THE IDENTIFICATION AND CULTURE OF SPORIDESMIUM BAKERI SYD.

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THE BIOLOGY DIVISION of the Soil Bureau was established to gain an understanding of the living organisms in soil. Since 1955 a team of workers at the Taita Experimental Station, Lower Hutt, has been studying the ecology of fungi, yeasts, bacteria, protozoa and earthworms in a number of soils. Two main projects have been undertaken:

(1) To study soil microorganisms under tussock grasslands and the introduced pastures that are replacing them.

(2) To study soil microorganisms under forest and the introduced pastures that have replaced them.

By the end of 1956 the first project had been completed (part published in the *N.Z. J. Agric. Res., 1,* 913-1005, 1958) and some initial work had begun on the second. At this stage there appeared to be an important relationship between the type of vegetation and the soil microbial population, while marked seasonal changes had been recorded both in numbers and species, particularly in summer. It was with this background that the team, in late 1956, began to consider seriously the possibility that soil microorganisms might play a part in the facial eczema problem. A microbiological cause for facial eczema had been considered by other workers in earlier investigations (Cunningham *et al.*, 1942; Simpson *et al.*, 1957) but no definite evidence had been obtained.

These possibilities were discussed with the Department of Agriculture who kindly offered the use of their experimental plots. In February, 1957, studies were initiated on the facial eczema experimental plot at Claudelands Showgrounds, Hamilton, in co-operation with Ruakura Animal Research Station. At the same time organisms were also examined from other areas being studied as part of the second major soil microbiological project.

In collaborative work with Drs di Menna, Ross and Stout, large numbers of soil and pasture herbage microorganisms were examined. Herbage microorganisms were included in the investigations to establish the relationship between soil and herbage microbial populations. Altogether some 6,000 organisms were isolated and cultured (4,500 from soil, 1,500 from pasture herbage), of which 1,600 were from the experimental plot at

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Claudelands Showground. Ten organisms from soil or pasture were selected for particular study because of their high numbers, marked seasonal changes, and growth under particular conditions. Three yeasts, five fungi and two bacteria were examined during 1957 in a series of trials in which 18 feeding experiments with guinea pigs were carried out at Taita Experimental Station. In addition, several "beaker test" experiments were conducted at Ruakura Animal Research Station. All these investigations were apparently negative as far as facial eczema was concerned. At this stage all that was hoped for was a clue indicating that a microorganism was implicated.

A general account of the investigations which culminated in the demonstration that a fungus is capable of producing facial eczema disease in lambs (Thornton and Percival, 1958) has already been given (Anon., 1958a).

Isolation

During 1957 soil and pasture samples were collected systematically from the areas being examined; samples from Claudelands Showgrounds being collected with the help of J. C. Percival.

From samples of turf containing fungal mycelium from Claudelands Showgrounds which Percival sent in March, 1958, a number of fungi, including *Sporidesmium bakeri Syd.* were isolated. Several days later Percival obtained scrapings from a gang-mower used on Claudelands Showgrounds. Microscopical examination of these scrapings showed the presence of large numbers of fungal spores in which those of *S. bakeri* were predominant. This material gave a positive beaker test (Percival and Thornton, 1958). This was the important clue implicating a microorganism in the problem.

Sporidesmium bakeri was also isolated from a sample of dead ryegrass sent by Dr G. W. Butler from experimental plots at Massey Agricultural College, Palmerston North, in April, 1958. Samples of grass clippings collected by Dr Butler from a ryegrass plot on which sheep had developed facial eczema, were freeze dried and when examined microscopically showed large numbers of spores of *S. bakeri,* particularly on dead grass.

Cultivation

Six cultures of *S. bakeri* were obtained from turf samples sent by Percival in March. These grew reasonably well on several

laboratory media with a suggestion that some organic amendments might be necessary. The cultures did not spore very profusely. One culture which spored a little more than the others was selected for further investigation. Initially, this strain, CL, gave a negative beaker test when grown on Raulin-Thom and Czapek-Dox solutions, and sent to Ruakura Animal Research Station for examination. Further cultures on these media were grown and fed to two guinea pigs at Wallaceville Animal Research Station. Results as far as facial eczema were concerned were apparently negative.

Because large numbers of *S. bakeri* spores had been observed on toxic grass samples sent by Butler, and because of the high content of spores in the mower scrapings obtained by Percival, means of enhancing spore production were sought. During the cultivation of strain CL another strain was isolated and found to be high sporing. It is now called strain C and was the culture examined by the Commonwealth Mycological Institute and is deposited in their herbarium as Culture No. 74473.

The growth and sporing capacity of strain C was studied on a number of undefined media. Different concentrations of media components were employed and in some experiments mineral salts, sugars, and growth factors were added. Growth was also studied on synthetic media. As a potato-carrot extract medium was easy to prepare, favoured uniform growth and gave good yield of mycelium with copious spore production, it was employed in further studies.

At this stage spores scraped off a culture of strain C on the potato-carrot medium gave a positive beaker test. Mycelial felts and culture fluids of *S. bakeri,* strain C, also grown on this medium were macerated and sent to Ruakura Animal Research Station for animal feeding experiments. Percival (1959) has already shown that this material and other cultures grown at Ruakura were toxic to animals.

To date four strains have been isolated, grown and tested for toxicity at Ruakura and Wallaceville Animal Research Station. A number of other media have also been tried to enhance toxin production.

Identification

Identification by the Commonwealth Mycological Institute, Kew, England, of the fungus as *Sporidesmium bakeri* Syd. estab-

lished that an early tentative identification of the fungus as a species of *Stemphylium* (after Barnett, 1955), was in error (Anon., 1958 a, b). *Sporidesmium bakeri* was first recorded and described on banana leaves from the Philippine Is. Records at the Commonwealth Mycological Institute show that this fungus has been described from various parts of Africa and from Malaya, Mauritius Is., Jamaica, Trinidad on such plants as rice, maize, sorghum, *Ipomoea,* bananas and tobacco. It has been isolated from air in Queensland, Australia. The fungus does not appear to have been recorded at Kew from grassland in any other country than New Zealand. The taxonomic position of this fungus is at present in doubt, and it is probable that the name *Sporidesmium bakeri* will be changed.

Mature spores or conidia are broad oval, pale brown to brown in colour, mainly with three transverse septa, or cross walls, minutely roughened, constricted at the septa and measuring 18 to 27 μ long by 9 to 15 μ (Hughes, 1953).

The conidia develop singly as the blown out ends of short fertile branches or conidiophores at first oval, roughened, pale brown, without septa, gradually expanding and becoming three-septate. In mature spores the centre two cells possess longitudinal septa, whereas the terminal cells do not. The conidia break off near the top of the conidiophore leaving the main portion of the conidiophores on the parent hyphae. The conidiophores are crowded and irregularly arranged on the hyphae and are between 4 and 6 μ long (Hughes, 1953).

Some Preliminary Field Observations

Sporidesmium bakeri occurs as small black spots or patches mainly on dead pasture plant material, usually in association with other fungi, but is not the only fungus to form such spots or patches. The fungus does not appear to be parasitic but lives on dead plant material. Spores closely resembling those of *S. bakeri* have been observed in pastures in the spring in both the North and South Islands and this suggests that the fungus overwinters as conidia. Actively growing and sporing colonies of *S. bakeri* have been found in the spring in dead grass leaves at Gisborne and Wellington.

At Manutuke Research Station, Gisborne, large numbers of spores of *S. bakeri* have been recorded in a paddock where quantities of litter have been produced by frequent mowing and

the return of the clippings. These spores are blown by wind, particularly when the surface of the ground is disturbed, on to leaves of grasses and clovers where they would be ingested by sheep.

Measures for control of the fungus will depend on detailed basic knowledge concerning the life history of the fungus and the stages of growth in the field at which it is likely to be vulnerable. Until this information is obtained, it will not be known whether the fungus can be controlled by management practices or by the use of fungicides.

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DISCUSSION

DR T. R. VERNON: It seems clear that this fungus has its place in the story of facial eczema. Dr Thornton has shown you that it is present on the litter in the field, and at first sight it seemed to be a fungus that was common. Some years ago I took part in a survey of the airborne spores over New Zealand and in certain cultures we classified quite a number of isolates as *Stemphylium*. Looking at the picture in those first few days after the discovery that facial eczema was caused by a fungus, one wondered why a fungus that was so widespread should be implicated at all in a disease that was so sporadic, that was located in specific areas and that seemed to depend on very special conditions. With greater knowledge and better identification of the fungus itself, the story seemed to sort itself out. And the present definition of *Sporidesmium bakeri* indicates a fungus

that requires certain special conditions for its growth, so that the sporadic nature of facial eczema certainly fits in with the specific requirements of this particular fungus.

A good deal of the work that has been done so far with *Sporidesmium* has been with a high sporing strain. A number of speakers have suggested that the spores are largely to blame in producing the disease. It seems to me that the toxin is not only distributed in the spore and the mycelium, but also in the culture filtrate. The mechanism of getting the toxin into the sheep is not quite clear, and I think this is a point that might still be taken up.

It is rather extraordinary that this fungus so far reported only in the tropics should be in New Zealand, and distributed over quite a wide area. This is something that needs a great deal further investigation. Just how far over New Zealand can we expect to find this fungus living? Work that has been done so far indicates that it grows over quite a wide range of temperatures and in places far removed from the areas where facial eczema occurs. Maybe, however, it requires some very special conditions under which it can produce its toxin. J. C. Percival showed in one table that the culture, after one day's incubation when no spores were produced at all, was capable of producing toxin. He showed also that after prolonged incubation, the toxin seemed to disappear. Are we then primarily concerned with the production of spores or can we expect facial eczema to occur when the fungus is in the mycelial stage? If that is so, why has a high sporing strain been selected for the experimental work?

A bottle neck seems to be the need for using animals to test toxicity but the fungus must be studied to the nth degree. *Sporidesmium* is one of the imperfect fungi. There are, no doubt, a multitude of variants. How are these distributed? How many of them are toxic? What are the required conditions for toxic production? I am sure much of this work is already under way.

Q.: *What is the toxicity of the low sporing strains?*

DR R. H. THORNTON: Two low sporing strains have been tested for toxicity and to date have been shown to be non-toxic. A moderately sporing strain has been shown to be toxic.

Q.: *Are there two sources of facial eczema—one derived from grass and the other through the consumption of the spores of* Sporidesmium bakeri? *J. C. Percival has mentioned that the mycelium has been proved to be toxic. Does the mycelium occur in the green grass or just in the dead leaf?*

J. C. PERCIVAL: Identical liver damage in guinea pigs has been produced by feeding either toxic grass or *Sporidesmium* spores. Toxic grass has numerous spores adhering to the green leaves but with invariably greater spore numbers on dead leaves.

Dr Brook has established that *Sporidesmium bakeri* is a quick-sporing saprophyte which colonizes the dead material of many pasture plants. Most of the toxicity of the green leaves is probably due to spore contamination derived from the dead leaves.

DR R. L. M. SYNGE: There is always the possibility of other species of fungi producing the toxin. It would be very dangerous if that possibility was not taken into account.

DR A. T. JOHNS: According to Percival's results, the toxicity of the culture went up to a maximum with increasing numbers of spores. Subsequent decline in toxicity accompanied a continued increase in spore numbers.

Q.: *Would Dr Thornton define the nutritive requirements of* Sporidesmium?

DR R. H. THORNTON: Dr D. J. Ross has been studying the nutritive requirements of *Sporidesmium bakeri.* It grows most rapidly on non-defined media but it also grows well on simple synthetic media containing inorganic or organic nitrogen compounds. Many different carbohydrates can be utilized. Added growth factors do not appear to be essential.

DR P. D. SEARS: I understand the fungus does not grow on sweet vernal and that there are some pasture plants whose waxes are toxic to the fungus. The back of the perennial ryegrass leaf is a suitable surface for the spores and this seems to be the reason for the high toxicity of a ryegrass pasture.

Q.: *Since the spores are mainly found on the dead leaves, how does this fit in with the recommended procedure of concentrating sheep on to a pasture during danger periods? Sheep which had been shut up would presumably, on this account, be more subject to facial eczema than those not shut up.*

DR C. P. MCMEEKAN: All we know about shutting up sheep is that it works. One possible explanation is that the intake of sheep is reduced so much when they are concentrated at the rate of 300 to 400 per acre that the dose rate of the toxin is at a level which the animal can handle.

Q.: *What are the prospects of growing the fungus in deep culture?*

DR R. H. THORNTON: In a preliminary trial the fungus grew moderately well although the yield of mycelium was less than from static surface cultures. Spores were not produced. Further work is planned on this aspect.

Q.: *Is the toxin anti-genic?*

DR R. H. THORNTON: That question is a little premature as yet. We do know from our own work with soil and mycorrhizal fungi that fungi are capable of producing anti-genic substances.

D. MCFARLANE: A year or two ago many of the Ruakura staff separated the green leaves from the dead leaves of toxic pasture. The green portion when fed to guinea pigs, was toxic. That finding now seems to be in direct contradiction to what is known of the growth habits of the fungus.

DR C. P. MCMEEKAN: The experiment referred to provides a very good illustration of the need for work to be checked and double-checked, the need for back-tracking in research and the need more than anything else never to take anything for granted even if it looks good. Perhaps even at this stage we should not accept that *Sporidesmium bakeri* Syd. is responsible for facial eczema. The guinea pig was accepted as an assay animal because we went back over the early work on small animals. Another illustration can be found in the fact that in 1938 one of the best mycologists in the Commonwealth and his colleagues spent a long time investigating the idea that a fungus may be involved in facial eczema. I think it would be fair to say that the fact that they had done so and got negative results helped to delay quite considerably the interest in a possible microbiological background to facial eczema.

It is because of these and other examples that I make no apology for the fact that 40 people for 12 hours a day for 2 days separated green leaf from dead leaf in a sample of grass of known toxicity and found animals fed on the green material developed severe lesions, while those fed on the dead material showed only microscopic lesions.

DR A. T. JOHNS: I see no reason why Dr. McMeekan should apologize for the results obtained in feeding green and dead grass. His team fed green grass and found it to be toxic. This is a positive result. The mycologists believe that fungus grows and spores on the dead material and hence consider that the dead material should contain the toxic fraction. However, from what J. C. Percival and his colleagues have done in culturing the fungus, it appears that some of the toxin can be in the culture medium. It seems possible that the toxic principle could be washed down from the fungus growing on dead material into the soil taken up by plant roots and translocated within the green plant.

Dr K. J. Mitchell has observed mycelial growth on the cut ends of grass under the special conditions when you get plenty of exudate. Dr McMeekan has stated that the shutting up of sheep to stop them obtaining much grass does prevent eczema.

All these observations do seem to me to at least leave the question open as to whether the animal obtains the toxin from the green or dead grass. I do not think, in view of the positive results obtained by feeding green grass, that the dead material can be considered to be the only culprit.

N. T. CLARE: It has been observed in guinea pig feeding trials that the animals will select the green material and leave the dead. The guinea pigs have shown facial eczema lesions under these conditions.

J. C. PERCIVAL: I believe that both the dead and green leaves of different pasture species can be toxic, their relative toxicities depending upon the amounts eaten and their respective toxic spore populations. Guinea pig variation could quite easily have influenced the results in the experiment in which the green portion of the toxic pasture sample was highly toxic and the dead portion only slightly toxic.

Q.: *Is any work being undertaken to find methods of control in the field?*

DR C. P. MCMEEKAN: A series of field experiments have been laid down in the Waikato on three facial eczema prone areas and another series are under way at Manutuke. Several approaches are being tried. Attempts are being made to encourage the development of the fungus to increase the incidence of toxicity. In other trials methods of inhibiting the growth of the fungus are being tried using fungicides which laboratory work at the Plant Diseases Bureau has shown to be capable of killing the fungus but are non-toxic to animals. An antibiotic approach is being used in one treatment. Harrowing of the pasture is also being done to see if this will disturb the micro-environment and thereby reduce the incidence of the fungus in the pasture. In addition to these trials, there are a larger number of plots in both districts where more fungicides are being examined in conjunction with the beaker test and the guinea pig assay, both of which should give a measure of success or failure with the fungicides.

P. D. SINCLAIR: At the Manutuke research area we get quite a strong positive beaker test on all control plots and also on all the fungicide treated plots.