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Investigation of Viability of Uredospores of Puccinia glumarum

by Johanna Becker

Hhman Archiv. 19: 353-411. 1928

The entire article was translated by SP G. H. Reitel. The following is an abridgment of the translation.
Abridged translation of

"Investigation of Viability of Uredospores of Puccinia glumarum"

Author: Johanna Becker

The only sufficient control measure for rust is breeding of immune varieties.

Because of uncontrolled variables in the field testing of varieties is best done in the greenhouse.

Immune varieties may be lacking in certain desirable attributes which may be included by proper crossing and the hybrid must then be tested for rust immunity. Difficulties are compounded in that immunity to one species or race of rust does not insure immunity to other species or races. Since immunity cannot be obtained for all rusts within a single variety, it is necessary to create a variety immune to the most disastrous rust within an area.

In Germany and in the northern countries of Europe (Denmark, Sweden, Finland, England and Northern to Central France) it is primarily yellow rust that causes the most damage.

Pesola has reported losses of 44.8 to 47.6% with one hybrid as compared to 23.6 to 34.6% with another hybrid attacked by stripe rust in Finland. But it is not only yield that suffers - quality is reduced as well. In 1926 the grain quality in some areas of Germany, e.g., Pomeran, suffered by a yellow rust epidemic so greatly that it was difficult for many farmers to sell their grain at a favorable price or to sell it at all.

Since P. glumarum is recognised as the greatest parasite in Germany
the Institute for plant culture and plant breeding of Halle University

deals principally with yellow rust of wheat.

It was noted that yellow rust was difficult to maintain in the
greenhouse. Since a source of viable inoculum is a prerequisite to
testing varieties, attention was focused upon methods of maintaining
spore viability to permit testing throughout the year. Periods during
which work with *P. glumaria* in the greenhouse are difficult are those
of high temperature (July and August) and those of low light (November,
December, January).

Longevity of spores also plays a role in overwintering.

A. Viability of spores of *P. glumaria* and of *P. triticina*

Kirchner deems the maintenance of viability of spores through winter
as improbable and Hiltner contends that they lose viability during winter.
Beauverie states that spores spend the winter in free air on the living
plant and Biffen deems it possible that spores over-winter in the mild
climate of England.

Overwintering in European countries would correspond to maintenance
of viability for 5 or 6 months (October to March).

Beauverie placed grain bearing uredia of *P. glumaria* in storage over
winter to temperatures as low as -12 °C. From hundreds of tests started,
he obtained germination in only three.

Dacomet and Faex report that spores remain viable for one month when
stored dry. Matha also reports spores of *P. glumaria* have short life
but can survive temperatures of 2.5 to 5° for one month.

Most authors recommend dry, cool locations for storage.
**P. glumarum** is especially sensitive to external factors as compared to other rust species.

In Hungerford's tests on effects of desiccation he noted that **P. graminis** avenae, **P. holicina** and **P. triticina** maintained viability for 81–82 days by 2 of 6 methods of storage, but **P. glumarum** showed traces of germination only after 20 days, and only once was there a trace after 63 days.

She tried storage temperatures of 0, -5, 15 and 25°C.

Cabinets were held in a cold room – water was circulated in jackets around cabinets and electric heating coils in jackets were thermostatically controlled. Cabinets were insulated with asbestos.

2. Atmospheric humidity.

R.H. of 80, 60, 50, 40% were maintained by H₂SO₄ - water mixtures. (Table of Stevens).

Leaves bearing media were collected in the field in June of 1926 – pieces 4 to 5 cm. long were cut from the leaves bearing thick stripes. Leaves were collected in the morning, wrapped loosely in moist tissue paper and placed in a moist ceramic dish at 21-22°C. This was covered by a glass bell. In the P.M. the material was freshly sporulating; therefore, the further development of spores had occurred on leaves detached from the plant. (This is counter to Schaffint's (1909) finding that spores removed from the fertile tissue can no longer be influenced in this germination and maturation. In his paper an attempt to induce spores to mature on excised leaves was unsuccessful.

Becker did following tests:

Leaves with media were shaken so that spores dropped off. Leaves then handled as mentioned above. In addition culms of wheat plants bearing media on the leaves were washed with water to remove spores and were placed in a water
vessel at the same temperature and canned with a bell jar.

In both cases spores developed and germination was comparable to that of spores obtained from intact plants.

Spores shaken onto black-shiny paper and covered with a bell-jar and stored in the greenhouse at 19-21° showed increased germination on the second and fifth day after separation from the plant on the date of removal and the first day thereafter germination was 12%; it rose to 41% on the third day and to 52% on the fifth day. Subsequently inability decreased gradually.

III. Test Procedure

Longevity of spores was measured at 8-day intervals and from January 1927 at 14 day intervals. Both germination and infection were checked.

2. Infection and its dependency on external factors.

Leaves with uredia were washed in 1% dextrose with a brush and the spore suspension was smeared on leaves with a brush. Leaves were first rubbed with fingers to remove waxy coating. Haerning's Dickkopf was the variety used; 5 plants per pot.

Inoculated plants were placed on moist sand and covered with a bell-jar for 2 days. Plants were then removed to the greenhouse at 12-21°C and RH 50-70%. Temperature could not be held down during July & August even with shade covers and water cooling of glass roof, after it reached 25°C at noon.

For yellow rust, it is important not to exceed 25°C. It is especially sensitive from 3 to 10 days after infection. At 24-25°C subsequent pustule formation is only weak and stops completely when exposed for longer periods above 25°C.

No injury was apparent during the period with morning temperatures as low as 10°C, but there was some delay in pustule break which occurred in
12 to 14 days at a temperature of 15-20°.

Insufficient light results in longer incubation time and in weaker infection.

The vigorous condition of the host plant is decisive for infection success—this previously pointed out by Gassner and Appel and Rudorf. A 750 watt Osram nitro-lamp was used to supplement natural light on dark days and in December and January.

3. Germination tests.

First tests done in 1% dextrose later used distilled water to reduce bacterial contamination. Diluted plant extract was not satisfactory. Stored samples at 18-22°.

Since Eriksson and Henning had reported a change in molecular structure from cooling which stimulated germination samples were also tried outside, but no positive improvement in germination resulted from this cooling.

Germination was checked on the 2nd and 3rd days. If spores were still brown to yellow, they were counted on the 4th day.

Averages were made of 3 counts of 100 spores each.

Usually the majority of spores were gray and shrunken by the 2nd day and on the 3rd day, no colored spores were seen.

IV. Discussion of tests.

1. Checking longevity by infection.

A. Explanation of table.

A clearer picture of longevity could be obtained from infection than from germination of spores.
Scale used very severe - 1
severe - 2
weak - 3
very weak - 4

Column 4 of table - mean of evaluation of number of all successful infection on a given day.

B. General opinions on course of infections.

C. Influence of External Factors on Results.
   a. General Discussion

   Appearance of attenuation of viability is compounded by external conditions, especially low light, mildew. Table III shows all successful infections as percent of conducted inoculations for each month.

   In August infections decreased because of high temperature.

   The slight rise in infection in November and December is due more to method of computation than to external factors, since in December the 15-40% and 15-60% were dropped out of the calculations, hence # of conducted inoculations was decreased while the # of successful infections was not changed significantly. January shows a sharp drop since some inoculations did not succeed at all.

D. Maintenance of viability.
   
   (a) P. glumarum

   Best f for storage new or under 0°C.

   Lowest humidity used (40%) was most effective, e.g. 0-5°C at 40% retained viability for 433 days.

   0°C-50% viable only 291 days.
Storage temperature somewhat more critical than humidity, e.g. little difference in early phase of test vs 0-50% and 0-60% and a very high temperature 27-28°C yielded no infections at all.

High RH-80% rapidly damages infection potential.

After 16-18 weeks no definite difference seen between 0°-40%, 0°-50% and 0°-60% lots.

Near 0° and 40% RH P. glumarum withstood 433 days or 61.9 weeks at 5°, 179 days or 25.6 weeks.

2. Testing viability by germination.

The germination tests were conducted on the same dates as inoculations.

It was noted that the floating spores germinated best. Beaverie reported similar results for P. triticina and P. graminis t. He found no germination of the submerged spores.

Often no germination or isolated germination was found when infection was successful.

Results do not warrant a conclusion (Table IV) that high germination parallels high infection.

At 5°-40% RH germination held up best. At 80% it abated rapidly. At 0°C the differences in germination of the 0-40%, 0-50% and 0-60% material was not as distinct as in infection.

She noted that the germinal power is generally greater than the "shoot-energy", i.e., spores may master the strength to germinate but not enough to penetrate the plant tissue. Pelletier obtained similar results with P.g.t. but his lower limit of germination percentages at which infection
still occurred is relatively high in comparison to that for P. glumarum—namely 10%.

At lower germination percentages infection was uncertain or did not occur at all.

A 10% germination is considered good for P. glumarum. It was observed only during the first 12 to 14 weeks.

3. Viability of spores stored without adjustment of environmental factors. (Temperature in the room often reached 25°C.)

Leaves bearing media were collected in the field in June 1927 and stored in glass containers with lids loosely seated.

Infections with stored material 7 days old gave good results—all inoculated leaves infected.

After 12 days infective power had abated—only one leaf was infected although 5% germination was still obtained.

After 18 days only isolated germination was noted and no infection.

After 26 days neither germination nor infection occurred.

Detached spores stored on shiny black paper under a bell jar in the greenhouse in 3 tests: 25 May, 6 September, 27 September, 1927, retained germinability for 15 to 17 days after high germination on the first 7 days, i.e., 87-65% in May, 52% in September. In the latter instance spores matured in the first 5 days. After 17 days the spores appeared grey and shrunken under the scope. Hungerford reported similar results—from storage of spores on a glass plate under a bell. Germination was high (76-50%) in the first 5 days but abated rapidly after 20 days—to only 1% after 23 days and to 0% after 25 days. Storage of material at 0 to -5°C and at RH of 40% permits testing a number of strains on differentials simultaneously.
B. Research upon germination potential of spores of *P. glumarum*.

I. Literature references.

Erikss and Henn stressed the poor and erratic germination of spores of *P. glumarum*. Beauverie, Lang and Schaffint reported difficulty in germination of the spores. Only Hungerford had no difficulty in germination of fresh spores.

Becker found that in the same solution germination might be high in one test and low in another. Germination fluctuated between 37% to a few or none at all.

In 1909 Schaffint gave a new perspective to the problem. He stressed the importance of full ripeness as the decisive factor. Fully matured spores in his tests germinated rapidly and almost completely. Simultaneous maturation of all spores requires a temperature of 20 to 25°C. This work of Schaffint gave impetus to the work on spore maturation at Halle. The following facets were to be investigated:

(a) How long can one and the same mycelium produce germinable spores?

(b) Is decrease in productive power of the mycelium matched by germinable power of the spores?

(c) To what extent do environmental factors during their formation (especially duration of sunshine and temperature) influence germinability of spores.

(d) If the fungus mycelium is directly or indirectly inhibited in its development can it still deliver fully matured and germinable spores?

II. The preliminary condition for research.

Pots bearing infected plants were placed on cardboards covered
with shiny black paper and covered with a bell jar. (Temperature under bell fluctuated between 16-22°.)

III. Test Procedure.

Germination of spores which fell off the leaves were compared with those which were released by jarring the same plants and with those removed by a scalpel (after jarring).

Germination tests were done in distilled water in a hollow ground slide. Only the floating spores were considered since past experience had indicated that these were the more viable spores.

Samples were checked after 7 hours. Samples were checked the following day. Samples were checked on the second day after initiation.

Germ tubes usually grow away from the water and break off easily; this is an important consideration if examinations are made after the 2nd day.

3. The germination power of spores is highly dependent upon maturity. In every case the highest germination was obtained from the spores which fell off naturally and therefore were the ripest and oldest spores.

There was some indication that spores from aging mycelium were lower in germinability than those produced at the peak of mycelial development (3rd or 4th day after eruption).

Believed that increasing age of the mycelium has less effect on germination ability as such than on the rate of development of the spore. Spores produced by aging mycelium would develop more slowly, i.e., require more time to reach maturity and hence maximum germinable capacity than spores produced by virorous young mycelium.

One cannot assume that spores separated from the leaf are always
equally and fully capable of germination at the moment of separation. Yet it is possible that such separated spores can continue to mature and a few cases of this were found.

Spores which fall upon shiny paper under the plant are not all of the same maturity, but the immature spores find a more suitable environment for development than that which exists in the uredia where the moist atmosphere of transpiration inhibits the separation of water from the maturing spores. Maturity is related to loss of water.

Maturation is believed to be affected by light. Whether the effect of light is direct or indirect was not clearly determined by her.

Spores imbedded in media were observed to be larger, lighter in color and not as mature as those that fall off.

Overwintering

Mycelium lasts for at least 2 months in leaves during cold weather. On 3 April, 1927, a few overwintered thick, dark pustules were found. About one month of favorable weather was required for transmission of overwintered spores to plant parts formed in the spring. Rust was active in the field from 15 June to early July.
General Summary

1. Spores of *P. glumarum* maintain their viability for 433 days at light frost temperatures and relatively low R.H. which is evoked in a limited space by a sulfuric acid concentration of 50% corresponding to a humidity of 38%.

2. Spores of *P. glumarum* are very sensitive to temperatures above 0. They lose their viability much sooner than at light frost temperatures—at 5° after 179 days; at 15° after 89 or 47 days.

3. Spores of *P. triticina* are less sensitive to temperature. Their viability could be maintained for 291 days at 5° for 110 days at 15°; 41 days at 25°.

4. Spores of *P. triticina* maintained longevity for the longest period at light frost temperatures and RH of 49 or 60.7%.

5. Both types of rust lose viability rapidly at high RH—80.5%.

6. *P. triticina* is less sensitive to environmental factors than is *P. glumarum*.
   a. During examination of viability of uredospores this property is expressed by longer viability in connection with all unfavorable types of storage.
   b. During infection in the greenhouse *P. triticina* proved less sensitive to such influences of environmental factors as light and temperature.

7. In the course of the year the development of infection fluctuates according to infection conditions (especially light) and this to a greater degree with *P. glumarum* than with *P. triticina*.

8. The preference of *P. glumarum* for cooler temperatures in comparison to *P. triticina* could be compared to the occurrence of this rust in the cooler seasons and in cooler regions.
9. The maturity of spores is decisive for their germinal power as also observed by Schaffnit.

10. There must be a certain relationship between maturation of spores and duration of sunshine aside from the thermal influence of the sun. It is not clear whether the influence of the sun acts directly on the maturation of spores or by way of the fungus mycelium.

11. The maturation of the spore does not necessarily progress in relation to the fungus mycelium but may occur separately from the leaf.

12. Certain conditions (lack of light or possibly aging of mycelium) may delay maturation of spores by several days, just as the germinal process proper slows down and lasts several days.

13. Mycelium of P. glumarum in the tissues of leaves can tolerate cold periods—spores produced by such a mycelium are infective.

14. An excessive infection in the fall is unfavorable for transmission from fall to spring since the severely affected leaves have little resistance to weather conditions.

15. It was observed that the dissemination proceeds in spring from mycelium located in the leaf and that it spreads initially in the affected leaf.
Table 1
Puccinia glumarum
Testing of longevity of spores which had been held at

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Table 1

*Jia glutarum* in held at 0°, 5°, 15° C. (through infection.)

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1) 0°--50% b-Material nicht mehr vorhanden, da durch H₂SO₄ zerstört.
2) 5°--50% a-Material nicht mehr vorhanden, da durch H₂SO₄ zerstört.
3) 5°--50% a-Material nicht mehr vorhanden, da durch H₂SO₄ zerstört.
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<td>Percent</td>
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### Table IV

Puccinia glumarum

Testing longevity of spores which were held at 0\(^{\circ}\), 5\(^{\circ}\), 15\(^{\circ}\) and 25\(^{\circ}\)C., through...

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</table>

B
| Date          | No. of days | 0°-40% | 0°-50% | 0°-60% | 0°-80% | 5°-40% | 5°-50% | 5°-60% | 5°-80% | 15°-40% | 15°-50% | 15°-60% | 15°-70% | 15°-80% |
|--------------|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 29 September | 96          | 2.0    | 1.0    | 7.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | 2.6    | 6.6    | 2.3    | -      | 5.3    | -      | -      | -      | -      | -      | -      | -      | -      |
| 5 Oktober   | 102         | 9.0    | 5.0    | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | 3.3    | 3.3    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 13 Oktober  | 110         | 3.3    | 1.0    | 3.0    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 21 Oktober  | 118         | 2.0    | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | -      | 3.3    | 4.0    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 27 Oktober  | 124         | 2.6    | 4.6    | 2.3    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | 2.6    | 1.0    | 2.3    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 3 November  | 131         | 1.6    | -      | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | 1.0    | 1.0    | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 10 November | 138         | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 16 November | 144         | -      | 1.6    | 1.3    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | -      | 3.0    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 24 November | 152         | -      | 3.0    | 2.3    | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | 0.6    | 11.2   | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 2 Dezember  | 160         | 0.6    | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
|              | b           | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 8 Dezember  | 166         | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| 15 Dezember | 173         | -      | -      | 1.3    | -      | -      | -      | -      | -      | 2      | -      | -      | -      | -      | -      |
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* = O reinzelt Keimung
### Table 5a
Germination Test of 2 May

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- #: radically; ?: not possible to unequivocally determine germination; SK.: spores removed with a scoop; eingstr.: dried.
## Table 5b
Coordination Test of 2 May

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* - sporeductly; ? - not possible to unequivocally determine germination; SK. - spores removed with a corbel; eingstr. - dried
### Table 6
Summary of average of germination research from 2 and 25 May - and the results of germinations from 6 and 16 of September

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### Series B - 25 May

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### Series C - 6 September

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### Series D - 16 September

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<td>27</td>
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? - not possible to determine germination unequivocally; eing. - dried up; *) - sporadically; [D] - restoring spores
Table 7

Stored Spores Separated from the Leaf

<table>
<thead>
<tr>
<th>Month</th>
<th>1 day</th>
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<td>9</td>
<td>12</td>
<td>?</td>
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