THE ASSOCIATION OF SPORIDESMIUM WITH FACIAL ECZEMA *

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ALTHOUGH MANY FARMERS and faddists have proposed numerous and often novel ideas as to the probable cause of facial eczema, in saner circles two main theories have been acceptable. One was that the toxin came from an aberration in plant metabolism and the second was that it was produced by a plant/microorganism association.

In September, 1956, members of the microbiological section of the Soil Bureau, located at Taita, discussed with the Ruakura facial eczema team a mutual interest in a further microbiological approach to facial eczema. Ruakura interest had been stimulated by its chemical extraction work by White (1959) which had reached the stage of suggesting a possible microbiological origin of the toxin.

As the Taita team had been conducting a broad investigation of forest, pasture and soil microbiology, it was decided to include pasture and soil samples from eczema prone areas.

Clare (1959) has mentioned that in 1957 a large scale collection and grass preservation programme was started on 11 areas in the Waikato in our attempts to overcome the shortage of toxic grass. The serial mowing of each area at two-day intervals, and assaying of samples by guinea pigs was also expected to provide more accurate data on the time of onset, duration and level of toxicity of pasture than had been possible by the lamb grazing technique. These data were also considered to be essential to any comparative toxicity/microorganism studies.

In 1957 most of the soil and pasture samples for microbiological examination were obtained from the Claudelands polo field, one of the grass collection areas with a bad history of facial eczema. Sampling was intensified when eczema was expected to occur and samples were obtained periodically throughout the year so that the seasonal population changes among the different species of yeasts, fungi, and bacteria could be followed.

* Earlier reports on this work have been published elsewhere (Percival and Thornton, 1958; Thornton and Percival, 1959).

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At Taita the predominant species that had been isolated were assayed with guinea pigs but with negative results. However, if a microorganism was to be incriminated, there was insufficient evidence to indicate whether the absence of toxicity was due to either failure to isolate or to incorrect cultural requirements of the organism.

Towards the end of 1957 other data already mentioned by Clare (1959) gave much more information about the accuracy of the beaker test/toxicity relationship. It was thought that location of the origin of the beaker test substance might provide a lead to the origin of the toxin. The microorganisms that had been bio-assayed at Taita were submitted to the beaker test at Ruakura but all samples were negative.

In 1958 the beaker test was introduced into the grass collection programme and the main objective was to collect large quantities of beaker test positive grass. This was only partly successful because of variations in the intensity of the test at the two-day sampling intervals, and to the fact that, although some "pilot" grass samples were positive the subsequently collected bulk samples of grass were often negative to the beaker test. This aroused interest as to the reasons for these anomalous beaker test results.

In 1958 the Claudelands polo field was not readily available, but it was kept under observation by beaker test checks at weekly intervals. On 13 March this area gave a strongly positive test, one of the few recorded for the season to date. All mowable grass was preserved from this area between 13 and 20 March in anticipation of it being toxic. During this period beaker test values varied greatly from the numerous samples collected from the different parts of the field each day. Further investigations revealed that the highest beaker test gradings were associated with the grass samples cut closest to the ground and these samples contained a very high proportion of dead grass. Even completely dead patches of grass gave high beaker test gradings.

On 15 March during the course of these investigations mycelium was observed around grass plants near ground level and turf samples were sent to the Soil Bureau.

On 23 March the operator of the gangmower noticed a black dust hovering over the mower as the area was being prepared for polo. A light dusting of the suspected spore material collected

from the mower and subjected to the beaker test was strongly positive.

Further samples scraped off the mower were also strongly positive and from one of these samples E. P. White (pers. comm.) established that the major component of the beaker test substance with a m.p. of 260°C was present at a high level.

A sample of the dust sent to the Soil Bureau was identified in April by R. H. Thornton (pers. comm.) as predominantly *Stemphylium* spores. This genus was reported by Dye and Vernon (1952) as being a very common fungus, widespread throughout New Zealand.

The identification in October of this fungus by the Commonwealth Mycological Institute at Kew as *Sporidesmium bakeri* Syd., an organism of much more restricted distribution, fitted more closely to the facts relating to the incidence and distribution of facial eczema in the field.

Further interest was aroused, however, when R. H. Thornton (pers. comm.) reported that this same species of fungus was predominant in the mycelium collected from Claudelands on 15 March at a time when the grass was suspected of being toxic. Lambs that had been grazing this area since 18 February were killed on 16 April and showed eczema liver damage. This indicated that the grass was toxic at some time between 18 February and 16 April. The guinea pig assays of grass collected between 13 and 20 March proved that the grass at Claudelands was toxic during this period.

A microscopic examination of grass samples from Claudelands showed spores were present on both green and dead leaves although they appeared to be more dominant on the dead material. An examination of non-toxic grass samples, however, showed that in some instances spores were also present.

A clinical outbreak (Butler, 1958) of facial eczema occurred in March, 1958, on an experimental area at Massey College, Palmerston North. When inspected by the writer on 11 April the incriminated paddock was a mosaic of dead and green areas. As only the green areas had been sampled for beaker testing and were positive it was suggested to Dr G. W. Butler that the dead grass areas should also be beaker tested to substantiate the Claudelands evidence, that strong beaker tests were associated essentially with dead grass material. This fact was confirmed and grass samples sent for examination in May to the Soil Bureau, also con-

firmed the presence of the same species of spores that had been observed in March at Claudelands as giving a positive beaker test.

To confirm that the substance giving the beaker test reaction was contained in the spores, it was essential to obtain a cultured sample of spores. The procedures used by Thornton and Ross (Thornton, 1959) to induce sporulation will be described in the next paper of this symposium. The high sporing Strain C of *Sporidesmium* grown on potato-carrot medium and sent to Ruakura gave a positive beaker test. On receipt of Strain C it was grown on potato-carrot-agar medium and the spores collected by washing. This pure spore sample was again shown by White to contain the beaker test substance and was the final necessary proof to substantiate the earlier claim as to the origin of the beaker test substance.

That spores of *Sporidesmium* contained the beaker test substance must be regarded as the first scientific evidence of a possible fungus/facial eczema toxin relationship.

The fact that the grass was toxic at the same time as the original mycelium was collected, reinforced the need to test this fungus for possible hepatotoxin production.

The initial assays were with the high sporing Strain C which had been isolated by Thornton from mycelium forwarded from the Claudelands area.

At Ruakura Strain C was grown on potato-carrot-agar medium at 24° C for incubation periods of 4, 7, 11, 14, and 21 days. Each incubation period was separately assayed except the 21-day batch which became contaminated.

The fungus was fed to guinea pigs as macerated cultures of mycelial felts and media at a daily level of 25 ml mixed with grass for a 35-day test period. To cover possible low level toxicity the fungus was also fed as ether extracts containing the equivalent of 80 ml of culture per day. These extracts were fed by the standard 28-day extract assay procedure as developed by Perrin (1957).

None of the guinea pigs fed daily the 80 ml extracts of culture survived the 28-day test period. All died within 7 to 14 days. The pig fed material incubated for 4 days, died on its 7th day but had a normal liver. The pig fed material incubated for 7 days, died on the 9th day on 29 August, 1958. This was the first guinea pig ever fed on fungal material to exhibit macroscopic liver damage characteristic of the lesions produced in guinea pigs fed toxic grass. The specificity of eczema liver damage was confirmed by histological examination.

Animals fed on 11- and 14-day cultures also exhibited macroscopic liver damage, subsequently confirmed histologically as eczema liver damage. These two pigs also had enlarged livers, a characteristic symptom often produced by the feeding of highly toxic grass.

Guinea pigs fed 25 ml a day of the macerated cultures survived for longer periods and also showed characteristic eczema liver damage. In this instance the 4-day-old culture was also toxic. A culture grown by Thornton and Ross also showed toxicity when assayed at Ruakura.

It was obvious from these series of assays that a toxin produced by the fungus *Sporidesmium bakeri* was capable of producing a lesion in the liver of a guinea pig typical of that associated with the disease known as facial eczema. It was also clear that cultures were toxic for incubation periods ranging from 4 to 14 days.

Preliminary attempts were also made to test separately the mycelium and spores for the presence of the toxin. The experiments carried out indicated that the toxin can occur both in mycelium and spores. To assess more accurately the effect of different incubation periods on the levels of toxin production, *Sporidesmium* was grown on potato-carrot media under the same cultural conditions as previously, but for incubation periods ranging from 1 to 21 days. For assay accuracy three pigs were fed on each culture. To enable pigs to survive the full test period only 10 ml of culture was fed daily. Some trace of toxin was present on the first day with a sudden rise in toxicity on the third day to a peak on the fourth day. Toxicity appeared to be slightly less even by the seventh day with a marked fall at the fourteenth day to a trace of toxin present at the twenty-first day. Liveweight gains progressively decreased and increased as toxicity rose and fell. Liver size was appreciably affected at the higher levels of toxicity.

Rapid sporulation occurred in the four-day culture which was toxic, but toxicity also occurred in the complete absence of spores, in the three-day culture.

Further cultures grown at the Soil Bureau and sent to Ruakura for assay suggest that both media, and strain of fungus, are also important in toxin production. There is evidence to suggest that high level toxicity can be attained on an inorganic medium.

Since facial eczema is primarily a disease of ruminants it was considered essential to reproduce the complete facial eczema syndrome by feeding cultures of *Sporidesmium* to sheep.

In the preliminary attempts, five lambs were fed different amounts of fungal cultures for one week. The livers of two only showed small macroscopic lesions characteristic of facial eczema liver damage, which were confirmed by histology. The data suggested that at the feeding level used, one week was too short a period for full lesion development. A lamb fed fungal fluid for 21 days became photosensitive on the 16th day, with a Van den Bergh reaction of over 2 mg/100 ml. When killed on the 35th day the carcass showed symptoms of icterus and the liver was at an advanced stage of fibrosis. This animal thus showed the complete facial eczema syndrome, namely liver damage, icterus and photosensitivity produced by feeding a culture of *Sporidesmium bakeri* Syd.

One of Dr J. F. Filmer's first comments after this discovery was that "if this is true, then it raises more problems than it solves". How right he was!

Whereas before "F.E. Day" a few workers had been toiling in a broad, unrewarding, and unpopular field of research, now many scientists have been attracted to what has become a narrow field of infinite length.

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Discussion: See p. 87.