AN IMPROVED TECHNIQUE FOR THE ISOLATION OF PHYTOPHTHORA INFESTANS

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Summary, Zusammenfassung, Résumé, p. 156

Breeding for blight resistance commenced at the N. Ireland Plant Breeding Station, Loughgall, in 1957. During collection of blight strains, by isolation of the fungus from diseased leaves and infected tubers, considerable difficulties were encountered.

Initially the methods described by MULLER et al. (1955) were used for isolation of the fungus from infected leaves. In many cases, however, fungal and bacterial contaminants were present and invariably these were transferred to media along with the blight fungus. Attempts were made to surface sterilize the leaves by dipping them in a 0.1 °o mercuric chloride solution for 2-3 min. They were then placed in airtight jars and after 24 hours abundant sporulation occurred. Unfortunately in many cases when mycelium was transferred to media, contamination was still evident.

It was then decided to discontinue the direct transfer of mycelium from leaves to media and to utilise either naturally or artificially infected tubers as the main source of the pathogen. Where the fungus was actively growing on leaves, several infected leaflets were harvested in the afternoon, stored overnight in air-tight containers and, on the following day, actively sporulating mycelium was washed off with sterile distilled water into a small beaker.

Tubers, from a known susceptible variety (Craig's Royal), which had previously been incubated at 18 °C for 6 days to ensure that they were free of blight, were inoculated with the suspension, using a 5 ml hypodermic syringe. To prevent the entry of other pathogens the inoculation sites were sealed with collodion solution. The inoculated tubers were placed in polythene bags, and incubated for 10-14 days at 18 °C. After this period infection had spread throughout the tuber.

In a few cases where leaves had been very heavily infected with other fungi, and with bacteria, it was found that these had been transferred during inoculation. In the use of polythene bags to enclose the inoculated tubers, conditions were set up which were more favourable for the development of the contaminants than for P. infestans. Latterly, for this reason, either paper bags have been used instead of polythene bags, or the inoculated tubers have been placed in open trays.

Cores were obtained from infected tubers, by using a 2 cm cork borer. These were received for publication 28th November, 1959.

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cut longitudinally to give two tuber "slopes". Surface sterilization was accomplished by dipping the slopes in 0.1% mercuric chloride solution for 3 minutes, and continuously agitating to remove air bubbles from the cut surface. They were then washed in sterile distilled water and transferred aseptically to previously sterilized 7 inches × 1 inch boiling tubes, the bottoms of which had been packed with 1½ inches of soaked cellulose wadding. These formed ideal moist incubation chambers, and after incubation at 18°C for 5 days a good growth of mycelium was obtained. This could then be transferred to a suitable synthetic medium.

Inoculum obtained from the tuber slope was deposited with a sterile paracentesis needle in a depression, (cut with the needle), in the surface of the agar slope. The test-tube was then tilted to allow free moisture to flood the depression. Excellent results have been obtained from the pea-meal, sucrose medium described by Keay (1953), abundant sporing mycelium being produced after 4–5 days incubation at 18°C.

In isolating *P. infestans* from naturally infected tubers, a similar method to that described above has been used. To reduce the possibility of contamination by surface organisms the infected tuber is washed and then peeled before coring. Very little contamination has been recorded from cores obtained in this way. Where bacterial contaminants have been present it has been possible to obtain pure blight cultures from the tuber slopes as, such contamination generally spreads downwards, whereas, blight grows up the slope. Usually a minimum of four slopes per tuber sample are prepared.

It is unusual for more than a single slope from a tuber to be contaminated, and the above method has the advantage that contamination cannot spread to other tuber sections as in the culture-dish method of Muller.

Boiling tubes closed with a sterile cork, then sealed with a "viskap", and wrapped with paraffin tape ("parafilm") have been used, to maintain cultures, without sub-culturing, for long periods. Initially, collodion was used in place of the paraffin tape but, after drying out, it tended to crack, and a complete seal was not obtained.

Isolates of the fungus, including races 1; 2; 1, 2; 1, 2, 4; and 1, 2, 3, 4 (Proudfoot 1959), have been stored by this method at room temperatures, for periods of 10 months without any reduction in their infective power. Cultures have also been retained at room temperature for 20 months by sealing a cotton-wool stoppered test-tube with molten paraffin wax.

Large quantities of inoculum can be produced on sterilized peas. This method was developed by Cruickshank in New Zealand (1953) and has been in use at Loughgall for the culture of flax and grass pathogens since 1954 (Wright & Wilson 1958). Other pathogenic fungi readily cultured by this means include *Streptomyces scabies* and *Fusarium caeruleum*. A similar method has been described by Thurston (1957).

**SUMMARY**

A method, in which tuber cores, obtained from either naturally or artificially infected tubers, are used for isolating races of *P. infestans* is described. The production of large quantities of inoculum, and the maintenance of cultures for long periods, are discussed.