PRELIMINARY INVESTIGATION ON THE DETERIORATION OF MAIZE INFECTED WITH *DIPLODIA ZEAE*, (Schw.) Lev.

By Paul A. van der Bijl, M.A.

(Communicated by Mr. I. B. Pole-Evans.)

(Read July 15, 1914.)

Sound maize and food products prepared from sound maize are recognized as most wholesome articles of food, but whether maize infected with this fungus is equally wholesome, or whether it can be considered safe for consumption is an altogether different question.

Smith and Hedges* state that possibly corn infected with *Diplodia* may have been the cause of the death of great numbers of negroes in the Southern States during the years 1906–1909 from a disease known as pellagra, which follows the consumption of mouldy corn meal and mouldy hominy.

They also consider it worthy of inquiry as to whether this fungus may not be the cause of the so-called "corn-stalk" disease prevalent among cattle in the Western States.

Evidence has also been brought by various farmers of the Province of Natal, who state that not only does this disease cause severe loss to their crops, but also produces paralysis and death amongst stock fed on infected cobs, especially if the cobs were damp and not properly dried out. Sheep especially have been reported very susceptible.

In August, 1912, Government Veterinary Surgeon Webb, stationed at Mooi River, Natal, submitted specimens showing this disease, and wrote: "I am also sending you some specimens of mealies taken from fields in which cattle have become sick, showing symptoms of intoxication and paralysis due, I believe, to poisoning with the Fungi on the mealies."

Mr. I. B. Pole-Evans (Chief, Division of Botany, Union Department of Agriculture), visited Natal in October, 1912, and referring to this

* E. F. Smith and F. Hedges, "Diplodia Disease of Maize (Suspected cause of Pellagra)," Science N.S. vol. xxx., No. 758, pp. 60–61.

disease as the cause of paralysis and death in stock, writes: "All the cobs examined from the various farmers prove to be infected with *Diplodia zeae*, (Schw.) Lev." *

Black and Alsberg † have devoted some time in finding an adequate, and, at the same time, simple test for determining whether corn products are fit for human food. Such a test they consider to be the determination of the acidity of corn—a test well known in both Italy and Austria, where the Governments have enacted stringent laws regulating the quality of corn and corn meal which may be sold or imported.

The authors, besides giving a description of the method of determining the acidity, give and discuss various other tests. Their work and suggestions have been closely followed and frequent reference will be made to it.

A.—Chemistry of Maize Inoculated with the Fungus in Pure Culture, Compared with the Chemistry of Control Maize not so Inoculated.

On the 11th of January, 1914, a large flask containing 1 lb. crushed maize soaked in 500 c.c. distilled water and autoclaved was inoculated with spores of *Diplodia zeae* and incubated at 25° C. (Fig. 1). A control flask which was otherwise similarly treated, but not inoculated, was incubated at the same time.

On the 23rd of January the contents of the two flasks were shaken out and placed in the sun on trays to dry. The inoculated flask showed a rich pure white cottony growth, the control was sterile.

On the 24th of January, the two sets were separately ground up in a coffee mill, the control being taken first.

With this ground material the following tests were made :—

(1) *Acidity.*

Method.—Ten grams of the inoculated and ground maize was placed in a 50-c.c. measuring flask which was then filled to the mark with 85 per cent. neutral alcohol (prepared by distilling 95 per cent. alcohol with the addition of quicklime). The flask was kept at room temperature for 24 hours with an occasional shaking.

At the end of the above period the contents were filtered into a measuring funnel; 25 c.c. of the filtrate was placed in a beaker and the

* Union Department of Agriculture, Division of Plant Pathology and Mycology, Annual Report 1912–1913.

† O. F. Black and C. L. Alsberg, "The Determination of the Deterioration of Maize With Incidental Reference to Pellagra, U.S. Department of Agriculture." Bureau Plant of Industry Bull. No. 199.

washings of the funnel added. 100 c.c. distilled water and a few drops of phenolphthalin was added. $\frac{n}{20}$ Sodium Hydrate from a burette was then run into this with frequent stirrings, and when the colour was pale pink the amount of Sodium Hydrate added read off. This multiplied by 10 gave the acidity of 1,000 grams of the substance in terms of cubic centimetres normal Sodium Hydrate.

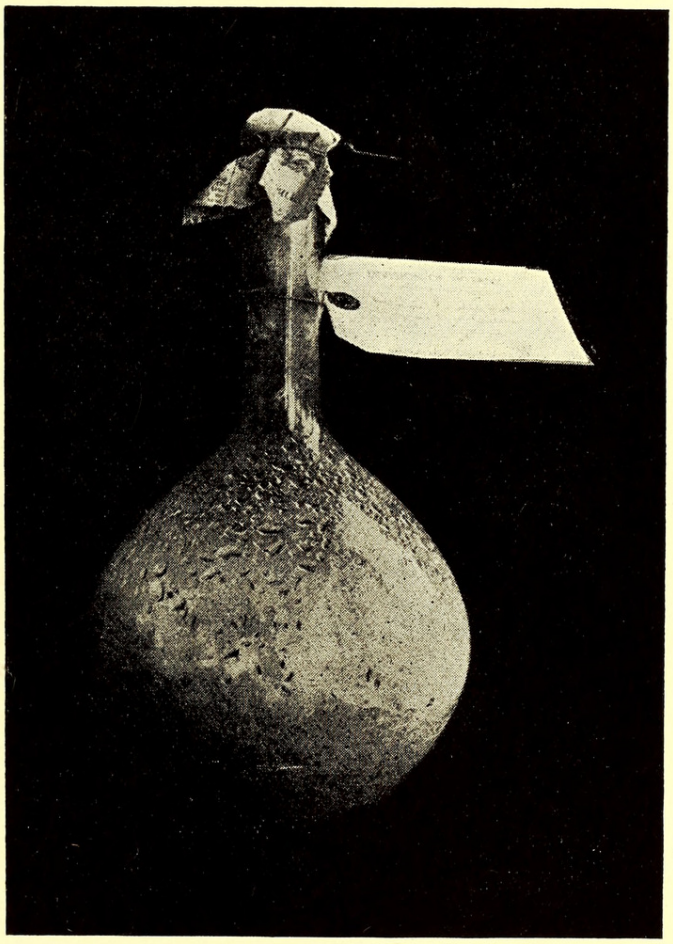

Fig. 1.—Flask-culture of *Diplodia zeae* 10 days at 25° C.

Result.—

	Acidity in c.c. Normal NaOH.
Control	53 c.c.
Inoculated	65 c.c.

Black and Alsberg advise that corn meal should not have a greater acidity than 30 c.c. They found that extraction for 24 hours while not giving maximum acidity, nevertheless gives uniform and comparable results which is all that is desired for practical purposes. Warming the

flask during extracting was found objectionable because much zein goes into solution, and unless a thermostat be used other differences are produced. The effect upon acidity determination of slight changes in the concentration of the alcohol, they found to be insignificant. Later experiments* carried out by the *Office of Grain Standardization, United States of America, Department of Agriculture*, found that the degree of acidity is directly proportional to the percentage of damage and to the commercial grading at terminal markets, but inversely to the percentage of germination.

(2) *Moisture and Ash Determination.*

These were determined in the usual way, following the suggestions made by Black and Alsberg :—

Result.—

	Control.	Inoculated.
Per cent. Moisture	6·8	13·5
,, Ash	1·61	7·42

Black and Alsberg state that in Italy the amount of ash is regarded as significant and over 4 per cent. considered a sure sign of deterioration. The ash content of good corn should be in the neighbourhood of 1·5 per cent.

Nearly all the ash is located in the germ, and the ash determinations, therefore, indicate how far a meal has been degerminated and the starchy endosperm layer removed.

(3) *Fat Determination.*

Five grams of the inoculated and control substance respectively were extracted with water-free Ether for five hours in a Soxhlet's Apparatus. The Ether was then evaporated, the residual fat dried and its weight determined.

Result.—

	Per cent. Fat in Dry Material.
Control	4·8 per cent.
Inoculated	5·6 ,,

As mentioned by Black and Alsberg, the greater part of the fat is situated in the germ, and the fat determination would enable one to determine whether meal has been adulterated with the germ.

* J. H. Besley and G. H. Baston, "Maize Acidity Investigation." Agricultural Journal of the Union of South Africa, vol. vii., No. 4, pp. 549-552.

The germ contains about three times more oil than ash, hence of the two determinations the latter would be the more delicate for determining the degree of degermination.

(4) *Nitrogen Determination.*

The Nitrogen was determined by Kjeldahl's process. One gram of the dried substance was treated with 30 c.c. strong Sulphuric Acid and heated until there were no free solid carbonaceous particles present. Eight grams dry Potassium Sulphate was then added and the heating continued. After a while a few grams Manganese Dioxide was added gradually and the heating kept up until all the carbonaceous material was oxidized. When the mixture had lost nearly all its colour the flame was removed and the mixture allowed to cool.

The cooled acid solution was next poured into a large flask containing 100 c.c. distilled water. The small flask was rinsed out three times with distilled water and its contents added to the large flask. 100 c.c. strong Sodium Hydrate (357 grams to the litre) was added to the contents of the large flask; and the cork, fitted with a bent glass tube through which steam was to pass, and a splash-head leading to the condenser, immediately inserted. Steam was then passed through the large flask and the distillate absorbed in 20 c.c. $\frac{n}{1}$ Sulphuric Acid. This passing of steam was continued until red litmus paper held over the exit was not changed.

The distillate was then removed and titrated with $\frac{n}{1}$ Sodium Hydrate using Methyl Orange as indicator.

Result.—

	Per cent. Kjeldahl's Nitrogen in Dry Material.
Control	1·404
Inoculated	2·8

(5) *Reaction of Ori.*

This reaction depends upon the fact that moulds contain enzymes known as catalases, which are capable of oxidizing Hydrogen Peroxide and thus liberating Oxygen. This enzyme also occurs in corn kernel, but is less abundant. Black and Alsberg found it located practically exclusively in the germ, and point out (1) meal from good whole corn will decompose Hydrogen Peroxide to a certain extent. (2) Thoroughly degerminated meal ought not to decompose Hydrogen Peroxide. (3) The extract of germ gives almost as powerful a reaction as spoiled meal.

(4) Meal might be made from corn spoiled in such a way that the moulds were situated mainly in the kernel. If in milling the corn be thoroughly degerminated and carefully bolted, the greater part of the moulds might be removed. Such meal might show a high acidity and yet give a weak reaction. (5) The action of the enzyme is destroyed at 60° C. and higher.

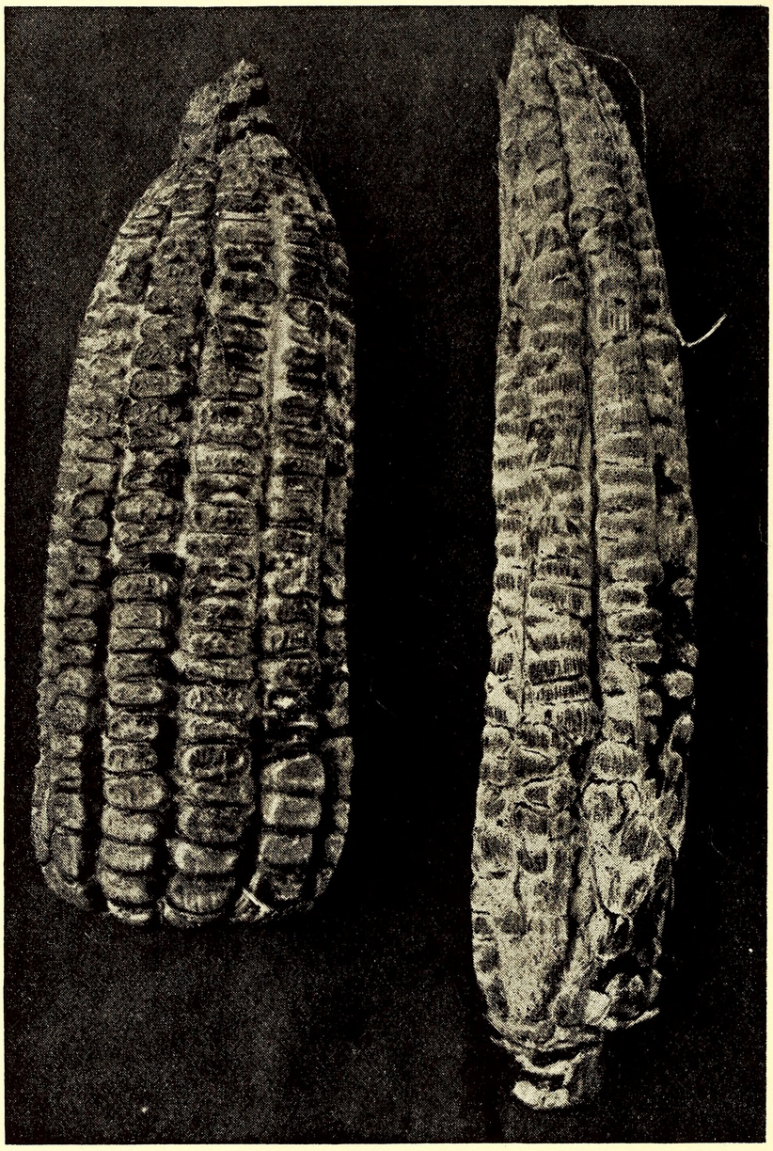

FIG. 2.—Maize inoculated with *Diplodia zeae* by puncturing through husks 49 days after inoculating.

Carelessly dried corn might therefore lose its power to decompose Hydrogen Peroxide. (6) With the above limitations the reaction of Ori has its value and might be developed into a useful and rapid quantitative method.

Method.—Five grams of the substance was extracted overnight with 15 c.c. of a 50 per cent. aqueous solution of Glycerin. The extract was then filtered through paper; 1 c.c. placed in a test tube and 4 to 5 drops of a 3 per cent. solution Hydrogen Peroxide added.

Result.—

Inoculated.....	*Very small bubbles after a little while.* The bubbles increase in size and the effervescence becomes more rapid.
Control	No bubbles.

B.—Chemistry of Maize Inoculated with the Fungus in the Garden, Compared with the Chemistry of Healthy Control Maize.

In view of the fact that Maize may undergo certain changes when sterilized in an autoclave, it was thought desirable to carry out the tests already mentioned, also on maize inoculated in the garden, and to compare the results with that obtained from healthy maize which had stood alongside the inoculated.

Maize cobs (Fig. 2) inoculated on the 16th of March, 1914, by puncturing through the husks with a sterilized needle dipped into water containing the spores of the fungus, were picked on the 5th of May, 1914, and placed in the sun to dry. Control cobs were picked at the same time and treated similarly.

On the 12th of May, 1914, the kernels of the diseased and control cobs respectively were ground up separately in a coffee mill, the control being ground first.

With the ground-up material the following tests were made, following the methods described in part A of this paper.

(1) *Acidity.*

	Acidity in c.c. Normal NaOH.
Control	25
Inoculated	53·71

(2) *Moisture and Ash.*

	Percentage Moisture.	Percentage Ash.
Control.....	5·5	1·48
Inoculated	7·5	1·94

(3) *Fat.*

	Percentage Fat in Dry Material.
Control	5·5 per cent.
Inoculated	3·4 ,,

(4) *Nitrogen Determination.*

	Per cent. of Kjeldahl's Nitrogen in Dry Material.
Control	1·404
Inoculated	1·755

(5) *Reaction of Ori.*

Control	Small bubbles after a while, but effervescence never vigorous.
Inoculated	Bubbles at once and effervescence becomes vigorous.

SUMMARY.

1. Though previous publications have dealt with the deterioration of maize generally, I believe this to be the first where attention is directed to one particular parasitic organism. These preliminary investigations have opened up a wide field for further research and investigation.

2. Maize infected with *Diplodia zeae* has a higher acidity than healthy maize.

3. Infected maize gives Ori's reaction distinctly. Healthy maize gives only a slight effervescence. The fact that the control of the crushed mealies inoculated after autoclaving gives no effervescence, is due to the destruction of the enzyme in sterilizing. In the inoculated material this enzyme originated from the fungus.

4. Infected maize has a higher percentage of ash brought about by the fungus using the organic substances as food, but leaving the salts, etc.

5. The percentage of Nitrogen is also higher in the inoculated material.

6. The fat content of maize inoculated in the laboratory and of maize inoculated in the field differ remarkably as compared with their respective controls. In the laboratory the inoculated has a higher percentage of fat, and in the field a lower, as compared with their respective controls. In the field the fungus lives in and destroys primarily the germ, *i.e.* the region containing the most fat. The cobs were inoculated while young, and hence the fungus hindered the development of the germ and as a result a lower percentage of fat. How far this fungus is able to live on fat or to convert it into Fatty Acids has still to be determined.

7. For practical purposes the acidity determinations appear the most useful, though with the limitations stated the reaction of Ori could be developed into a delicate test.

The Botanical Laboratories of the
Union of South Africa,
P.O. Box 1294,
Pretoria.

June 23, 1914.

Note.—Since writing this article a letter has been received from the principal Medical Officer, Basutoland, who writes:—

"In reply to your (the Agricultural Officer, Maseru) inquiry as to the prevalence of Pellagra in Basutoland, I beg to inform you that except in the Leribe District, no cases of that disease have been notified. The supposed cases of Pellagra above referred to were attributable to the consumption of damaged or unripe grain.

"Scurvy is common at times, and its prevalence is, as above, attributable to the same cause."