Transmission by Snails of the Species of *Phytophthora* which causes Foot Rot of *Piper nigrum* L. in Sarawak

Achatina fulica Bow., the giant African snail, was first introduced into Sarawak in 1928[1]. It is now a relatively common pest. In infested pepper gardens as many as 10–20 snails per vine have been counted.

Prior to the onset of the 1962–63 north-east monsoon season, pepper vines in widely scattered localities in western Sarawak, attacked by *A. fulica*, developed a wilt and die-back which could not be accounted for by simple mechanical damage. Attacked vines exhibited one or a number of wilted, necrotic lateral or terminal branches, the leaves of which remained attached, but were desiccated and yellow or necrosed. Lesions, similar to those found in normal subterranean attacks of foot rot disease, were invariably found at the bases of these wilted branches. However, the juncture between the necrotic and healthy tissues, as described by Holliday and Mowat[2] and Holliday[3] for subterranean attacks, was not always clearly defined. Where a lesion had developed in a branch-fork or on a leader, low down in the vine, one-quarter to one-half of the canopy wilted. In the majority of cases examined, the snail, *A. fulica*, was associated with the lesions and wilted branches. Where snails were not found, signs of recent infestation in the form of fæces and trails were present.

The species of *Phytophthora* which causes foot rot of pepper has been consistently isolated from aerial-lesion material. Using fresh apple tissue baits, the pathogen has been isolated from the fæces of the snail species. Fæces were obtained from snails infesting healthy vines and vines with die-back, and, in addition, were collected from vines directly. After moistening, the fæces were inserted into 0·5 cm bore-holes in fresh apples, which were then kept in damp-chambers for 4 days. The pathogen was then readily isolated from the invaded apple tissue, on to plain agar, and has been obtained from 75 per cent of all isolations performed. Zoospore suspensions, prepared from the isolates, were pathogenic to three-week-old, single-node pepper cuttings.

Fæces of *A. fulica*, collected from gardens with vines exhibiting the die-back, when examined microscopically, have been found to contain sporangia of the fungus.

On first consideration it would seem improbable that the thin-walled sporangia could survive digestion by the cellulytic enzymes in the alimentary tract of the snail. However, resistant spores are rare, oospores having been found only in pure culture[4]. Proof that sporangia remain viable after passing through the alimentary tract has been demonstrated under laboratory conditions. Batches of snails of the species *A. fulica*, collected from sites where pepper is not grown, were caged and starved for periods of 24 h. They were then fed with either zoospore-inoculated pepper leaves, on which sporangia had developed, or with four-day-old, 0·5 cm, oatmeal agar, culture disks of the *Phytophthora* species. The disks, prior to ingestion, had been placed in Petri's solution to induce sporulation. Only those fæces which fell outside the cages containing the snails were collected for testing for the presence of the fungus, to avoid contamination with spores from the food materials. On examination, sporangia were found in all fæces collected and the fungus was isolated by baiting with apple tissue. Zoospore inocula prepared from the isolates were found to be pathogenic to pepper cuttings.

The composition of fæces from snails fed with pepper leaves in the laboratory and fæces collected from pepper gardens appeared to be identical, and it is therefore considered that the snails become vectors by ingesting naturally infected leaves.

The role of *A. fulica* in the spread of foot rot is limited. It only assumes importance at times when climatic conditions are not conducive to the normal rapid spread of the disease, as during the south-west monsoon when the weather is dry. During these times the snail has been found to transmit the disease from infected gardens to surrounding healthy gardens and to healthy vines within infected gardens. Vines attacked by infective snails will recover after pruning out the wilted branches and lesions, providing the roots are not diseased.

As all other records of plant disease transmission by slugs and snails have been made under artificial conditions in the greenhouse (Commonwealth Mycological Institute, personal communication, 1963), it is considered that this is the first record of the transmission of a plant disease by a snail species in the field.

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G. J. TURNER

Department of Agriculture,
    Kuching,
        Sarawak.

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Probable Function of Macromolecules in Tissue Culture

THE role of macromolecules in cell cultures remains obscure. For this reason tissue culture media are supplemented with serum or with purified protein fractions. In some instances other macromolecules, for example, carboxymethyl cellulose, are used[1].

A method for preparing an active macromolecular factor showing intense growth-promoting activity from the previously described α-globulin[2] has been devised in our laboratory. The growth-promoting α-globulin was fractionated by column chromatography on DEAE-cellulose. 400 mg of protein was adsorbed on a column of 21 mm × 105 mm and eluted first with 0·1 M phosphate buffer, pH 7·6 (inactive protein), and afterwards with 0·02 N hydrochloric acid (active factor). The solution with the active factor was adjusted to pH 9 by the addition of 3 N sodium hydroxide and then dialysed twice for 12 h against 100 vol. of demineralized water.

The activity of the factor was estimated by the method described previously[3]. As shown in Table 1, 80γ of the active factor in 1 ml. caused an attachment and flattening of the cells in the absence of any additional compounds in the synthetic medium.

Table 1.   HeLa CELLS INCUBATED IN A SYNTHETIC MEDIUM (REF. 4) SUPPLEMENTED WITH THE ACTIVE FACTOR (20 h, 37° C)

Amount of the factor (γ/ml.)	Percentage of flattened cells (inoculum = 100 %)
5	10
10	27
20	54
40	85
80	97
160	95

Further experiments indicate that the active factor differs from the complete growth-promoting α-globulin in that it is composed, according to an ultracentrifugal analysis, of two components, one of which represents about 10 per cent of the total amount only. It gives a positive reaction to hexoses and a positive direct Ehrlich reaction[5]. Amino-acid analysis by paper chromatography of an acid hydrolysate of the active factor (a butanol–acetic acid–water mixture was used) revealed the presence of at least ten amino-acids.

The attachment and flattening of HeLa cells in synthetic medium was found to depend on the form of the active factor. A comparison was made of the activities of the factor dialysed directly after chromatography on DEAE-cellulose column (pH value of the solution after dialysis, 5–6) and after alkalization with sodium hydroxide (pH value after dialysis 7·3). If not rendered alkaline, the factor is inactive and the cells are destroyed in 20 h at