The Germination of Ergot Conidia as Affected by Host Plant, and the Culture of Ergot on Excised Roots and Embryos of Rye

By

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The relation between rye and ergot may be investigated from two different point of views: 1) What is the effect exerted by infection on host plants? 2) In what is shown the effect of the host plant on the life of parasites? I have already given an account of investigations concerning the first point (Garay 1955). The present discussion deals with the second question. Earlier experiments on the relation between rye and ergot were intended to determine the stage of development at which rye is most susceptible to infection by ergot (Engelke 1902). Kirchhoff (1929) in particular treated this question in detail, and stated that the infection by ergot is generally more successful in the first stage of flowering than later. Bonns (1922) endeavoured to culture ergot on rye kernels in vitro. Similar experiments have been reported by Kreitmair and Kussner (1931) and by Schweizer (1941). On the basis of their work Michener and Snell (1950) added to saprophytic cultures of ergot an extract of rye seddlings, filtered through a Seitz filter, and stated that in this case the growth of fungus was accelerated. The growth stimulating effect of rye homogenate was investigated first of all by Berman and Youngken (1954). According to them an ear extract generally stimulates the growth of saprophytic cultures of ergot. The extract from mature ears had a more marked effect, than that of ears in the budding or flowering phases, which is somewhat contrary to the above mentioned data of Kirchhoff. The

active substance may be extracted by acetone and ether. Partly on the basis of these investigations I started my experiments.

Materials and methods

Test plants: Petkus rye and Bánkuti 1002 wheat; ergot race number 184. Ergot cultures were prepared in the usual way, and were allowed to grow on malt agar culture medium. For both infection and germination experiments (in hanging drops) conidia from 20—25 day old cultures shaken with sterile glass-beads were used. Germination took place at 20° C on the culture medium shown below:

KH_2PO_4	1.0 g	Asparagine	2.0 g
$MgSO_4 \cdot 7H_2O$	0.6 g	Saccharose	30.0 g
$CaCl_2$	0.5 g	Dist. water	1000.0 ml

The culture medium was adjusted to different pH values with citric acid and sodiumphosphate in order to determine the pH optimum for germination of conidia. The pH of tissues slices was determined by different indicators, the osmotic pressure was determined by plasmolysis methods. In view of the extraordinary variability of ergot I used ten replicates for each experiment, and the experiments were repeated at least three times.

Results

The pH. Figure 1 shows the pH dependence of germination of ergot conidia and the length in microns of hyphae developed during 48 hours.

As can be seen from the graphs, the maximal values are attained at pH 4.8 both in percentage of germinated conidia and in the length of germ tubes.

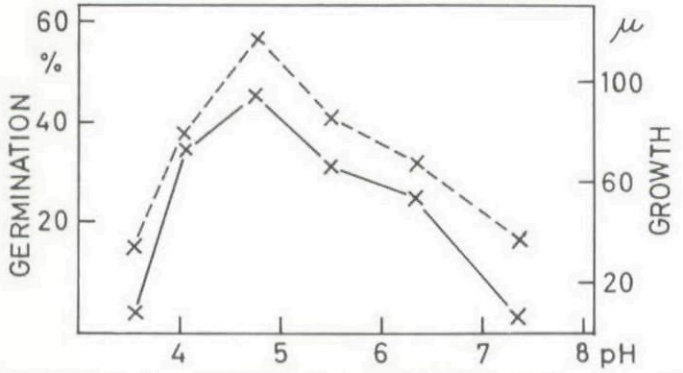

Figure 1. *Effect of pH on the germination* (———) *and the growth of germ tubes* (- - - - -) *of ergot conidia.*

Table 1. *The pH and osmotic pressure of different organs of rye.*

Material		pH	Osmotic pressure
Seedling	coleoptile	5.2—5.7	9.07
	root	5.2—5.7	9.07
Fully developed plant	node	5.2—5.7	3.35
	internode	4.0—5.0	9.28
	style	5.4—6.2	—
	glume	4.0—5.0	—

This value is pH 0.4—0.6 lower then estimated by others. The explanation for this may be sought, in my opinion, in the extraordinary variability of ergot and, on the other hand, in the circumstance that only the pH optimum for the growth of saprophytic cultures, and not that for germination, was determined by the authors mentioned. Growth and germination are not dependent on pH in quite the same way. If we examine the values of the graphs from this viewpoint, then we are able to state that only 1.5 per cent of conidia germinate at pH 3.3, *i.e.* only 3 per cent of the optimal value. At the same pH the length of germ tubes is 38 microns, *i.e.* 32 per cent of the optimal value. At other pH levels the same comparison held. This means therefore that curve showing the relationship between germination and pH is much steeper, then that for the growth of germ hyphae.

Table 1 shows the pH of different tissues of rye. As may be seen, all values fall into a range in which ergot conidia germinate well, although only pistils, stamens, lodiculae (Békésy 1939) internodes and nodes are susceptible to infection. Stoll and Brack (1944) give an account of the susceptibility of nodes, while I performed experiments showing the susceptibility of internodes. Figure 2 shows sclerotia growing on nodes and internodes.

Osmotic pressure. The optimal osmotic pressure for germination of conidia was determined on pure cane sugar solutions. The results are presented in Figure 3.

The second column of table 1 shows the osmotic pressure of different tissue sections of rye. That of the node, as may be seen, approaches the optimum for germination of conidia.

Effect of rye extract on germination of ergot conidia and on the growth of saprophytic cultures of ergot. In a series of experiments it has been proven that the extract prepared from detached parts of rye [fresh matter] with dist. water in the proportion of 1 : 25 does not stimulate the germination of ergot conidia, even if sterilized by autoclaving or filtration. This contradict the above mentioned data (Berman and Youngken 1954) showing that the extract stimulates the growth of saprophytic cultures. Results of my own

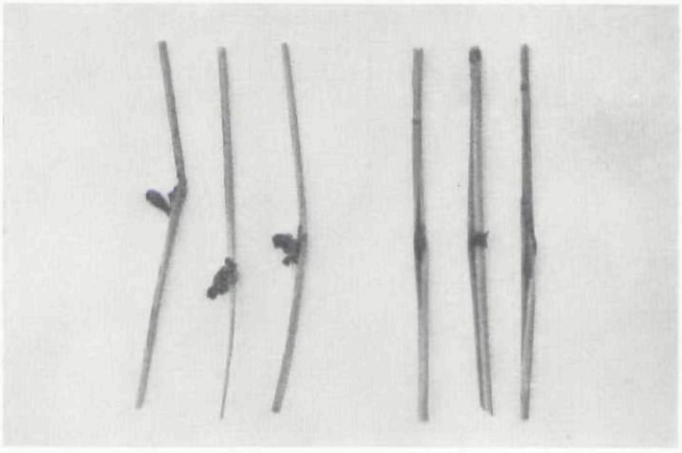

Figure 2. *Sclerotia growing on internodes and nodes of rye.*
[Photo P. Szalay]

experiments carried out on this question are shown in table 2. In this case saprophytic cultures were cultured on 100 ml, diluted malt-extract in Kolle flasks. To the experimental cultures 2 g powdered rye (dry matter) was added. The media were sterilized in an autoclave (1 atm., 20 min).

As may be seen from the table the growth of ergot is initially stimulated by the extract of ears, leaves, stems, nodes and roots. The strongest effect was obtained in the case of ears. On cultures older then 20 days, it was obviously not possible to observe stimulating effect. It is noteworthy that neither in the treated nor in the control cultures could any alkaloid be found by the van Urk reaction.

There emerges the question, whether infection produces in rye a substance

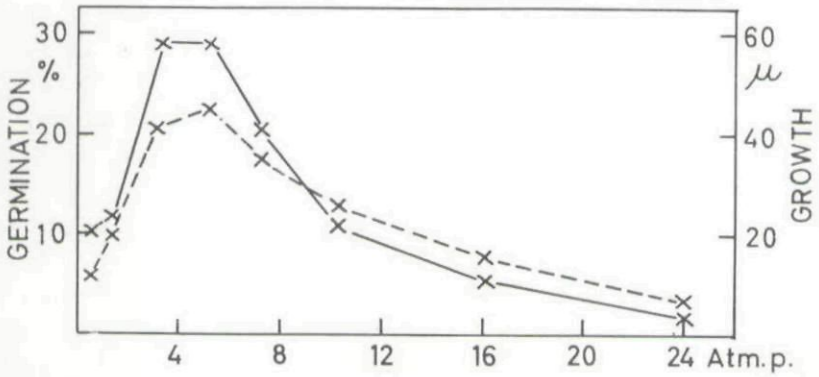

Figure 3. *Effect of the osmotic pressure on the germination* (———) *and the growth of germ tubes* (- - - - -) *of ergot conidia.*

Table 2. *The effect of rye extracts on the growth of saprophytic cultures of ergot.*

Organs	g. dry matter			
	after 10 days	after 20 days	after 40 days	after 60 days
Control	0.361	1.082	1.383	2.109
Ear	0.723	1.122	1.420	2.141
Leaf	0.585	1.057	1.281	2.250
Internode	0.572	1.088	1.265	2.118
Node	0.576	1.160	1.360	2.282
Root	0.560	1.087	1.350	2.202

which is inhibitory to the growth of ergot. To decide this problem the malt culture medium was enriched with the extract of infected and healthy rye. On both extracts the ergot developed at a similar rate.

It is of interest to note, that although the growth of saprophytic cultures is stimulated by the extracts mentioned, no higher yield of ergot could be obtained when the conidia before inoculation of rye were suspended in malt+ear extract in comparison with the control (malt). Using the above mentioned suspensions, 36 m^2 plots of rye were infected in five replications and in every case the ergot yield remained the same within the limits of error. The infections were carried out according Békésy's method (Békésy 1938).

Growth of saprophytic cultures of ergot on excised roots of wheat and on embryos of rye. Rye embryos have been cultured in the usual way by the method of Gautheret (1935). The cultivation of isolated roots of rye being very difficult, wheat roots were cultured by Gautheret's (1935) methods. Attempts were made to infect isolated roots and embryos. Ergot grows equally well in the presence of roots and embryos; its hyphae grow around the roots and embryos without penetrating into them, even when roots and embryos were injured with a needle. It was therefore impossible to infect isolated roots and embryos with ergot. In such »complex cultures» no kind of micro-sclerotia (Engelke 1902) or sclerotia formation could be observed. The cultures did not contain any alkaloid detectable by the Urk reaction.

It may be stressed that the growth of roots was inhibited when the inoculated ergot culture developed to a certain degree. The cause of this is not, as I have already indicated (Garay 1956), that the saprophytic cultures of ergot contain substances inhibitory to the growth of roots, but as we may assume, that the culture formes a complete impermeable layer excluding oxygen from the roots. So far this assumption has not been proved experimentally.

Summary

1. The optimal pH value for the germination of ergot conidia is 4.8 in the presence of citric acid sodiumphosphate buffer. The growth of germ tubes is not as sensitive to pH as the germination percentage.

2. Ergot is not only able to live as a parasite on ears and nodes but also on internodes.

3. Germination of ergot conidia proceeds most satisfactorily at an osmotic pressure of 3.8 atm.

4. Extracts from different parts of the rye plant did not stimulate germination of ergot conidia.

5. Autoclaved extracts of different organs of the rye plant stimulated the growth of saprophytic cultures of ergot. The strongest effect was shown by extracts of ears.

6. Extracts of infected as well as of healthy rye stimulated the growth of saprophytic cultures of ergot. It was thus impossible to show by this method that in ergot as a result of infection substances injurious to the parasite might be produced.

7. Saphophytic cultures grew well in cultures of rye embryos and on isolated wheat roots, but did not affect the host-plant. In such cultures it was not possible to detect the presence of alkaloids.

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