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ON THE MODE OF INFECTION AND PERENNATION OF THE SMUT OF "DOOB" (CYNODON DACTYLON PERS.)

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Introduction

Magnus (4) has recorded as many as three different smuts on *Cynodon Dactylon* Pers, caused respectively by *Ustilago paraguariensis*, *U. Dregeana* and *U. cynodontis* P. Henn, which can be easily distinguished from one another.

Out of these three *U. cynodontis* P. Henn is the only one, as far as the author knows which occurs in the plains of India and does great damage to "doob" an important fodder grass in this country. The author has noticed the presence of smutted "doob" at many places in the United Provinces of Agra and Oudh, the Punjab, Central Provinces and Bihar, and keeping in mind the distribution of the host, it seems highly probable that the disease occurs also in other parts of India. In waste places and on lawns under cultivation one can easily find smutted specimens during the greater part of the year.

From his unpublished notes, I learn that S. K. Basu, assistant to the Imperial Mycologist at Pusa, carried on some inoculation experiments in the year 1906 with the object of finding out whether the host is infected in the seedling stage. Unfortunately he did not get any positive results. In the year 1916 I took up this piece of work at the suggestion of Dr. E. J. Butler, to whom I am very much indebted for casual advice and for the loan of literature from his library at Pusa.

The work was commenced at Agra and, during the period September 1916—August 1920, numerous inoculations on seedlings of

different ages and healthy flowers were made, but none of the experiments were successful. After my arrival in Cambridge, the work was continued at the Botany school, with air-dried material of diseased grass obtained from India by post. At last, in July 1921, after repeated trials, I succeeded in finding out the right stage for seedling infection. So far I have had no success with blossom infection of this host.

The author has attempted in this paper to give a fairly complete description of this species—*Ustilago cynodontis*. Out of a very large number of experiments, details only of the more important ones, have been recorded which indicate that it is only in the very young condition that the seedlings of doob are susceptible to infection by *U. cynodontis*. The present work also shows that the smut of doob furnishes another clear case of the perpetuation of a disease through a perennial mycelium hibernating in the underground parts of the host.

A short description of the parasite

The disease manifests itself in the region of the inflorescence as a black sooty mass emerging from between the enclosing leaves. As a general rule the whole of the young inflorescence is destroyed, and is replaced by a collection of powdery spores while still enclosed within the enveloping leaves and seldom yields any grain. Sometimes a few spikelets near the tips look quite healthy, but a microscopic examination of such parts reveals in most cases an active mycelium inside. In addition to the spikes, the main axis of the inflorescence within the enclosing leaves often becomes smutted over the whole surface, or at least the portion just below the panicle. In this smut, it is interesting to note that spore formation on the leaf blade and the stem is also quite common.

A transverse section through the smutted part of the main axis of the inflorescence shows, here and there on the periphery, sporiferous layers which have replaced the outer tissues of the host. At some places one finds a complete destruction of the epidermis, cortical parenchyma and even a part of the sclerenchymatous pericycle, at others it is only the epidermis and the outer layers of the cortex which suffer. In some parts however all the structures are quite intact, but under the higher powers of the microscope one finds a copious supply of the mycelium, both in the outer and the more deeply placed tissues and very often in the pith itself.

In the case of a plant bearing a diseased tip or an inflorescence, the roots, rhizome and the aerial branches, though looking quite healthy externally, show in suitable preparations fungal hyphae here

and there, particularly in portions of the ground tissue near the vascular bundles. The hyphae lie between the cells but are occasionally also intracellular. They are hyaline and measure 2·5–3μ in diameter with knob-like haustoria projecting into the neighbouring cells. Inside a cell of the host the hyphae are seen to be copiously branched and rather intricately fused with their ultimate tips frequently lobed.

The sori in the region of the inflorescence occur very often in a group of five more or less tangled threads, each representing a spike practically all the flowers of which have been replaced by spores. After brushing off the powdery spores one finds only the remnants of the glumes here and there and the short pedicels.

The spores are sub-globose measuring 7–8μ in diameter with a smooth margin and, as sketched by Magnus (4) with a distinctly reticulate epispore.

The spores retain their viability for a considerable length of time, as McAlpine (6) has already pointed out. The author collected some in November, 1916 and, in August, 1918, after a year and nine months, they still showed fairly good germination.

The germination of Spores

The author has tried several times the germination of fresh as well as old material in distilled water and also in a weak solution of an extract of horse dung but has failed to observe the formation of a 3 celled promycelium, as stated by McAlpine (6). In horse dung extract the spores show very good germination, producing a typically 4-celled promycelium, as observed by Brefeld (1).

The origin of two promycelia from a single spore does not take place very frequently. If allowed to remain on a slide in horse dung extract for a week or so the spores show a marked increase in the production of sporidia and in subsequent sprouting as the liquid in the solution dries up. It is interesting to note that sometimes the spores of this smut, when allowed to germinate in distilled water and even in horse dung extract, do not produce promycelia but develop more or less elongated germ tubes instead. This happens more frequently when there is plenty of liquid on the slide. Very often one sees both types of germination going on side by side. Blackman (9) has stated that the teleutospores of rusts also may not produce any sporidia or even the characteristic transverse walls of the promycelium if their germ tubes are submerged.

Brefeld (2) has quite definitely brought out an important fact concerning the connection between the mode of infection of the host

and the type of germination of the spores of the smut affecting it. He has stated that a smut which infects its host in the seedling stage shows a copious production of the germs of infection following the germination of spores in manured earth, and that such spores retain their viability for a very long time.

The facts that the smut of "doob" infects its host in the seedling stage and that the normal type of germination of spores in a nutrient solution yields a profuse formation of conidia, and also that the spores of this smut retain their viability for a long time are in complete agreement with Brefeld's observations.

Again McAlpine (6) has remarked that in cases of blossom infection of the host the spores produce no conidia at all. It is very difficult to say how far this fact suggests the possibility of a blossom infection as well being effective in this case. It is not impossible, but my inoculation experiments with healthy blossoms have so far given only negative results.

Seedling infection

Brefeld has done a considerable amount of work on cereal smuts and has obtained 30—40 per cent. infection in the case of Oats by covering the seed grain with infected compost and humus soil mixed with half its amount of horse dung.

He has concluded that in Oats, infection by smut takes place in the seedling stage immediately after germination. Similarly Butler (3) has remarked that in Oats the greatest number of successful inoculations is obtained just after the primary shoot has emerged from the grain and that after the shoot has reached a length of one inch infection is rare.

The following is a summary of experiments conducted by the writer on seedling infection of "doob" at different times from October, 1916 to July, 1919 with the object of finding out the length of the period for which the host remains susceptible to an attack by the smut.

1. Aerial parts of seedlings of different ages (from 21 days down to 7 days after sowing) were inoculated with moist spores and kept covered with bell jars or glass cases for 2—3 days. In two of the experiments the soil was also dusted with spores.

2. Some seeds were sown in saw-dust and seedlings about three weeks old were inoculated at the underground parts and then grown on Knop's solution for a week. The inoculated parts were kept above the surface of the liquid. The seedlings were transplanted into a pot after that.

3. Seeds mixed with moist spores or soaked together over night were sown in pots on four different occasions in October, February, July and August. Spores used for these experiments were either collected fresh or such as had been kept dry in the laboratory for a variable period (2 weeks—5 months). As already pointed out none of these experiments were successful although the germination of spores was always found to be quite satisfactory.

4. In view of the possibility of infection taking place only in very young stages some more inoculations were attempted in July, 1921. Seeds soaked previously over night were skinned on the 6th of July and allowed to germinate on moist filter paper in a covered petri dish. Young seedlings were inoculated with spores on the 11th of July and were transplanted into a pot on the 20th of the same month. The germination of spores was found to be very good. A control experiment was also arranged. It is interesting to note that no smutted inflorescence appeared in that pot.

5. Seeds soaked previously for 2 days were skinned and dusted with spores on the 21st of July and were allowed to germinate on moist filter paper in a covered petri dish. They were dusted again with spores on the 23rd (2 days later) when just sprouting. Seedlings were transplanted into a pot on the 28th. Out of 15 seedlings transplanted 4 did not germinate much further and died. Two young shoots were tested for mycelium in October following and one was found to be infected. One diseased inflorescence was observed on the 6th of January, 1922. After that five more smutted inflorescences were noted on four different plants.

The pots in the last two experiments were removed to the tropical pit at the Botanic gardens in October 1921.

These experiments show clearly that the aerial parts of seedlings even when they are tender are not susceptible to attack by the smut.

The underground parts too are not infected after some time as indicated by experiment (2).

It is difficult to account for negative results in experiments where seeds and spores were sown together. Walker and Jones (8) have shown that soil temperature is a factor of great importance with regard to infection of onion seedlings by smut (*Urocystis cepulae*).

It is quite possible therefore that temperature of the soil in those experiments was not favourable for a simultaneous germination of seeds of "doob" and the spores they were dusted with. It may be pointed out that the spores of this smut show fair germination even at 29—30°C.

Most probably the seedling remains susceptible for a very short time, certainly not more than three or four days from the time of the

sprouting of the seed. Otherwise there seems to be no reason why, in experiment No. 4, where the seedlings were inoculated on the 5th day after the soaked seeds were put on moist filter paper for germination, there should have been no infection. It may be pointed out here that in warm weather seeds which have been previously soaked sprout in 2—3 days.

In experiment No. 5 undoubtedly, conditions for infection were most favourable, since the seeds had been soaked for 2 days before being dusted with spores, to allow a simultaneous germination to take place so that by the time the seeds sprouted sporidia might be available.

Blossom infection

Among the cereals, the loose smuts of Wheat and Barley furnish clear examples of infection through the stigma. Brefeld (2) has remarked that blossom infection is the ruling form of infection, if not the only one, in the case of the loose smut of Wheat.

Having observed no diseased inflorescences on seedlings inoculated in October, 1916, the author attempted inoculation on healthy blossom of "doob" in April, 1917. One cannot be altogether sure of hitting at the time most favourable for infection of the stigma, but care was taken to inoculate flowers about the time when their pollen was being shed. Some healthy inflorescences showing ripe anthers in many of the flowers were removed with the main stalks and their cut ends dipped in a bottle containing distilled water. In some cases pot plants bearing healthy inflorescences were used. Spores were sprayed with an atomizer, or the spikes were dipped in a watch glass full of spores which had been allowed to germinate for two or three days in water, and the plants were kept covered for 2—3 days.

From April, 1917 to July, 1920 as many as seven trials were made with blossom infection. About two weeks after inoculation the inflorescences were dried and the seeds collected. Seeds from inoculated blossom were sown about four months after the date of collection and after disinfection with 2 per cent. Formalin. Seeds collected after the last experiment conducted in India in July, 1920 were sown on the 11th of May, 1921. As already stated above none of the inoculations with healthy blossom have been successful so far.

Brefeld (2) has remarked that we have to reckon in the case of most of the smuts with two places of infection *i.e.*, the young seedling and the blossom, and have therefore to consider the possibility of both these forms of infection being effective in individual cases. As regards the possibility also of blossom infection in the case of Oats, he has not come to any definite conclusions, but has expressed that

it is of lesser significance. Keeping in mind the marvellous success that has been achieved in checking the smut of Oats in different countries by the disinfection of the seed grain, one can safely say that Brefeld's observations are quite conclusive.

It is possible though very unlikely that blossom infection in the case of "doob" may be proved later on to be equally effective.

A perennial mycelium

The presence of fungal hyphae in the rhizome of a diseased plant of "doob" suggests to one the possibility of the perennation of this disease. In order to find out how far the mycelium inside the underground parts was effective in producing smutted blades and inflorescences year after year the following experiments were conducted.

1. Diseased "doob" (bearing smutted inflorescences) was transplanted in four tubs on the 10th of September, 1916. The tubs were kept in the open and observed occasionally. During a period of nearly four years only smutted inflorescences or shoots with smutted tops were noticed. The largest number of smutted shoots appeared during the summer rains. No healthy inflorescence was ever noted in them.

2. Diseased grass was transplanted in a tub on the same day as No. 1. The aerial parts were cut on November 20th and the tub was kept covered with a glass case for two weeks. Fresh shoots on examination invariably showed the mycelium inside. Only diseased inflorescences were noticed as in No. 1.

3. Healthy doob (inflorescences without any trace of smut) was transplanted in two pots on the same day. Only healthy inflorescences appeared in them.

4. Healthy grass transplanted like No. 3. The aerial parts were cut on November 20th and the pot kept covered for 2 weeks. Fresh shoots showed no mycelium.

5. Healthy grass transplanted in a pot showed two diseased inflorescences on November 25th. The plant bearing such inflorescences was dug out and on examination its rhizome as well as aerial shoots showed the mycelium inside. Other plants in the pot showed only healthy inflorescences.

The unexpected appearance of two smutted inflorescences in the last experiment was evidently due to the fact that a diseased rhizome bearing only young shoots (certainly without flowers) was somehow transplanted along with grass bearing healthy flowers.

From the observations recorded above it is clear that the perpetuation of this smut does not depend very much upon fresh

infection either of the seedlings or the blossom. Undoubtedly the mycelium in the underground parts is responsible for the perennation of the disease and its appearance in an epidemic form during the summer rains. a period so favourable for a luxuriant growth of the host. During the earlier part of summer (May and June), in the Punjab and United Provinces, one finds, on account of intense heat and drought only dried blades of this grass above the ground. The mycelium during such periods may well be said to be in a state of hibernation within the underground parts. Obviously young shoots arising from a diseased rhizome (Expt. 2.) are infected from the same source and ultimately end either in diseased inflorescences or in such shoots as show the formation of spores at their apices and terminal leaf blades.

It is unfortunate that cultivators use the underground parts rather than seeds of "doob" for the purpose of raising a lawn and that is the reason why one seldom sees a lawn altogether free from smut. An indiscriminate transplantation of diseased rhizomes, which are indistinguishable from the healthy ones except when in flower, is very undesirable.

It is possible however to avoid diseased rhizomes by making a careful selection at a time when the grass is in flower and by using only such stuff as has no diseased plants within a yard or two.

It seems likely that by the use of seed which has been disinfected the disease may to a considerable extent be checked.

I wish to express my warmest thanks to Mr. F. T. Brooks, under whose supervision I worked at the Botany School, for some very useful suggestions in connection with this work. I am also very grateful to Mr. C. C. Calder, of the Botanical Survey of India, for a very reliable collection of healthy seeds that he sent to me on request.

Summary

The smut of "doob" caused by *Ustilago cynodontis* P. Henn is of very common occurrence on the plains of India. Besides destroying the whole of the inflorescence which seldom yields any grain the smut frequently makes its appearance at the apices of short aerial shoots and their terminal leaf blades. The latter fact makes this disease far more serious because "doob" is an important fodder grass in this country.

The host is infected in the earlier stages of germination of the seed and ceases to be susceptible very probably within three or four days after its sprouting. So far blossom infection has not been found to be effective.

THE SMUT OF "DOOB" (CYNODON DACTYLON PERS.)

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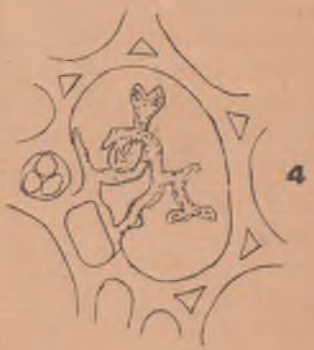

Ustilago cynodontis P. HENN, or the Smut of "Doob".

The perpetuation of this disease is brought about also by a perennial mycelium which hibernates in the rhizome beneath the surface.

The use of rhizomes in cultivation is therefore responsible to a great extent for the spread of this disease, and should be discouraged.

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Explanation of Plate

Fig. 1. A photograph showing two smutted inflorescences, × 3.

Fig. 2. Spores of *Ustilago eynodontis* and their germination without the formation of promycelia.
(*a*) and (*b*) spores, (*c*), (*d*) and (*e*) germination of spores after 48 hours, (*f*) and (*g*) germination of spores after 72 hours × 1000.

Fig. 3. Spores showing normal type of germination and promycelia × 750.

Fig. 4. Transverse section of a diseased shoot showing intracellular hyphae × 1000.

Fig. 5. Longitudinal section of a diseased shoot showing hyphae and haustoria × 1000.