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VIRUS DISEASES OF PLANTS [1, 2]

BY

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Plant Pathology or the study of plant diseases is, in a sense, one of the most modern of the applied sciences. So modern is it that it is still largely in the leading strings of the mycologist and, in fact in India, the term mycology is considered as synonymous with plant pathology. This is to be regretted because many pathological conditions in plants are produced by agents other than fungi, so the terms mycology and mycologist do not rightly indicate the nature of the work and the training required by an investigator of plant diseases. The person who is a mycologist and nothing more is, in fact, very ill-equipped for a study of the problems connected with diseases affecting plants.

In no field of plant pathology is this more strikingly illustrated than in the field of virus diseases, for whatever may be the character of the primary cause or causes of these diseases they cannot be attributed to the action of fungi in any usual acceptation of that word.

The use of the term virus as applied to the diseases of both animals and plants is really a confession of our ignorance, for it can be defined but vaguely. A virus, as we understand it to-day, is an element or principle of unknown origin and character which is the agent for communicating infection or infectious diseases. As common examples, more or less familiar to all, may be cited the virus of smallpox, the virus of rabies, the virus of rinderpest and the virus of foot and mouth disease. In the case of none of these diseases has it been possible as yet to isolate any organism which can be multiplied in pure culture and can thereafter produce the disease when inoculated into a healthy animal.

As in the case of diseases in general so in the case of virus diseases, those attacking animals and more especially human beings have received much more extensive and concentrated attention than those attacking plants. Nevertheless in recent years the economic importance of the virus diseases of plants as well as their absorbing scientific interest has led to a steadily increased attention being paid to them. As an example at our very doors may be cited the 'Spike' disease of sandal which seriously threatens our most important source of forest revenue and which is at present under investigation by a considerable number of scientific workers in South India.

As the subject of my address to-night indicates, I shall not attempt more than a very general survey of the virus diseases of plants largely because my work is of such a character as to prevent my taking an active part in the investigation of any of these diseases. My reasons for dealing with the

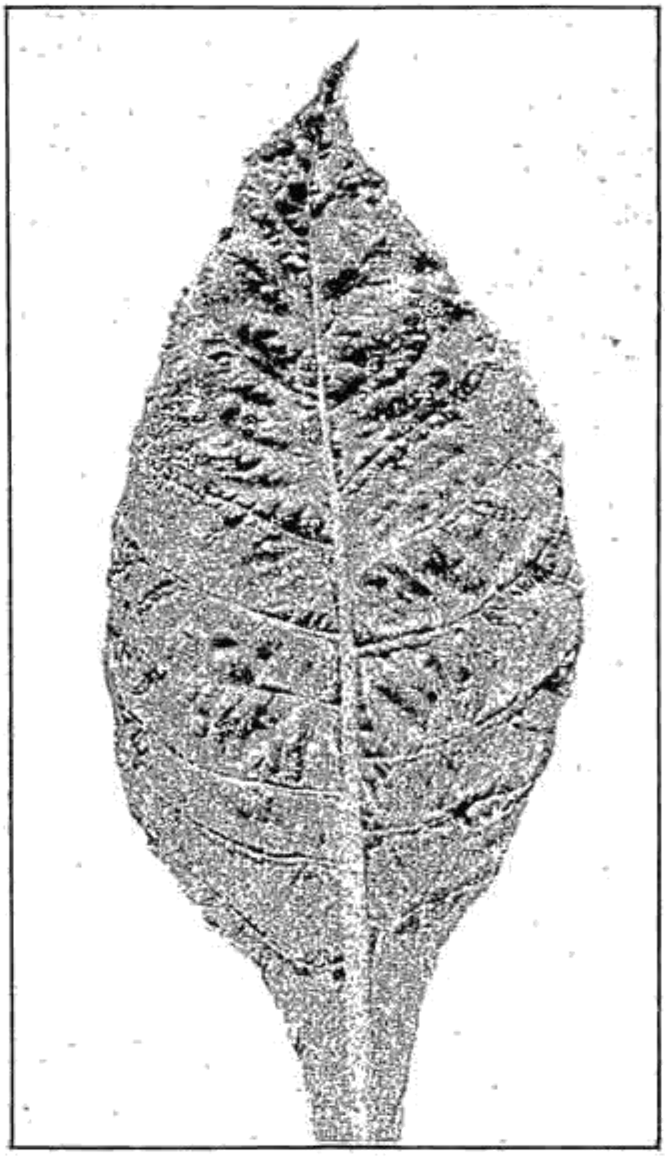
[1] Address to the South Indian Science Association.

[2] The photographs reproduced in this paper are by the courtesy of *The Mysore Agricultural and Experimental Union.*

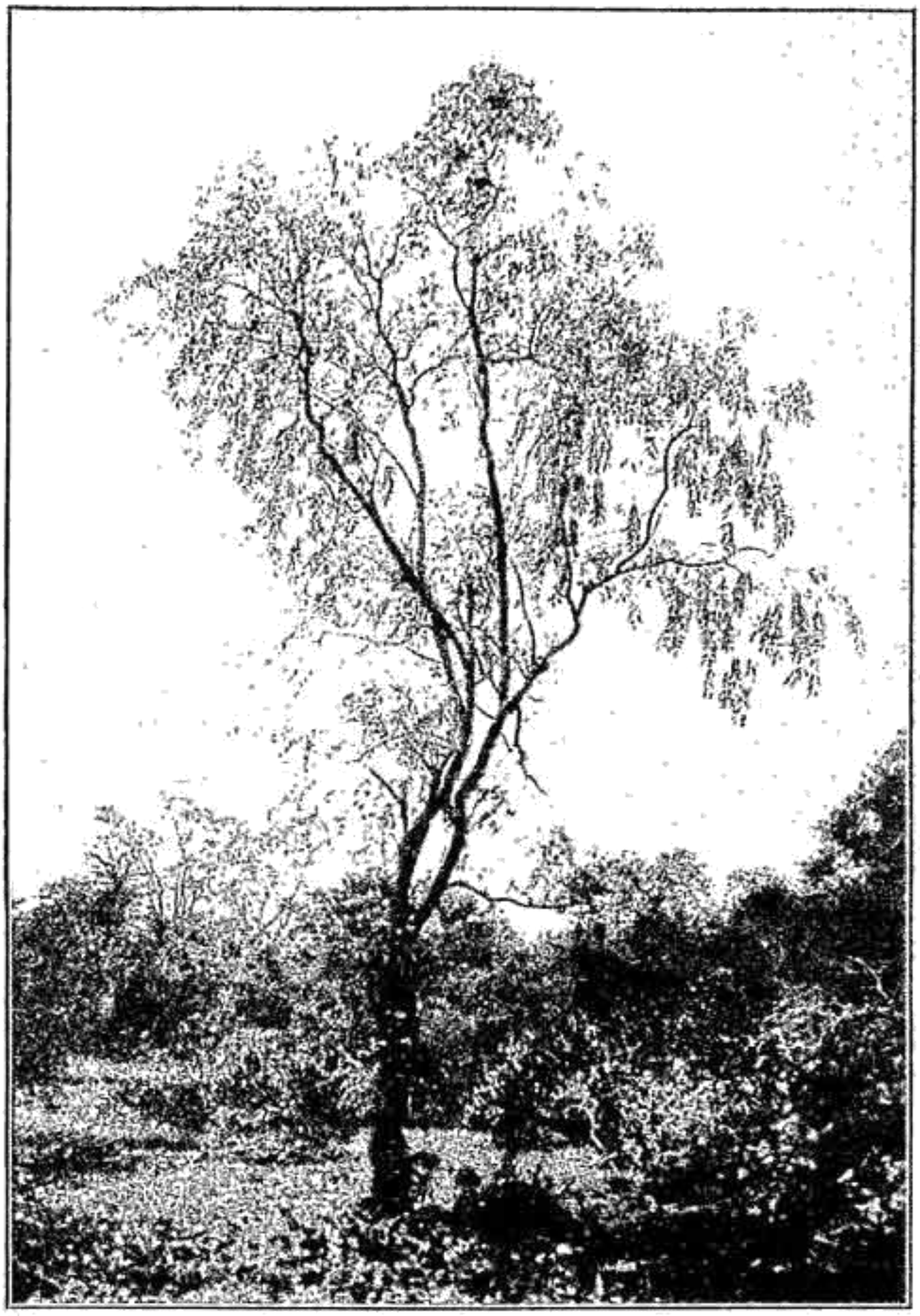
subject are three-fold. In the first place there is no doubt that the virus diseases of plants are continually levying an increasing toll in the agricultural and forest products of the world. In the second place there seems to be some connection between the virus diseases of plants and the more strikingly important diseases of human beings and domesticated animals; it seems, therefore, probable that continued investigation of the former may throw some light on the latter. The third reason is a personal one. While, as I have said, I have not myself in recent years contributed to our knowledge of these diseases—nor can I hope to do so in the near future—I have had opportunities of observing a goodly number of them in other parts of the world, such as have not been afforded to most workers in India. I should like further to preface my remarks by the statement that as I am a biologist, I shall confine myself to a consideration of the biological aspect of the question leaving a consideration of the bio-chemical and other aspects to those much better qualified than I in those fields.

The first virus disease of plants to attract scientific attention was the mosaic disease of tobacco and this may be used as a suitable starting point for our hurried survey of the field. This disease is present in all parts of the world where tobacco is grown including India though in this country it has not yet been studied. As the name indicates it is characterized by a mottled appearance of the leaves in which rather irregular areas of lighter green alternate with areas which show the darker green colour typical of healthy leaves. The lighter areas are at the same time somewhat thinner. Where the disease breaks out in a field it is almost always the younger leaves which show the symptoms. The disease is highly infectious in nature. In fact it can be transmitted by simply rubbing a diseased leaf between the fingers and thereafter rubbing the leaves of a healthy plant with the same fingers. The handling of tobacco in the field in the processes of topping and removing secondary shoots is considered in Sumatra to have been one of the regular means of spreading the disease. A microscopic study has revealed no fungus or bacterium which could be looked upon as the causal organism. The expressed juices are highly infectious even in very great dilutions (1 : 10,000). Even when this juice is filtered through a filter candle which prevents the passage of ordinary bacteria, it still remains infective. One of the most striking features of the infective principle is its resistance to drying out for long periods. I have personally transmitted the disease by grinding up dried diseased leaves, which have been kept in the herbarium for a year in distilled water and injecting the extract by means of a hypodermic syringe into healthy plants. Others have found such dried leaves infective after preservation in an herbarium for over twenty years.

The filterable nature of the virus is characteristic not only of this disease but of a large number of virus diseases of both plants and animals. However, that it is intimately associated with particles which are very large as compared with, for instance, a molecule of water is shown by the fact that filteration through the finer filters such as those made of celloidin leads to a filtrate which is no longer infective. An attempt has been made to measure the size of these particles by comparing their filterability with that of colloidal particles of which the size has been more or less accurately estimated. In this way Duggar and Karrer have come to the conclusion that the particles carrying the virus of tobacco mosaic are approximately of the same size as the colloidal particles of 1 per cent hæmoglobin or 30 millimicrons. Such particles



Tobacco mosaic showing mottled appearance of leaf having more light green areas than dark green ones. In addition, the leaf shows a peculiar crinkled appearance.



Sandal tree affected by spike disease. This type, showing a pendulous orientation of the affected branches, is met with occasionally.

are far below the limits of visibility with the most powerful microscopic magnifications. To give one an idea of the size of these particles relative to that of bacteria it may be stated that half a micron is about the lower limit for the diameter of known organisms such as micrococci. In other words these particles seem to have a volume not more than 1/4000 of that of our smallest bacteria. As a matter of fact, however, the method of judging size by ultrafiltration, is so beset by pitfalls, such as those associated with absorption and electric charge, that any estimates of size made by this method must be accepted with a great deal of reserve.

Another striking feature of the virus of tobacco mosaic in which it resembles many other plant and animal viruses is its marked resistance to disinfectants. Thus it can withstand concentrations of alcohol, thymol, corrosive sublimate and other disinfectants which are far in excess of that required to kill bacteria except when they are in the spore stage. These two characteristics of viruses, *viz.*, small size and great resistance to disinfectants, have been used as arguments against the supposition that we are here dealing with living organisms. As to size, no one knows what the lower limits of size for living organisms are; as to resistance to disinfectants, Olitsky has recently shown in his studies of Foot and Mouth disease that this resistance is not a real one but is due to the association of the virus with protein materials which serve as a protection, through their coagulability or their electric charge or both. Thus the virus was found highly resistant to such coagulating disinfectants as corrosive sublimate where as to such non-coagulating disinfectants as antiformin and sodium hydrate it was just as susceptible as living microorganisms such as staphylococci. It seems probable that the observed greater resistance of virus extracts in general to certain common disinfectants is not due to any greater resistance in the virus itself but to the protective action of the proteins from which it has in no case, up to the present, been separated.

For a long time it was considered that there was one and only one disease of tobacco included under the designation mosaic. Work of the past four or five years, however, has indicated that this is not the case, but that there are a large number of such diseases. One author claims, for instance, that tobacco is susceptible to no less than six distinct strains of mosaic virus. A Dutch scientist has quite recently discovered and described a new virus disease of tobacco in Sumatra distinct from the common mosaic. Similarly workers on mosaic of potato have reported as many as eight strains of mosaic virus on that plant. These strains are distinguished by the different symptoms they produce, their different reactions under varying temperature conditions and by the fact that in some cases the inoculation of a plant with two different strains has led to symptoms very different from those produced by either when used separately.

A very similar state of affairs has quite recently been found in the case of Foot and Mouth disease of cattle where these strains have been found. Here, the differentiation is on perhaps a surer basis, for Vallee and others have shown that an animal after being attacked by one type of Foot and Mouth disease and having acquired thereby the temporary immunity of about 4 weeks that results from attack, is immediately susceptible if inoculated with one of the other two virus strains. It is quite possible that this condition occurs in the case of other virus diseases as well. This is a possibility which we shall have to keep in view in our investigations of Sandal Spike.

It must be clear that the method of disease transmission already described is one that does not usually occur in nature, for while in the case of mosaic diseases of economic plants transmission through the handling or rubbing by human hands or through the use of cultivating implements or pruning tools might be possible, this could hardly be a factor in the case of the mosaic diseases of wild plants. Investigators, therefore, very early sought for some other factor in disease transmission. The most natural place to look for such a factor was in the insect world and as a matter of fact, it was here that it has been found and among those insects which obtain their nourishment by sucking up the juices of plants such as mosquitoes suck the blood of human beings. In a very large number of the mosaic diseases transmission through sucking insects such as plant lice and leaf hoppers has been established and the insect vector has also been demonstrated in a number of other virus diseases as well. I shall refer to this later on.

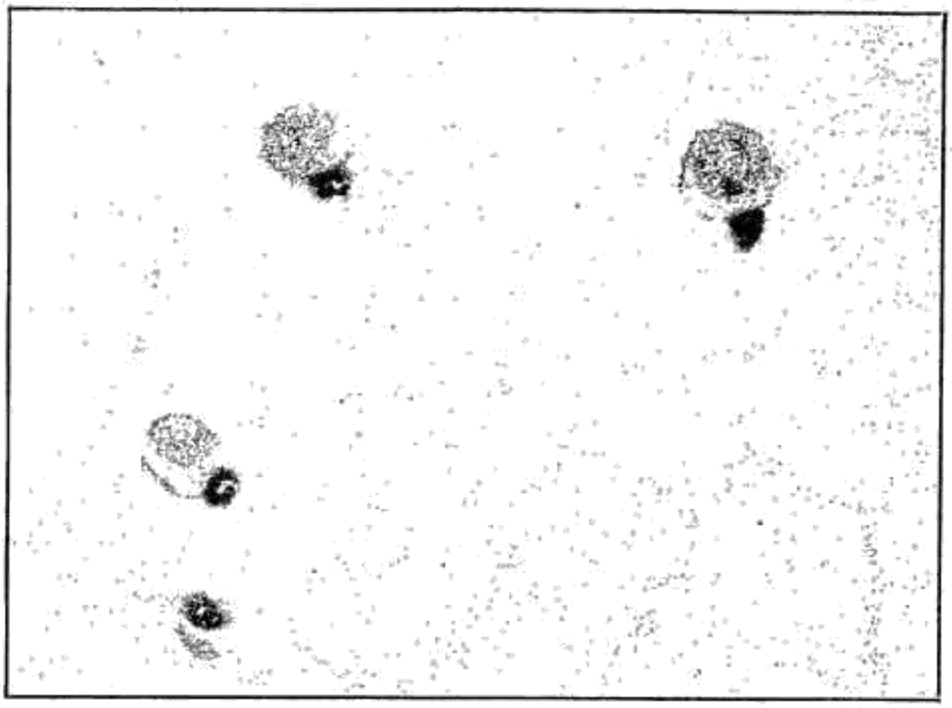
Transmission of virus diseases through seed from diseased plants has been established in only a very few cases. It does not occur, for instance, in tobacco mosaic, nor does it apparently occur in the case of Sandal Spike. Transmission of disease through the soil has not yet been established in a single case, so I think we may take it that this sort of transmission must be rare indeed.

Lastly there are quite a number of virus diseases in which transmission has been brought about only through grafting a scion or bud from a diseased plant on to a healthy stock and as you are aware, Sandal Spike and a number of similar diseases found in South India belong to this group. We know, however, that these diseases spread in nature where no such grafting takes place. Therefore there must be some other means of transmission. It seems highly probable that insects are responsible in the case of these diseases also.

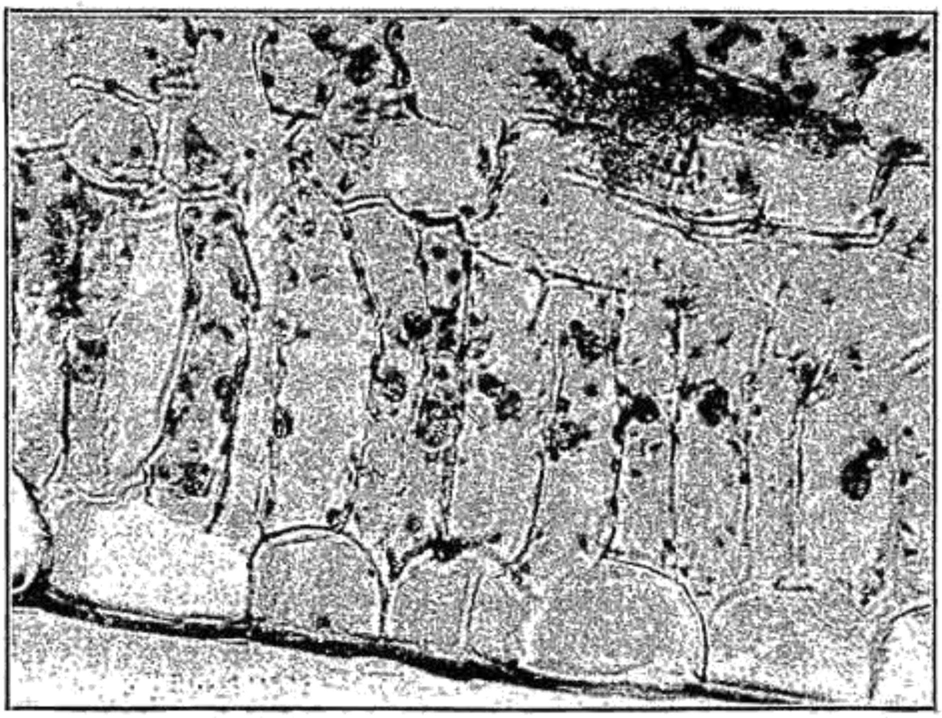
In respect of insect transmission, virus diseases of plants resemble many human and animal diseases such, for example, as plague and malaria. The question naturally arises whether the insect vectors are simply the carriers of these virus diseases or whether the virus must pass through a stage in the insect itself before it is able to infect a healthy plant, similar to the conditions we find in malaria.

One of the most interesting pieces of work on virus diseases of plants (that by Kunkel on Asters) throws a most interesting light on this very question. Kunkel found that the disease known as Aster Yellows which attacks the China Aster and a number of other plants is transmitted by a leaf hopper *Cicadula sexnotata*. He found further, however, that infection did not take place if an interval of less than ten days elapsed between the time when the insect fed on the diseased plant and the time when it fed on the healthy plant. In other words, an incubation period of ten days has to elapse before the Cicadula becomes infective. A similar but shorter incubation period has been established in the case of three other virus diseases: the curly top of sugar beet, the streak disease of Indian corn and the leaf roll disease of potatoes. One is almost tempted in these cases to draw an analogy with malaria and one can hardly escape the conclusion, in the case of Aster Yellows at least, that the virus does develop in the insect. The important experiments of Kunkel seem to me to furnish the most convincing evidence yet advanced that we are here dealing with a living organism. It is almost inconceivable to

Periwinkle (*Vinca rosea*, L.) showing the lower branches affected by spike disease, the top ones being apparently healthy.



Photomicrograph showing vacuolated intracellular bodies attached to the nuclei present in the cells of a spiked sandal leaf. Intracellular bodies are large, the nuclei smaller and dark coloured.



Photomicrograph showing the intracellular bodies in the cells of a spiked Vinca leaf. In the middle of the photograph one is shown attached to the dark coloured nucleus.

me that an unbalanced sap circulation or a plant enzyme out of control or a lethal gene escaped from the plant cell could propagate itself in the body of an insect. I need hardly point out that Kunkel's work greatly complicates the problem of insect transmission, for we shall have not only to seek out from among the large numbers of insects which are found on, for example, the sandal tree the one insect which is capable of transmitting the disease, a difficult job in itself; we shall also have to take into account the possibility that even that one insect may not be capable of transmitting the virus till the latter has passed through an incubation period of unknown duration in the insect's body.

While I must point out to you that the views of workers on virus diseases are very divided on the subject of the nature of the virus, there seems to me no escape from the conclusion that in a large number of these diseases at least the virus is a living organism. As I have already stated, in no case has any organism yet been observed which on inoculation could produce any one of these diseases. However, evidence is steadily accumulating in connection with virus plant diseases that certain definite structures are present in the cells of diseased plants which are not to be found in healthy ones. While there is as yet no valid evidence that these bodies are themselves the organisms of the virus (they seem much too large for that), there are workers who believe that they are either stages in an organism which has also an ultramicroscopic stage or that they hide within them the ultramicroscopic organism which causes the disease. To my mind the most important recent contribution to our knowledge of Sandal Spike has been the discovery by Mr. M. J. Narasimhan of the presence of intracellular bodies of this character in diseased sandal leaves as well as in the leaves of vinca suffering from a similar disease. Mr. Narasimhan has been good enough to allow me the use of some of his micro-photographs here.

I trust that the very hurried and imperfect survey which I have been able to present to you will convince you that we are here dealing with a subject of immense practical and scientific importance and that the investigation of our very important virus diseases of plants demands the very best in brains and investigating genius that this country can produce.

It would perhaps be going too far to speculate upon the possibility of coming, through a study of these diseases, to a knowledge of the most elemental forms of life existing on earth. Whatever the cause of these diseases may be, if it is a living organism it is one that cannot live by itself. It is, in other words, an obligate parasite whereas the most primitive forms of life must be organisms which can live free. There is also the possibility of our having to do here with organisms whose structure has been degraded and simplified through their parasitic mode of life. Who is to say, however, that there are not free living organisms to-day which are similar in their minuteness and their simplicity of structure to those which we believe to be the causes of virus diseases? If such exist, they stand very close to the line separating the animate from the inanimate world.

Before proceeding to the consideration of the lantern slides you may wish my views as to the lines of future work which are most likely to lead to practical results in the control of these diseases. Apparently they are in many, if not most, cases insect-borne and a control of their insect vectors must always be kept in view as a means of controlling the diseases themselves.

This, as you know, is the most effective means of controlling many of our insect-borne human and animal diseases. However, in the case of plants where the economic factor is much more prominent than in the case of human diseases, at least, I believe that our chief hope for the future lies in the discovery of, or the breeding of, resistant varieties. Much progress has already been made in this direction in connection with quite a number of the virus diseases of plants and while the breeding of a sandal strain resistant to spike will probably be very difficult, still it seems to me to be our chief hope for the future.—*The Journal of the Mysore Agricultural and Experimental Union.*

THE CINEMATOGRAPH IN AGRICULTURAL EDUCATION

A Leicestershire Experiment

BY

F. V. MILLINGTON

The Empire Marketing Board supplied lists of films applicable to agriculture and allied subjects, and arranged to provide most of the films free of charge. Amongst them the following were selected as most suitable for the experiment:

Commercial Potato Growing (British)
Co-operative Marketing of Eggs (Canadian).
Aspects of Modern British Poultry Farming (British).
Soil Physics (Canadian).
The Life of a Plant.

No difficulty was experienced in obtaining other films of interest and educational value, although those which dealt with home practice were not so readily available as films produced overseas.

Procedure.—The van commenced its itinerary in October 1928, and visited villages in which a suitable room could be obtained. The more modern elementary schools, with their movable screens, usually provided sufficient space for seating accommodation and the fireproof cabinet which enclosed the projecting apparatus. Schools or village halls in which electric lighting had been installed were used where possible.

In spite of the fact that a "cold light" was used in the projector, the lamp being of the filament variety, the County Police Authorities required to be notified several days in advance of the display, and a permit was issued after the local police officer had reported as to the suitability of the premises. The permit required that proper gangways should be provided, exit doors readily accessible and labelled, and sand, water and blankets available for use in case of fire.

The van was drawn up outside the building and placed in a convenient position as near as possible to the room in which the films were displayed. A