BIOLOGY

A Strain of *Peronospora tabacina* Pathogenic to Tobacco Lines with Resistance derived from *Nicotiana debneyi* and *N. goodspeedii*

The occurrence of strains of *Peronospora tabacina* Adam. (blue mould of tobacco) as suggested by Hill[1] has now been confirmed.

Conidia of *P. tabacina*, from susceptible varieties growing at Canberra, A.C.T., and at Myrtleford, Victoria, were used as inoculum for separate lots of plants of the susceptible variety Virginia Gold and the resistant variety SO_1 (ref. 2). After 4 or more days, depending on temperature, symptoms of the disease were obvious on leaves of the susceptible variety and extensive sporulation occurred on the following morning. Small chlorotic flecks that failed to sporulate appeared on young leaves of the resistant variety. Three or more days later, one to three sporulating lesions appeared on some of the resistant plants that had been inoculated with conidia from either source.

Conidia produced on SO_1 plants following inoculation with the pathogen from Canberra were used to inoculate alternate leaves of three SO_1 plants from which the flowers, and all except 4–5 leaves, were removed. The remaining leaves, inoculated with conidia from a susceptible variety, served as controls. Results at 8 days after inoculation are shown in Table 1. An SO_1 plant destroyed by blue mould is shown in Fig. 1.

Table 1

Plant *A*		Plant *B*		Plant *C*	
Leaf size (cm)	No. of sporing lesions	Leaf size (cm)	No. of sporing lesions	Leaf size (cm)	No. of sporing lesions
30 × 18	24	26 × 16	18	27 × 16*	0
31 × 18*	0	29 × 18*	0	34 × 20	42
35 × 18	12	32 × 19	19	41 × 20*	1
34 × 17*	0	36 × 19*	0	41 × 19	28
		38 × 18	56	43 × 21*	0

* Leaves inoculated with conidia from Virginia Gold plants. Other leaves inoculated with conidia from SO_1 plants.

In the susceptible variety, the disease appeared earlier, and its progress was more rapid, when the plants were inoculated with conidia from susceptible varieties than when inoculated with conidia from SO_1 plants. Rates of development were similar in SO_1 seedlings inoculated with conidia produced on SO_1 plants and in Virginia Gold seedlings inoculated with conidia produced on Virginia Gold plants.

Seedlings of the resistant lines *Bel*-61-12 (ref. 3) from the United States, Fixed *A*2 hybrid[4] and two lines[2] with resistance derived from *N. goodspeedii* Wheeler were all susceptible when inoculated with conidia produced on SO_1 plants.

Resistance in the SO_1 variety is derived from *Nicotiana debneyi* Domin. In another test, a line with resistance derived from *N. goodspeedii* was inoculated with conidia from a susceptible variety, and sporulating lesions indistinguishable from those reported for SO_1 were produced.

The results show that the *P. tabacina* used in these experiments is a mixture of a major and at least one minor component. When propagated on the normal susceptible commercial varieties, the major component appears to have a selective advantage over the minor component. However, propagation on genotypes resistant to the major component excludes the operation of this selective advantage and the minor component builds up rapidly.

As *P. tabacina* in Europe is considered to be of Australian origin, results obtained in Australia may be applicable in Europe. In Australia, resistant lines of tobacco have not been released for commercial production, consequently the situation in the fields will remain unchanged. However, it would appear that extensive use of resistant varieties now becoming available could be followed by a build-up of the strain to which they are susceptible.

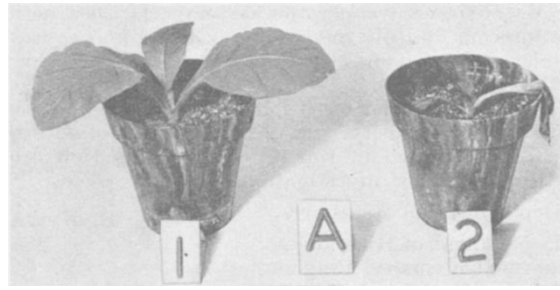

Fig. 1. Resistant variety SO_1 photographed 11 days after inoculation with conidia from: (1) Virginia Gold plants; (2) SO_1 plants

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[1] Hill, A. V., *C.S.I.R.O. (Austral.) Div. Plant Indust. Tech. Paper* 9 (1957).
[2–4] Seed supplied by D. Wark, C.S.I.R.O., Canberra, Australia. *Bel*-61-12 was originally from the U.S. Department of Agriculture and Fixed *A*2 hybrid from Mr. H. Lea.

Nitrogen Fixation by the Leaf-nodule Endophyte of *Psychotria bacteriophila*

Investigations of the symbioses in root-nodules of legumes and non-legumes have established that nitrogen fixation occurs in the nodular tissue[1]. Although some reports have appeared describing the leaf-nodule symbiosis in *Psychotria*[2–4] no isolate of the endophyte is at present available, and it has not been unequivocally established that nitrogen fixation is involved. We now report that the isolated endophyte fixes atmospheric nitrogen in pure culture and, apparently, in the plant as well. It is known that plants either naturally or experimentally freed of the endophyte are dwarfs[2,3]. Nitrogen fixation does not fully account for these observed effects.

The endophyte has been repeatedly isolated in pure culture from germinating seedlings on either nitrogen-free mineral agar or trypticase soya agar. Although the isolates obtained from both media were identical the N-free medium is preferable for isolation purposes, since contaminating non-fixing micro-organisms are thereby eliminated. The isolate, which we have identified as a species of *Klebsiella* by serum agglutination tests, was a Gram-negative, motile, non-acid fast, non-spore forming rod with terminal refractile granules.

Growth in nitrogen-free glucose broth was delayed for 4 or 5 days until the evolution of gas was observed. When an 80 per cent nitrogen–20 per cent helium mixture was used as the gas phase there was little delay in growth and both the growth rate and the total crop were greater than that obtained with cells grown in air.

Nitrogen fixation was demonstrated directly using a nitrogen-15 enriched gas phase. Cells were grown on nitrogen-free mineral agar for 4 days, aseptically suspended in sterile 0·1 M, *p*H 7 phosphate buffer and inoculated into 4–5 l. of the nitrogen-free culture solution in an 11-l. carboy. High-purity nitrogen was sparged through the culture for 48 h. The cells were collected aseptically by centrifugation and 10 ml. of a thick suspension of cells (≈3 mg nitrogen) in a mineral salts solution were treated with various gas mixtures as indicated in Table 1. Leaf homogenates were made by cutting small pieces of surface sterilized leaves with a razor blade followed by grinding in an all-glass TenBroeck homogenizer in 0·1 M, *p*H 7 phosphate buffer. After exposure to the labelled gas, the cells were digested by conventional Kjeldahl method using a mercury catalyst. The digests were distilled into hydrochloric acid and converted to nitrogen