RESEARCH PROJECT P74-6-06

A METHOD FOR THE RAPID IDENTIFICATION OF RATOON STUNTING DISEASE IN SUGAR CANE

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Recent work with the electron microscope has established that a slender, coryneform-type bacterium is constantly present in the sap of known ratoon stunting diseased plants and is absent from healthy plants. The purpose of this project was to determine whether a reliable method of diagnosing doubtful cases of the disease could be based on the findings of such organisms in the extracted sap, either by electron or phase-constrast microscopy.

The electron microscopy (EM) work in this project was carried out by Dr. D.S. Teakle of the University of Queensland, Microbiology Department.

Results were compared with those obtained from inoculations into Q28 and bana grass at Eight Mile Plains. Subsequently, studies were carried out to determine if the organism could be detected by the phase-contrast microscopy and to find the most reliable technique for extracting and concentrating the organism associated with the disease. Findings with phase-contrast microscopy were also compared with results obtained from inoculations into bana grass.

Electron Microscopy Versus Inoculations into Bana Grass and Q28

Specimens of cane with doubtful rsd status were requested from all major areas for this study. A total of 17 specimens was received. Extracts from each were examined with the electron microscope and inoculated into bana grass uprights, while some were also inoculated into Q28. However, it had previously been established that there was very good agreement between results from bana grass and Q28. Results from the above 17 specimens showed only one discrepancy between electron microscopy and bana grass inoculations. In this, the EM showed a very small number of particles which could have been rsd but the bana grass gave a negative result. In one other case, where the EM and bana grass gave negative results inoculation into Q28 gave a positive result.

Phase-Contrast Microscopy

Vascular Sap Extracts

Vascular sap was extracted from portions of diseased Q28 stalks about four to six inches long, by fitting a tapered rubber sleeve (Gooch crucible adapter) over one end, then inserting it firmly into the neck of a vacuum flask to obtain an air-tight seal. When the vacuum was applied, approximately three millilitres of sterile water were pipetted onto the top of the cutting, and sucked through the vascular bundles to flush out the bacteria. Portions of stalk both with and without nodes were used.

Examination of these extracts with phase contrast (see below) showed very few bacterial cells, and it was apparent that the extract needed to be concentrated before a reliable diagnosis could

be made. Extracts from rsd-infected stalks of Q28, Q71, Q87 and Q94 plant cane about 10 months old, grown at the Pathology Farm, were concentrated by centrifugation for one hour at 550 g in a Gallenkamp Junior centrifuge with an angle head holding six 10 ml tubes. The supernatant liquid was discarded and the pellets re-suspended in a few drops of sterile water. A small smear of this suspension was placed on a microscope slide, covered with a cover slip and examined with a Leitz Ortholux microscope, using oil-immersion phase-contrast equipment with a magnification of 1 250 X.

Because of the nature of these preparations it was virtually impossible to make accurate counts on the rsd particles; consequently only comparisons of the numbers of particles in the various preparations were made. Large numbers of slender filamentous particles, many of which were bent or twisted could be seen in each preparation, however, a considerable amount of plant debris was also present, so attempts were made to clarify the vascular extracts by filtration before centrifuging.

Since the Q94 extracts showed a larger number of particles than those of other varieties examined, it was decided to use this variety for further studies. In addition it was found that the movement of water through internodal sections of stem was much faster than through sections containing a node, and the extracts from the former usually contained larger numbers of bacteria. This was probably because the water was able to pass through and extract the bacteria from a greater number of vascular bundles in the internodes.

Filters used included Whatman papers Nos. 41,2 and 42 and Equip pads Nos. 0, 1, 2 and 4. All filtrations were done under

vacuum. Filtrates were centrifuged as above, then examined under phase contrast. These filters reduced the amount of extraneous matter in the order listed, but the numbers of rsd particles were not noticeably reduced until the No. 1 Equip pad was reached. There was a very marked falling off in particle numbers passing the Nos. 1 and 2 pads, and none could be found after the No. 4 pad. The most satisfactory preparations for both freedom from plant debris and numbers of particles present, were those filtered through a No. 0 Equip pad, closely followed by those filtered through a No. 42 Whatman paper. Because of their easier handling the No. 42 papers have been used in routine work.

Tissue Diffusates

Lamina tissue and midribs of mature leaves, mature leaf sheaths and stems from rsd-infected Q94 as above were washed and finely sliced with a sharp scalpel to prevent crushing the cut ends of the vascular bundles. They were then soaked in sterile water for approximately three hours to allow the bacteria to diffuse out. As much tissue as could be immersed in about 15 ml of water was used. After soaking, the tissues were removed with a strainer and the liquid was filtered, centrifuged and examined as for vascular-sap extracts.

The rsd particles could be found in all these extracts, but were less numerous than in vascular-sap extracts. Of these extracts, those from leaf sheaths appeared to have the most particles and lamina tissue the least.

Phase-Contrast Microscopy Versus Inoculations into Bana Brass

In order to compare the reliability of phase-contrast studies with inoculations into bana grass, both methods were

used on approximately 40 specimens of different varieties which had been sent from country areas for diagnosis. Vascular-sap extracts from mature stalk tissue of each specimen were used both for examination with phase-contrast and inoculation into bana grass uprights. In all cases the two methods gave the same results. The numbers of particles found in the various specimens which gave positive results varied greatly but was not generally correlated with varieties. Individual specimens of Q63, Q87 and Q90 gave very high counts, but others of the same varieties gave low or medium counts.

Conclusions

The above results indicate that phase-contrast microscopy provides a rapid and reliable diagnosis for ratoon stunting disease in mature cane stalks. Because of sampling difficulties, the presence of discoloured 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 disease is suspected for some other reason, then the phase-contrast test can give a quick decision.

The tissue diffusate method of extraction has not been fully examined, but it is believed that it could be used with young cane which has not formed a mature stalk. Mature leaf sheaths would probably be the most suitable material for making these preparations.