postulated that the extract contained a nematotoxic substance. It is assumed that the fungus secretes a chemical substance which paralyzes or kills nematodes after they are caught by its adhesive trapping organs, because only the filtrates from fungus-parasitized nematodes contained the nematode-inactivating substance. The relatively low toxicity of the filtrates may be attributed to dilution effects. This, apparently nematotoxic, substance is probably an unstable chemical compound because a nematode population in a Petri dish culture with *A. oligospora* will usually build up again soon after the natural decline of the nematophagous activity of the fungus.

This work was supported by the National Research Council of Canada.

TH. H. A. OLTROF

Experimental Farm,
Research Branch, Canada Agriculture,
Scott, Saskatchewan.

R. H. ESTEY

Department of Plant Pathology,
Macdonald College of McGill University,
Montreal.

¹ Drechsler, C., *Mycologia*, **29**, 452 (1937).

² Shepherd, Audrey, M., *Nature*, **175**, 475 (1955).

³ Duddington, C. L., *Biol. Rev.*, **31**, 152 (1955).

⁴ Soprunov, F. F., and Galiulina, E. A., *Microbiology* (Moscow), **20**, 489 (1951).

Isolation and Maintenance of Single Spore Lines of *Peronospora tabacina* (Adam.)

WHILE germination of conidia of the obligate parasite *Peronospora tabacina* Adam. has been effected on tobacco leaf disks^{1,2}, it has not previously been possible to complete the life cycle of the fungus on such disks, because of their rapid deterioration. Sporulation investigations have been possible only by taking disks from leaves which had been infected previously and in which mycelium was well established³. Izard⁴ has described a method for the growth of *P. tabacina* on leaves and leaf disks in the laboratory, but in our experience this method has been unsuccessful with the Australian varieties of host and pathogen. Accordingly, we have devised the following technique.

Following the report by Richmond and Lang⁵ on the effect of kinetin on the survival of detached leaves of *Xanthium pennsylvanicum*, leaf disks (15 mm diam.) from *Nicotiana tabacum* cv. Virginia Gold were floated, ventral side uppermost, on an aqueous solution of 1 µg/ml. kinetin. On this medium, the disks remain green and apparently healthy for approximately three weeks in the absence of infection. When kinetin is omitted from the medium, the disks show discoloration and yellowing after 5-7 days. Approximately 2×10^3 conidia of *P. tabacina* were applied in a drop of water to the centre of each disk. The disks were then incubated at 20° C under 800 ft.-candles of fluorescent illumination and a daily light regime of 16 h light and 8 h darkness.

Some necrosis occurs under the drop of conidial suspension after 2 days incubation and this afterwards extends over the leaf disk surface in an irregular manner. This necrotic reaction does not occur on control disks under a water droplet and in the absence of conidia. Sporulation occurs after 6-7 days, the number of conidiophores produced increasing to a maximum over a period of 2-3 days from their first appearance. The conidiophores appear to be confined to the green portions of the disk and have not been observed to emerge from the necrotic areas.

After 7, 8 and 9 days incubation at 20° C the average number of conidiophores produced per leaf disk was 5.6, 20.9 and 49.6 respectively (averaged over 48 disks). After

incubation of a similar number of disks for 9 days at 15° C and 25° C, the average number of conidiophores per disk was 0.3 and 19.0 respectively.

When 2 µg/ml. each of *p*-aminobenzoic and nicotinic acids were added to the kinetin solution, the number of conidiophores finally produced was approximately double that occurring when these vitamins were omitted.

The infection of disks from single spores was achieved following the isolation of single conidia on agar, using a De La Rue cutter. The percentage of successful 'takes' was increased from 21 per cent to about 50 per cent when such spores were applied to leaf disks in a drop of the complete medium described by Shepherd and Mandryk⁶. Clones, isolated in this manner, have been passaged three times successfully, but some loss of viability of the organism as measured by a reduction in the number of conidiophores produced appears to occur during such serial passage on to leaf disks. This difficulty has been overcome by transferring the first leaf disk infected by the original single spore isolate to the ventral side of a tobacco leaf. If the disk is attached closely to the leaf surface by means of a thin layer of 1 per cent Difco 'Bacto' agar, the mycelium from the disk grows readily into the leaf and abundant sporulation occurs afterwards on the leaf surface. Alternatively, the first crop of conidia produced on a leaf disk following single spore isolation may be used to inoculate tobacco seedlings grown in sterile culture according to Steinberg⁷.

This technique allows the ready isolation and maintenance of clonal lines of the pathogen. It opens the way to studies on the occurrence of physiological strains and to the possible isolation of biochemical mutants of this organism.

C. J. SHEPHERD
FREDRIKA STUART
M. MANDRYK

C.S.I.R.O.,
Division of Plant Industry,
Canberra.

¹ Darnell-Smith, G. P., *N.S.W. Agric. Gaz.*, **40**, 407 (1929).

² Henderson, R. G., *Virginia Agric. Exp. Sta. Bull.*, No. 62 (1937).

³ Cruickshank, I. A. M., and Müller, K. O., *Nature*, **180**, 44 (1957).

⁴ Izard, C., *C.R. Acad. Sci. Paris*, **251**, 3063 (1960).

⁵ Richmond, A. E., and Lang, A., *Science*, **125**, 650 (1957).

⁶ Shepherd, C. J., and Mandryk, M., *Trans. Brit. Mycol. Soc.*, **45**, 233 (1962).

⁷ Steinberg, R. A., *J. Agric. Res.*, **75**, 81 (1947).

MICROBIOLOGY

Lysogeny in *Providencia* Strains and the Host-range of *Providencia* Bacteriophages

BIOCHEMICALLY *Providencia* strains are closely related to *Proteus* organisms, especially *P. rettgeri*, and on these grounds the name *Proteus inconstans* has been suggested for them¹. The guanidine + cytosine content of *Providencia* strains is very similar to those of *P. hauseri* and *P. rettgeri*², and recently it was found that many *Proteus* (especially *rettgeri*) phages productively lyse *Providencia* strains³. It was decided to investigate the reciprocal relationships, that is, to test phages primarily isolated on *Providencia* strains for action on *Proteus* organisms. Media used were those previously used⁴. Thirty-seven *Providencia* cultures comprising 25 different serological groups were used. They are all National Collection of Type Culture strains and were kindly supplied by Miss Shona Wright of the Department of Bacteriology, University of Edinburgh. In addition, 25 *P. rettgeri* cultures (17 locally isolated, 8 N.C.T.C. strains) 17 *P. morgani* cultures (11 locally isolated, 8 N.C.T.C. strains) and 31 *P. hauseri* strains previously described⁵ were used in host-range experiments. The *rettgeri* strains can all be differentiated on the basis of susceptibility to a series of *P. rettgeri* phages. The *hauseri*