves to be susceptible. Thus, in order to combat attack by this form, it becomes necessary to search for a variety of wheat which is resistant to it, and then make further crosses and carry out the necessary selection work.

The new form, whose true designation by Stakman and Levine is not yet available, resembles Form 34 excepting that the three durum wheats Arnautka, Mindum, and Arnautka C.I. 6236 (Spelmars), instead of being very susceptible, are markedly resistant. With all the Australian forms that have been found, these three differentials have been all susceptible or all resistant. Therefore in differentiating Australian forms, nothing has been gained by utilizing all three hosts. Their use has been continued, however, because of the possibility of forms turning up similar to other known forms which are separable by these three differentials. In the new form derived from the barberry, the resistance of these three hosts, which is a characteristic of Form 43, has taken the place of the susceptibility characteristic of Form 34.

The complete answer to the question as to how the two new Australian forms actually originated will not be given until further monosporous infections are obtained and cytological investigations carried out. It is significant that barberry infections caused by the Werribee and Bathurst rusts—both of them Form 34—produced aecidia and that some of the aecidial cultures from each were in turn Form 34, although others were Form 11. On the other hand, the Grafton rust comprising Forms 34 and 43 produced spermagonia only, until after the mixing of the spermatia. The aecidial culture then obtained proved to be no one of the known forms, but an altogether new form.

Craigie (1927) has shown that *Puccinia graminis* is heterothallic. Even so the abundant multiple infections produced on the barberries by the Werribee and Bathurst rusts would render easy the admixture of spermatia on the shoots, with consequent production of aecidia. Form 34 would be expected to turn up in the subsequent form determinations. In addition to these aecidia, other aecidia might be expected to result from the intermixing of the spermatia with those from the Grafton rust, and thus account for the origin of Form 11. It is of course just possible that another form was present in the teleutosori used, and that a cellular fusion of adjoining hyphae, which had a diverse origin, led to the production of Form 11, which had never before been found in Australia.

In the case of the barberry infected by the Grafton rust, Forms 34 and 43 were present in the inoculum. The infections produced were merely of a spermagonial nature up to the time of intermixture of spermatia. Only consequent upon this were produced the aecidia from which the new form was determined. It is this occurrence with the Grafton rust which most strongly supports the belief that spermatial intermixing gave rise to the new forms. If so, Craigie's discovery of the importance of the spermatia takes on an added significance. So far no effort has been made to trace cytological happenings following upon a mixing of spermatia. Investigations in this direction have been planned, but extreme difficulty is being met in the attempt to grow to maturity wheat plants infected with known forms of rust, because of the ravages of *Erysiphe graminis* D.C. Other studies involving the two new forms are in progress.

Craigie has called attention to the importance of insects in transferring spermatia and leading to aecidial production. If it be correct that such transference leads to the production of new physiologic forms, it would be expected that physiologic forms would be numerous where barberries occur, and fewer where they are scanty. In North America barberries are widespread, and physiologic forms are numerous. In Australia they are uncommon and physiologic forms are few.

Whether the spermatial intermixing be the prime cause of the origin of specialization, or whether it be due to hyphal fusions at the base of the aecidia, it seems certain that physiologic forms originate on the barberry. This gives the strongest possible reason why this alternate host should be eradicated.

SUMMARY.

Wheat straw infected with the uredospore stage of *Puccinia graminis tritici* 34 and later with its teleutospore stage, was collected from Werribee, Vic., and from Bathurst, N.S.W., and similar material infected with a mixture of *P. graminis tritici* 34 and 43 from Grafton, N.S.W.

Successful barberry infections were obtained in the spring of 1928 by mass inoculation with teleutosori attached to the straw.

The Werribee and Bathurst rusts producedaecidia normally on some shoots, in addition to spermagonia. The Grafton rust gave rise to spermagonia only.

Spermatia from the Werribee and Bathurst rusts were respectively intermixed with those developed by the Grafton rust.

Consequent upon the spermatial intermixing, the Grafton rust developedaecidia, as did the other two.

Aecidiospores from the Werribee and Bathurst rusts developed uredospore cultures on wheat which proved to be mixtures of Forms 11 and 34.

Aecidiospores from the Grafton rust gave rise to a uredospore culture which is a new form as far as available records show.

Form 11 and the new form have never before been in culture, although hundreds of form determinations of Australian rusts have been made.

The two rusts which are new to Australia had their origin in the phase of the life history on the barberry.

It is believed that in this phase, the mixing of spermatia of Form 34 with those of Forms 34 and 43 led to the development of the two new rusts. Further work is planned to test this belief.

Insect transmission of spermatia under natural conditions with consequent production of new forms would account for the abundance of the latter in North America where barberries are widespread, and for their paucity in Australia where barberries are uncommon.

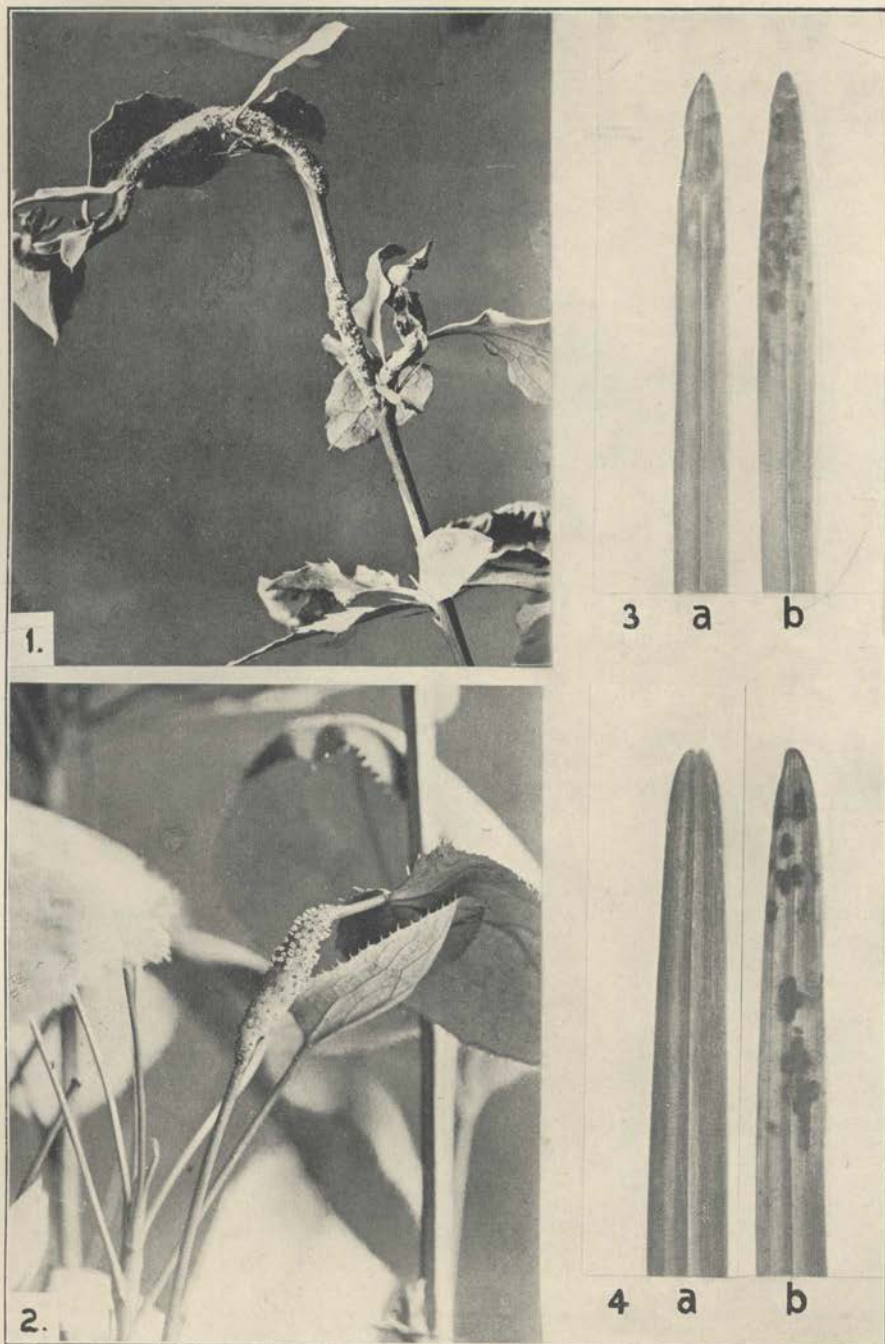
The strongest reason for eradicating barberries is afforded by this work.

EXPLANATION OF PLATE IV.

- Fig. 1.—Young shoot of *Berberis vulgaris* showing heavy aecidial infection and distortion with rust from Bathurst. Natural size.
 Fig. 2.—Petiole of *B. vulgaris* showing aecidial infection produced by rust from Werribee. $\times 3$.
 Fig. 3.—Two leaves of Einkorn wheat showing on the under-surface the resistant reaction and flecking produced by Form 34 in "a", and the susceptible reaction produced by Form 11 in "b". $\times 2$.
 Fig. 4.—Two leaves of Mindum wheat showing on the under-surface the resistant reaction and flecking produced by the new form in "a", and the susceptible reaction produced by Form 34 in "b". $\times 2$.

References.

- CRAIGIE, J. H., 1927.—Experiments on Sex in Rust Fungi. *Nature*, Vol. 120, Part 3012.
 ———, 1927.—Discovery of the Function of the Pycnia of the Rust Fungi. *Nature*, Vol. 120, Part 3030.
 ———, 1928.—On the Occurrence of Pycnia and Aecia in Certain Rust Fungi. *Phytopathology*, Vol. 18, No. 12.
 LEVINE, M. N., 1928.—Biometrical Studies on the Variation of Physiologic Forms of *Puccinia graminis tritici* and the Effects of Ecological Factors on the Susceptibility of Wheat Varieties. *Phytopathology*, Vol. 18, No. 1.
 NEWTON, M., and JOHNSON, T., 1927.—Colour Mutations in *Puccinia graminis tritici* (Pers.), E. & H. *Phytopathology*, Vol. 17, No. 10.
 RIDGWAY, R., 1912.—Colour Standards and Colour Nomenclature. *Press of A. Hoen and Co.*
 STAKMAN, E. C., and LEVINE, M. N., 1922.—The Determination of Biologic Forms of *Puccinia graminis* on *Triticum* spp. *University of Minnesota*, Tech. Bull. 8.
 ———, ———, 1928.—Supplement to preceding Bulletin, still in mimeograph.
 WATERHOUSE, W. L., 1921.—On the Production in Australia of the Aecidial Stage of *Puccinia graminis* Pers. *Journ. Proc. Roy. Soc. N.S.W.*, Vol. lv, p. 278.



1, 2. Young shoot (1) and petiole (2) of *Berberis vulgaris* showing acedial infection with rust.
3, 4. Leaves of Einkorn wheat (3) and Mindum wheat (4) showing resistant reaction and flecking (a), and susceptible reaction (b).

