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Abundant production of chlamydospores by Fusarium oxysporum f. sp. melonis in soil extract with glucose

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Most species of *Fusarium* exist and survive in the soil in the form of chlamydospores. Since no information was available about the production of chlamydospores in artificial medium by *Fusarium oxysporum* Schlecht f. sp. *melonis* Snyder & Hansen, required in our ecological work, the following work was undertaken.

The isolate M-15 D of *F. oxysporum* f. sp. *melonis* was maintained on potato dextrose agar and single conidium cultures were transferred at monthly intervals. Conidia for inoculation, were harvested from 3–4 weeks old cultures by shaking in distilled sterile water and washed three times by centrifugation (2,000 g) and resuspension.

Soil extract was shown by preliminary experiment to be the best medium for the production of chlamydospores by our fungus. Similar results were also obtained by Ford et al. (1970b) with *F. solani*. The soil extract was prepared by mixing greenhouse organic soil of pH 6.4 and distilled water (1:3 w/v), shaking for 2 hours and clearing by filtration through glass wool and centrifugation. Glucose was added in the soil extract to give the concentration varying from 0 to 10,000 ppm. Fractions of 50 ml solution were dispensed in 250 ml Erlenmeyer flasks and autoclaved. The pH after autoclaving was between 6.7 and 7.1. Flasks were inoculated with 1 ml of the washed conidial suspension, to give a final concentration of about 250,000 conidia per ml, and incubated at 28 °C.

Chlamydospores were counted by a slight modification of the method used by Ford et al. (1970a). After various days of incubation, 10 drops of aniline blue stain (Jones and Mollison, 1948) were added to each flask and shaken for 1 hour. The content was homogenized in a Sorvall omni-mixer at full speed for 1 minute to disrupt the mycelium and separate chlamydospores. 1 or 0.5 ml suspension was filtered through a 0.45 μm millipore filter of 13 mm diameter.

The filters were dried in air and made transparent by mounting in immersion oil. The number of chlamydospores in 10 oil immersion microscopic fields was recorded. The results are reported in Table 1; each value is the mean of four replicates. The experiment was repeated twice.

As Table 1 indicates, large numbers of chlamydospores were produced in the soil extract within 3 days and their number increased with increasing incubation period and glucose concentrations. After longer incubation time higher glucose concentration yielded more chlamydospores than the lower ones. The mycelium issued from germi-

Table 1. Number[1] of chlamydospores per ml soil extract as influenced by different concentrations of glucose and time of incubation.

Glucose	Incubation time in days			
concentration (ppm)	3	7	15	30
0	4,750	31,920	39,900	52,720
10	12,350	38,380	45,600	92,720
100	10,830	43,440	86,640	97,280
250	2,280	69,160	136,040	161,120
500	380	83,220	141,360	166,560
1,000	0	56,240	135,280	187,720
2,000	0	10,640	68,400	81,320
5,000	0	950	5,320	53,200
10,000	0	380	3,040	–

[1] Each value gives the mean of four replicates.

Tabel 1. Aantal chlamydosporen geproduceerd per ml grondextract, onder de invloed van de glucose-concentratie en de incubatietijd.

nated conidia either was converted into chlamydospores or lysed. In treatments having 2,000 ppm or more glucose, the mycelium remained viable even after 30 days, forming few intercalar chlamydospores, and caused problems in counting.

In the first 3 days round or pear shaped, terminal chlamydospores, single or in chains of two were formed. Later on, ellipsoidal to globose or round intercalar chlamydospores developed very commonly in chains of 2 and 3 and more rarely of 4 and up to 6. The size varies from 5.30–15.90 μm and average 8.34 μm.

By a judicious choice of carbon concentration in soil extract medium regulating the amount of mycelium formed, and of the incubation period during which the mycelium converts into chlamydospores, this method permits the production of large numbers of chlamydospores. In suitable fresh medium or in sterile soil they begin to germinate after 3 h and within 7 h of incubation up to 98% germination was observed.

These results also show the similarity in reaction of our isolate of *F. oxysporum* f. sp. *melonis* and the *F. solani* used by Ford et al. (1970b). As found by these authors, the conditions for maximum production of chlamydospores have to be determined for each strain or species of Fusarium.

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Samenvatting

Overvloedige chlamydosporenproduktie van Fusarium oxysporum f. sp. melonis in grondextract met glucose

In grondextract waaraan een kleine hoeveelheid glucose werd toegevoegd, vormt *Fusarium oxysporum* f. sp. *melonis* overvloedig chlamydosporen. Gedurende een in-

cubatieperiode van 30 dagen neemt het aantal chlamydosporen toe met stijgende glucoseconcentratie (10–1000 ppm). 98 % van de gevormde chlamydosporen kiemde na 7 uren incubatie in vers medium of in steriele grond.

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Book review

Stover, R. H.: Banana, plantain and abaca diseases. Commonwealth Mycological Institute, Kew, Surrey, England, 1972. XII + 316 pp., 190 fig., 8 coloured plates, 25 tables. Price: £ 5.50 (cloth bound).

This book is concerned with disease problems of banana produced for export on estates (about 15 % of world production), rather than on small farms. The author draws upon 15 years of experience with the United Fruit Company. The book is very efficiently written and much attention is given to the economic consequences of each disease and its control. The text is concise and yet very lucid and up to date. The illustrations and the general layout are excellent. Besides four short general chapters (19 pp.) an introduction (2 pp.) and two appendices (5pp.), there are fourteen chapters on diseases grouped according to cause. There are chapters on diseases caused by: nematodes, fungi (4 chapters), bacteria, viruses, non-infectious and unknown causes, chemical injuries, nutritional deficiencies, climate, soil conditions, and genetic abnormalities. The fungus diseases are divided over four chapters into those of the foliage, pre-harvest fruit, post-harvest fruit, and of root, corm and pseudostem. Each disease is systematically described under subheadings: symptoms, causal organism, factors influencing disease incidence, economic importance and control.

Major references up to 1970 with emphasis on the literature since 1961 are cited after each disease. For a more complete list of the older literature the reader is referred to C. W. Wardlaw's book of 1961.

In the preface the author draws attention to the tremendous changes in the banana industry since the appearance of Wardlaw's last book (1961). These changes have altered the entire field of banana disease research and were one of the reasons for writing this book. In Wardlaw's book nematodes were only treated in an appendix. Here nematodes merit a special chapter because replacement of 'Gros Michel' by mainly Cavendish varieties, have meant a corresponding replacement of fusarial wilt by Radopholus root rot. The new ways of harvesting and shipping, the introduction of fruit-branding (Chiquita) with high quality standards, etc, made control of pre-harvest and post-harvest fruit diseases more important than ever. New methods of control, such as the advent of systemic fungicides are other developments of great consequence. These and other changes and developments have been dealt with in two short chapters on the relation of disease to: varieties and breeding (3 pp.) and cultivation and shipping practices (7 pp.), but have especially been integrated with the treatment of the various diseases. This book is a very clear, efficiently written and designed, concise, accurate, well-illustrated and up-to-date treatise on diseases of bananas produced on large estates and on their control.

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