THE USE OF PHASE-CONTRAST MICROSCOPY IN THE IDENTIFICATION OF RATOON STUNTING DISEASE

By
D. R. L. STEINDL
Bureau of Sugar Experiment Stations, Brisbane

Summary
Phase-contrast microscopy has been found to provide a satisfactory method for the identification of ratoon stunting disease.

The method appears to have a similar degree of accuracy to bana grass inoculation and electron microscopy, but none of these methods may be as sensitive as inoculations into Q28.

Introduction
Four methods used for the identification of ratoon stunting disease have been described previously (Steindl and Teakle 1974) and the relative merits of these methods discussed. An additional method using phase-contrast or dark-field microscopy has also been described (Gillaspie, Davis and Worley 1973). In view of the fact that this can be carried out in approximately the same time as the electron microscope method, but with much less expensive equipment, it was decided to test phase contrast under Queensland conditions, using the extraction technique developed for electron microscopy.

The Phase-Contrast Method
Vascular sap is extracted from the internodal portions of cane stalks by fitting a tapered rubber sleeve over one end, then inserting it firmly into the neck of a vacuum flask, so an air-tight seal is obtained. The vascular sap drops into the flask when the pressure is reduced. A few millilitres of sterile water pipetted on to the top of the cutting are also sucked through to flush the vascular bundles. Another rubber sleeve can be fitted on top of the cutting to make a small reservoir for the water.

If possible, freshly-cut internodes from sound, mature cane should be used. They should be well washed to prevent contamination of the extract. Stalks which are hollow, or have severe rind cracks, can be plugged with plasticine, otherwise air will be sucked through them. Stalks which are oval in cross section may have to be ground off to make an air-tight seal in the neck of the flask.

Extracts prepared in this way are relatively-free from plant debris when compared with extracts obtained by crushing the cane. Nevertheless they are passed through a No. 42 Whatman filter paper before centrifuging to precipitate the bacterial cells.

In these experiments the extracts were centrifuged in a Gallenkamp Junior centrifuge at 3 000 r.p.m. for one hour. A high-speed centrifuge would reduce this time considerably. The supernatant liquid is discarded and the pellet suspended in a drop of sterile water. A small smear of this is placed on a microscope slide, covered with a cover slip and examined under a Leitz “Ortholux” microscope, using oil-immersion phase-contrast equipment with a magnification of 1250 X.

Extracts from ratoon stunting diseased plants show varying numbers of characteristic minute bacterial cells, many of which are bent or twisted. They show a great deal of Brownian movement, due to their small size. Even when all possible precautions are taken the extracts frequently show bacteria of other species, but these are bigger and much less numerous than those associated with r.s.d.

As well as carrying out tests with a range of canes known to be both diseased and healthy, examinations were made of extracts from approximately 40 specimens of different varieties which were received from country areas for diagnosis. Parallel bana
grass inoculations of all these were made for comparison and a few were inoculated into Q28 or examined with the electron microscope by Dr D. S. Teakle of the Queensland University Microbiology Department.

In every instance the phase-contrast diagnosis agreed with the bana grass inoculation results. The reliability of this latter test is indicated by the fact that of seventeen specimens in which electron microscope examination and bana grass inoculation were compared, there was only one discrepancy. In this, the E.M. gave a positive result and the bana grass negative. In one other instance, where the E.M. and bana grass gave negative results inoculation into Q28 gave a positive result.

Conclusions

It is considered from the above results that phase-contrast microscopy provides a rapid and satisfactory diagnosis for ratoon stunting disease in mature cane stalks when field identification is in doubt. The presence of discolored vascular bundles in the nodes must still be used as the main diagnostic tool in the field, but if doubtful symptoms are present, or if the presence of the disease is suspected because of poor growth or other reasons, then the phase-contrast test can give an apparently reliable quick decision.

REFERENCES