AUSTRALIAN RUST STUDIES.

VIII. PUCCINIA GRAMINIS LOLII, AN UNDESCRIBED RUST OF LOLIUM SPP. AND OTHER GRASSES IN AUSTRALIA.

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Synopsis.

A stem rust which attacks *Lolium* spp., *Dactylis glomerata* L. and about 30 other grasses is described. Aecidial, uredospore and teleutospore stages show that it is a type of *Puccinia graminis* Pers. Spore morphology and cultural studies make it clear that it is not one of the known subspecies of the cereal stem rusts, nor is it one of the described subspecies which attack grasses.

Its host range has been studied and its distribution determined in all the States of Australia and in New Zealand. The occurrence of physiologic races of the rust has been shown by differential reactions of plants of *Lolium perenne* L., *L. rigidum* Gaud., and *Arrhenatherum elatius* (L.) J. et C. Presl. In many grasses there are wide differences between the resistance and susceptibility of individuals within the species.

The name *Puccinia graminis lolii* is proposed for the rust.

Introduction.

Stem rust of cereals and grasses is caused by the highly specialized fungus *Puccinia graminis* Pers. The following subspecies (or varieties) are generally recognized: (i) a group of three which attack cereals, viz., *P. graminis tritici* E. and H., *P. graminis avenae* E. & H., and *P. graminis secalis* E. and H.; and (ii) a group of subspecies which attack grasses. Three of these have been studied intensively in U.S.A., viz., *P. graminis poae* E. and H., *P. graminis agrostidis* E., and *P. graminis phlei-pratensis* (E. and H.) Stak. and Piem. In addition, European workers have described four others, viz., *P. graminis airae* E. and H., *P. graminis calamagrostis* Jacz., *P. graminis aperae* Jacz., and *P. graminis arrhenatheri* Jacz. Little is known of these.

McAlpine (1906) recorded *P. graminis* on cereals and a number of grasses, but made no inoculation studies or determinations of the subspecies present. More recently Australian investigations of *P. graminis tritici* and *P. graminis avenae* have been carried out (Waterhouse, 1929). It has been shown that barley and rye are attacked by the former: *P. graminis secalis* is not present in Australia. A number of grasses were also found to be common hosts for one or other of these cereal rusts. But many other stem rusts occur on grasses and very little is known about them.

Of these a common type which is specially notable for its attack of *Lolium* spp. has been under observation since 1918. It quickly became apparent that it was not one of the cereal rusts: no lesions were produced on wheat, very occasional resistant reactions were shown on rye, tiny reactions occurred on a few varieties of oats at very favourable temperatures, and 260 barley varieties showed sharp resistance with the exception of two varieties in which a "2+" reaction was given. In no case were the reactions comparable with those caused by *P. graminis tritici* or *P. graminis avenae*. Detailed studies were therefore undertaken to determine its identity, host range and distribution.

Morphological Studies.

Large uredosori occur on stems and leaves, rupture of the epidermis being a noticeable feature. Lesions on glumes and rachises are not uncommon. Those on the stems frequently give place to black teleutosori late in the season, and sometimes these also occur on the leaves. The aecidial stage has not been found in nature but has been produced in the plant house on *Berberis vulgaris* L. from teleutospores on *Lolium perenne* L. that had been exposed to winter conditions on the tablelands at Glen Innes, New South Wales. Inoculations with these aecidiospores produced uredosori on *L. perenne* and *Dactylis glomerata* L. but on none of the cereals. All spore forms show characteristics which agree with those described for *P. graminis*. It has been shown (Levine, 1923, and Waterhouse, 1930) that statistical examinations of the spore morphology may reveal significant differences between subspecies.

Measurements were made of 200 aecidiospores and 200 uredospores lightly shaken from leaves of the susceptible host, and of 200 teleutospores obtained by scraping sori on the stems. The standard methods previously described (Waterhouse, 1930) were used.

The results are set out in Table 1, in which the name *P. graminis lolii* is used for the new rust, and the measurements are grouped with those determined for the Australian subspecies *P. graminis tritici* and *P. graminis avenae* (Waterhouse, 1930), and the U.S.A. determinations for the remaining grass subspecies and *P. graminis secalis* (Levine, 1923).

TABLE 1.

Comparison of Measurements of Spore Forms of Several Subspecies of Puccinia graminis.

Rust Subspecies.	Length in μ.			Width in μ.		
	Mean.	Standard Deviation.	Range.	Mean.	Standard Deviation.	Range.
Aecidiospores.						
P. graminis lolii	16·92	0·47	12·5–23·2	16·41	0·50	11·6–19·3
P. graminis avenae r.1	21·18	2·29	14·9–27·9	16·53	1·91	11·6–20·46
P. graminis tritici r.46	19·08	1·95	14·9–24·2	16·63	1·54	14·9–20·46
P. graminis secalis	17·10	2·0	12·0–22·0	13·46	1·17	11·0–16·0
P. graminis agrostidis	16·46	1·93	12·0–22·0	12·98	0·97	11·0–15·0
P. graminis poae	15·07	1·35	12·0–18·0	13·23	0·97	11·0–15·0
Uredospores.						
P. graminis lolii	27·61	0·56	22·3–31·6	18·08	0·48	14·9–24·2
P. graminis avenae (all races)	29·99	3·14	20·5–39·1	18·33	1·59	13·1–22·3
P. graminis tritici (all races)	31·04	3·70	20·5–44·6	18·25	1·88	11·2–22·3
P. graminis secalis	27·14	2·91	21·0–36·0	17·19	1·26	14·0–21·0
P. graminis agrostidis	22·37	2·61	15·0–30·0	15·68	1·12	13·0–18·0
P. graminis phlei-pratensis	23·95	2·41	18·0–30·0	16·88	1·32	13·0–20·0
P. graminis poae	18·64	1·51	15·0–23·0	15·78	1·06	13·0–18·0
Teleutospores.						
P. graminis lolii	48·75	1·78	32·8–63·7	16·93	0·56	11·6–21·2
P. graminis avenae r.1	46·95	6·54	29·8–67·0	15·27	1·85	11·2–20·5
P. graminis tritici (3 races)	44·32	7·40	29·8–74·4	15·90	2·27	11·2–22·3
P. graminis secalis	47·35	6·65	35·0–65·0	14·77	1·83	10·0–19·0
P. graminis agrostidis	40·30	5·87	25·0–55·0	14·64	1·72	10·0–19·0
P. graminis phlei-pratensis	41·30	4·78	30·0–55·0	15·63	1·46	12·0–19·0
P. graminis poae	36·90	9·13	20·0–60·0	15·52	2·07	11·0–21·0

The aecidiospores resemble those of *P. graminis poae* in being sub-spherical, but are much larger. In making the measurements, the uniformity in outline was striking, together with the tendency for the spores to remain in chains.

The uredospores are quite unlike those of *P. graminis poae*, resembling most closely those of *P. graminis secalis*. They are much larger than those of any of the other grass rusts.

The teleutospores agree in general with those of *P. graminis avenae* and again are quite unlike those of any of the grass rusts, being much larger. In the course of making the measurements, teleutospores having either a single cell or a series of three cells were noted.

On these morphological grounds the rye grass rust is different from any of the described subspecies.

Cultural Studies.

The recognized subspecies on grasses gave reactions in side-by-side comparisons as shown in Table 2, in which the new rust is again listed under the name *P. graminis lolii.* The designations of the reactions are those proposed by Stakman and Levine (1922).

Table 2.

Comparison of Reactions of Grass Subspecies of P. graminis *on Grass Hosts.*

Rust.	Grass Hosts and Their Reactions.			
	Poa compressa.	*Agrostis alba.*	*Phleum pratense.*	*Lolium perenne.*
P. graminis poae	4	0	;	;
P. graminis agrostidis ..	0	4	;	;
P. graminis phlei-pratensis ..	0	0	4	0
P. graminis lolii	0	0	;	4

On the basis of these grass reactions, *P. graminis lolii* is different from the recognized subspecies.

Turning next to the subspecies on cereals, inoculation experiments gave the reactions described hereunder:

P. graminis tritici (10 physiologic races) gave no attack on *Lolium* spp., and *P. graminis lolii* produced no lesions on any of the very many varieties of all the *Triticum* spp. tested.

P. graminis avenae (three races) attacked some individual plants of *Lolium* spp., but with a low frequency: the usual reaction was a tiny fleck. *P. graminis lolii* gave flecks on more than 100 varieties of oats: in the case of a few varieties like "Victory" it gives a "2–" reaction at very favourable temperatures.

P. graminis secalis does not occur in Australia, but side-by-side comparisons using three isolates of this subspecies which comprised two physiologic races showed that it is quite different from *P. graminis lolii.* Inoculations of 120 varieties of barley with the *P. graminis secalis* cultures resulted in 17 of them giving large semi-susceptible reactions ("2+"), 41 gave semi-resistant ("2") reactions, whilst the remainder were resistant (";") and ("1"). With *P. graminis lolii,* all gave resistant (";" and "1") reactions. An extensive series of inbred ryes which have been selfed for 17 years and which were fully susceptible to the two races of *P. graminis secalis* gave resistant reactions when inoculated with *P. graminis lolii.*

The cultural comparisons with the six subspecies of *P. graminis* indicate that *P. graminis lolii* must be regarded as a different subspecies.

Distribution of the Rust.

Serious damage is done to rye grass and cocksfoot when conditions are favourable for rust development, and other grasses of lesser importance are also damaged. Stems and leaves are attacked and not infrequently sori occur on the rachis and glumes.

In the course of the investigations the rust was isolated with the stated frequency from the grasses set out in Table 3. Many of the specimens were sent in for study by co-operators in scattered areas, whilst others were collected as opportunities offered. But it has not been possible to make an exhaustive survey of its occurrence, which may well be larger than indicated herein.

The practice has been to test each grass rust isolate on the four cereals and *Lolium perenne*: whenever an infection of the latter is shown, it is used to inoculate the original grass host in order to check its pathogenicity.

It will be noted that a number of these grasses have already been recorded as natural hosts for *P. graminis avenae*. This rust was found together with *P. graminis lolii* many times on a number of the grasses listed above. Determinations showed that it was either race 2 (not separated from race 1) or race 7 (not separated from

TABLE 3.

Naturally-occurring Grass Hosts of P. graminis lolii *with the Number of Isolates Examined from Each.*

Tribe.	Grass.	Number of Isolates.
Phalarideae	*Phalaris tuberosa* L.	9
	P. minor Retz	4
	P. paradoxa L.	1
	P. aquatica L.	1
Agrostideae	*Agrostis avenacea* Gmel.	8
	Echinopogon caespitosus C. E. Hubbard	18
	Dichelachne spp.	17
	Amphibromus Neesii Steud.	2
	Alopecurus pratensis L.	2
	Gastridium ventricosum (Gouan) Schinz et Thall.	1
	Stipa variabilis Hughes	1
	Lagurus ovatus L.	1
Aveneae	*Holcus lanatus* L.	5
	Aira cupaniana Guss.	1
	Trisetum flavescens (L.) Beauv.	1
	Trisetum sp.	1
	Arrhenatherum elatius (L.) J. et C. Presl.	2
Festuceae	*Festuca elatior* var. *pratensis* (Huds.) A. Gray	12
	F. elatior var. *arundinacea* (Schreb.) Wimm.	2
	Scleropoa rigida (L.) Griseb.	1
	Vulpia bromoides (L.) S. F. Gray	43
	V. Myuros (L.) Gmel.	3
	Dactylis glomerata L.	78
	Spartina alterniflora Loisel	1
	Lamarckia aurea (L.) Moench.	5
	Koeleria phleoides (Vill.) Pers.	5
	Poa annua L.	3
	P. iridifolia Hauman.	1
	Bromus Gussonii Parl.	2
	B. Benekeni G. Beck.	1
	B. breviaristatus Buckl.	1
Hordeae	*Lolium perenne* L.	99
	L. multiflorum Lam.	4
	L. loliaceum (Bary et Chaub.) Maz.	3
	L. rigidum Gaud.	17
	L. temulentum L.	12
	Hordeum leporinum Link.	1

race 3), and in one instance *Lamarckia aurea* (L.) Moench was infected by both these oat rust races in addition to *P. graminis lolii*. The details of these occurrences are set out in Table 4.

Apart from the joint occurrence on the one host of these two stem rusts, there were numerous cases in which the grass host of *P. graminis lolii* was also attacked by its leaf rust: *Lolium* spp. carrying *P. coronata lolii* E., and *Bromus* spp. attacked by *P. bromina* E. were notable examples.

In addition to the natural occurrence of the rust on grass hosts, studies were made of the plant house behaviour of many other grasses when inoculated under controlled conditions. These have shown that there are susceptible members within the following species, although in certain cases resistant members predominate.

Lolium remotum Schrank, *Briza maxima* L., *Bromus racemosus* L., *B. hordeaceus* L., *B. madritensis* L., *Ehrharta longiflora* Sm., *Phleum pratense* L., *Aegilops ovata* L., *Elymus paboanus* Claus., *E. canadensis* L., *E. junceus* Fisch., *Arrhenatherum elatius* (L.) J. et C. Presl., *Hordeum marinum* Huds., *Poa annua* L.

These plant house tests brought to light two important happenings, viz., that physiologic specialization occurs within *P. graminis lolii*, and that there are striking differences in the resistance and susceptibility of individuals within a species when inoculated with the same rust isolate.

TABLE 4.

Frequency Occurrence of the Two Races of P. graminis avenae *in Addition to* P. graminis lolii *on Various Grasses.*

Grass Host.	Frequency of Occurrence of Oat Stem Rust.	
	Race 2.	Race 7.
Lolium perenne	8	
L. multiflorum	1	
L. loliaceum	2	
L. rigidum	3	
Dactylis glomerata	29	2
Vulpia bromoides	30	2
V. Myuros	1	1
Echinopogon caespitosus	3	
Dichelachne spp.	1	
Festuca elatior var. *pratensis*	2	
Phalaris tuberosa	4	2
P. minor	2	
Agrostis avenacea	5	
Koeleria phleoides	3	
Lamarckia aurea	2	1

PHYSIOLOGIC SPECIALIZATION IN P. GRAMINIS LOLII.

Ten clumps of *Lolium perenne* growing in the University grounds were potted and tested with the stock culture of the rust. One gave sharp flecks (resistance), whilst all the others gave "4" reactions (susceptibility). The resistant plant and two of the susceptibles were retained. Later the plants were inoculated with a different isolate: the "resistant" clone produced "4" reactions in a side-by-side comparison in which the stock culture still gave flecks. A further interesting observation was that whilst the "resistant" clone was heavily attacked by *P. coronata lolii*, one of the two "susceptibles" was quite resistant to this leaf rust. It is thus clear that the breeding of a type with the combined resistance to stem and leaf rusts should not be difficult.

Further evidence of specialization came from *L. rigidum*. In a stand of rusted Wimmera rye grass at Tichborne, New South Wales, Dr. I. A. Watson collected a plant which was resistant. Seedlings from it segregated for resistance (fleck reactions) and susceptibility ("3" reactions) when inoculated with the stock culture. Typical plants of each sort were grown to maturity without bagging, and seed saved for further testing. These seedlings again segregated for resistance. Ten of these resistant ones were inoculated with the different isolate of *P. graminis lolii* and proved to be susceptible, thus giving proof of specialization of the fungus.

In yet another case, 16 seedlings of *Arrhenatherum elatius* were grown to maturity and each plant divided into separate entities for inoculation tests. Two of them, which gave susceptible "3" reactions with the stock culture, produced resistant "1" reactions with the second isolate.

Differential Resistance of Individuals within Species of Grasses.

In addition to the occurrence of resistance in *Lolium* spp., other cases of individual plants showing resistance were found in the following: *Agrostis avenacea* Gmel, *Hordeum leporinum* Link, *H. marinum* Huds., *Briza maxima* L., *Phalaris tuberosa* L., *P. minor* Retz., *Bromus racemosus* L., *B. Gussonii* Parl., *B. hordeaceus* L., *B. madritensis* L., *Lagurus ovatus* L., *Lamarckia aurea* (L.) Moench., *Ehrharta longiflora* Sm., *Phleum pratense* L., *Arrhenatherum elatius* var. *bulbosum* (Willd.) Spenner., *Dactylis glomerata* L., *Poa annua* L., *Lolium rigidum* Gaud., *L. multiflorum* Lam.

These inoculations showed that in some of the cases, a pot of 30 to 40 seedlings would give 3 or 4 resistant and the remainder susceptible plants, whereas the reverse was found in other cases. As a safeguard, seedlings showing the unusual reaction were grown to maturity in order to check their identity.

Inheritance studies were made in a few cases. In one, a collection was made of typical heads, nearing maturity, of *Bromus madritensis* growing in the Sydney University grounds. Pots of seedlings inoculated with the stock culture gave varying reactions: some were "X=", others a mixture of "X=" to "X+" and others "X‡". Eleven of the "X=" and 10 of the "X‡" were grown to maturity in the open.

In the next generation about 40 seedlings from each plant were tested. Of the 11 resistant ("X=") plants, one gave "X‡" (susceptible) reactions, five gave reactions varying from "X=" to "X‡" (heterozygous), and five gave "X=" (resistant) reactions. Of the 10 original susceptible ("X‡") plants, five produced seedlings giving "X=" to "X‡" reactions and five gave "X=" to "X" reactions—notably lower than the preceding.

For the next generation testing, representative resistant and susceptible plants from each group—72 in all—were grown on. Seedling tests of their progenies showed that three gave "X‡" (susceptible) reactions, nine gave "X=" to "X" (resistant) and the remainder "X=" to "X‡" (heterozygous).

Pure-lining of the grass was not carried out, and observations at flowering time indicate that natural crossing is to be expected. A genetical interpretation of the foregoing results was not possible, but the evidence shows that within a species there are marked differences between individuals in their resistance and susceptibility.

In the course of these seedling tests, the occurrence of albino seedlings was noted in the following grasses: *Lolium perenne*, *L. multiflorum*, *Agrostis avenacea*, *Hordeum marinum*, *Phalaris minor*, *Vulpia bromoides*, *Koeleria phleoides*, *Dactylis glomerata*, *Phleum pratense*, and *Agropyron trichophorum*.

Variegation was found in seedlings of *Lolium perenne* and *L. multiflorum*. These seedlings produced normal green leaves in later stages of growth, and their seedling progenies in no case showed inheritance of chlorophyll deficiency.

Resistant Grasses.

Apart from the occurrence of resistant and susceptible individuals within the species mentioned, resistance only was found in many species. This was of two sorts.

In the first, small hypersensitive flecks were produced. The grasses concerned are *Aegilops ovata* L. (some strains), *Phleum pratense* (some strains), *Briza maxima* (some strains), *Poa annua* (some strains).

In the second, resistance was complete, since inoculation produced no reaction whatever. These grasses were *Briza maxima* (some strains), *B. minor*, *Phleum pratense* (some strains), *Secale montanum* Guss., *Agrostis gigantica* Roth, *Poa compressa* L., *Agropyron scabrum* (Labill.) Beauv. (long and short glumes), *A. repens* (L.) Beauv.

Distribution of P. graminis lolii.

In respect of time, the isolates examined were distributed as follows: 1918 (2), 1921 (11), 1922 (2), 1923 (3), 1924 (7), 1925 (4), 1926 (13), 1927 (21), 1928 (8), 1929 (20), 1930 (24), 1931 (19), 1932 (18), 1933 (9), 1934 (45), 1935 (22), 1936 (21), 1937 (11), 1938 (19), 1939 (16), 1940 (9), 1941 (10), 1942 (2), 1943 (3), 1944 (2), 1945 (4), 1946 (4), 1947 (17), 1948 (9), 1949 (8), 1950 (6).

The source of the isolates was as follows: New South Wales, 249; Victoria, 4; South Australia, 25; Queensland, 2; Tasmania, 30; Western Australia, 1; Australian Capital Territory, 27; New Zealand, 31.

Discussion.

Delimitation of the units of classification of living things is necessarily difficult. It is particularly so in the organism under consideration because of the very wide variations it shows. The characteristics of *Puccinia graminis* Pers. as a species are clear as to general morphology and life history. Detailed studies, however, bring to light differences which have led to the setting up of groups which are called "subspecies" or "varieties". Statistical studies of the spore morphology reveal differences between them. Certain of them, e.g., *P. graminis phlei-pratensis*, may differ from others in their capacity to attack the barberry. The parasitic behaviour shown on certain hosts further emphasizes the differences between the subspecies and is, indeed, the main basis for their determination.

Within these subspecies further separation is made into physiologic races. These are characterized by the differential reactions they show upon groups of selected varieties of the host plant. Thus *P. graminis tritici* is split into more than 200 physiologic races which differ in the reactions produced on 12 particular varieties of wheat. The initial choice of these differential varieties is an empirical one based upon numerous "trial and error" tests. By more detailed study, as, for example, by using additional differential host varieties, isolates which appear to be the same physiologic race are separable further on the basis of the resistant or susceptible reactions now shown. These entities have been styled "biotypes" and may be regarded as individuals or groups of individuals which have the same genetic constitution.

In the present study no problem arises as to distinctions between biotypes and physiologic races. The question has to be considered, however, whether the stem rust on rye grass can rightly be considered as a physiologic race of one of the established subspecies (or varieties) of *P. graminis*, or whether it should be regarded as a new subspecies.

In his early work, Eriksson (1918) records as hosts of *P. graminis avenae* in Europe a number of grasses including *Dactylis glomerata, Lamarckia aurea, Vulpia bromoides*, and *Briza maxima*. These all have been found in Australia infected by *P. graminis*. In many of the cases the infection has been shown to be caused by one or other of the recognized physiologic races of *P. graminis avenae*, e.g., race 2 and race 7, of which the chief feature is their capacity to attack oats, even if some varieties are resistant. In many other cases the stem rust present has been unable to infect oats, or to do so very weakly in the case of only a few varieties in which a resistant reaction is shown. In yet other instances both these rusts were present on the grass host: one attacks oats and not *Lolium perenne*, and the other attacks *L. perenne* but not oats. A detailed examination has shown that both types can infect the barberry. Differences in the size of the spore forms is revealed by statistical examination of the measurements. In itself this would not be sufficient to warrant establishing a new subspecies, since significant differences are known to occur between certain well-recognized physiologic races within a subspecies. But when, in addition, the grass rust is unable to attack the cereal, then, instead of being regarded as a physiologic race of that cereal rust, it must be considered to be more widely divergent and to belong to a different subspecies.

The question then arises whether it is one of the recognized subspecies of grass stem rusts. Here again the rye grass rust is morphologically different when spore measurements are examined, and it differs also in its capacity to infect the normal hosts of these subspecies. Hence it must be regarded as a different subspecies.

Because of its frequent occurrence on *Lolium* spp. and the economic importance of these grasses, the name *P. graminis lolii* is proposed for this rust.

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