

LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) ON TOMATO IN THE TROPICS

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Of all the pathogens of economically important plant species, *Phytophthora infestans* (Mont.) de Bary can rightly claim its place as the most notorious. Its occurrence in Ireland during the last century caused both the potato famine of 1846–49 and the establishment of plant pathology as a scientific discipline by Rev. M. J. Berkeley and others. Even today, late blight frequently decimates potato crops during wet summers, despite significant advances in disease prediction, crop protection (usually with phenylamide or dithiocarbamate fungicides) and the introduction of more resistant cultivars.

Although late blight has traditionally been associated with potatoes, in tropical climates with periods of high rainfall the same pathogen can be a significant factor limiting yields from tomato crops. In many developing countries, tomato represents an important food crop, not only because of the high price it commands compared to staples such as rice, but also because it provides a valuable source of vitamins when fresh and improves the flavour of meals when cooked.

The requirement of tomatoes for moderately low night-time temperatures for efficient fruit-set limits production in the lowland tropics to the cooler seasons. Consequently summer production is centred upon cooler upland areas (e.g. in the Cameron Highlands of Malaysia and the Central African massif), where high rainfall levels are optimal for late blight infection. In such areas, late blight represents a serious problem, since application of costly imported fungicide is often ineffective due to dilution by rainfall and the potential occurrence of fungicide-resistant strains of the pathogen.

Research at the University of Wales, Bangor being funded by the UK Government Overseas Development Administration and in collaboration with the Asian Vegetable Research and Development Centre (AVRDC) in Taiwan has two

objectives. Firstly, we are identifying promising sources of resistance to late blight by screening material from tomato germplasm collections; promising accessions are currently being field-tested in Taiwan, Tanzania and Costa Rica prior to the initiation of a breeding programme. Secondly, we are examining levels of genetic variability within populations of the pathogen from tropical areas; it is vital to know the extent of geographical variation in the aggressiveness and virulence within pathogen populations to maximize the possibility that resistance factors introduced into planting material will provide durable disease protection over a wide geographical range.

An important component of our research therefore involves the acquisition of samples of the pathogen from a range of countries, both temperate and tropical. These will permit testing the resistance of promising tomato accessions against pathogens from the countries where the resistant cultivars will ultimately be grown. A further reason for wishing to assess *P. infestans* populations is the increase over recent decades in the international potato trade. The concomitant migration of the fungus in diseased tubers is believed to have led to significant population changes. We hope that by obtaining isolates of the fungus from around the world it will be possible to more accurately monitor these changes.

Wherever tomatoes are grown without irrigation and without heavy use of fungicides, it is likely that late blight is present unless conditions are very dry. The symptoms include blackening of the leaves (Fig 1a; often spreading lesions originating from the tip of the leaf) and death of whole branches of the plant (Fig 1b). Infected green fruit become marbled brown in appearance (Fig 1c) without becoming soft (not to be confused with nutritional disorders such as blossom end rot, which usually affect the area around the tip of the fruit). Sporulation of the fungus (only under

conditions of high humidity) can sometimes be seen as a zone of grey-white 'fluff' around the edge of necrotic lesions, especially on the lower surface of leaves Fig 1d.

Should any readers of the *Mycologist* encounter this disease on tomato (whether in the UK or abroad), we would be most grateful if they could send us samples, using the simple method des-

cribed here for transporting diseased material. The procedure is:

- (1) Obtain infected tissues (preferably leaves) from blighted tomato and incubate at high humidity until sporulation of the fungus is visible. Shake a leaf with a sporulating lesion in a small bottle with a little water (ca 5 ml).



Fig 1 Symptoms of infection by *Phytophthora infestans* on tomato. (a) Spreading black lesions develop on leaves. (b) Under suitable conditions whole branches (and eventually the whole plant) are killed. (c) Green fruits are susceptible to infection and become marbled brown in appearance. (d) Sporulation of *P. infestans* occurs when infected leaves incubated under high humidity and is usually visible as a zone of grey-white 'fluff' around the edge of darker necrotic lesions, especially on the lower surface of leaves.

- (2) Dip a household fork in the sporangial suspension and stab a medium sized (2–4 cm diameter) unripe, green tomato twice. Incubate the inoculated tomato for 2–4 days at high humidity. Faint brown lesions should develop around the points of inoculation.
- (3) Once symptoms have developed, wrap the inoculated fruits individually in dry newspaper and pack in a crushproof container. Send by airmail (in a crushproof packet) to: Dr G.W. Griffith, School of Biological Sciences, Memorial Building, University of Wales, Bangor, Gwynedd LL57 2UW, United Kingdom. To fulfil UK quarantine requirements, it is necessary for diseased material to be accompanied by a copy of our MAFF import licence (no. 1571/1156/84). We will gladly send copies of the licence in response to any requests.

If diseased fruits are encountered in the field, these too can be sent to us. The inoculated tomatoes should remain in good condition for several weeks. In the absence of any fruits, leaves bearing small lesions can be lightly pressed in dry newspaper and posted; we have found that the fungus can survive for a week or more under these conditions. Please note, however, that it is critical to avoid any build-up of free water around the diseased fruit or leaves, otherwise bacterial rots will set in rapidly to produce a soggy, smelly mess by the time the material arrives in Bangor! Please include details about the geographical origin of the blight, tomato cultivar etc.

P. infestans is not among the easiest of microorganisms to culture axenically. Since it exists in nature as a near-obligate biotroph, in agar culture it is prone to contamination, and requires frequent subculture and incubation at 15–20°C. However, since *P. infestans*, along with other oomycete fungi, is only distantly related to the true fungi, a number of anti-fungal and anti-bacterial antibiotics are effective in selectively suppressing the growth of contaminating microorganisms. We routinely used an antibiotic cocktail (RAN – 50 mg/ml each of nystatin and rifamycin, and 25 mg/ml ampicillin; dissolved in dimethyl sulphoxide and stored at –20°C). This cocktail is used to supplement standard media (2 ml per litre of medium) immediately before dispensing the agar into Petri dishes.

Our favoured medium at Bangor is ryeA, originally devised by Caten in the 1960s. It

contains the trace elements, vitamins and fatty acids required for growth and sporulation by *P. infestans* but is sufficiently nutrient-poor to prevent the rapid growth of contaminating microorganisms. It is prepared by soaking 60 g of rye grains in 300 ml distilled water for 36 hours at 15°C. The supernatant is decanted and the grains disrupted in a food processor with a further 500 ml water. The mash is incubated at 50°C for 3 hours before straining through muslin. 20 g sucrose is added to the supernatant which is then made up to 1 litre (including the original supernatant in which the grains were soaked). 6 g/l agar is added and the medium is sterilized by autoclaving (15 min, 15 psi). In other laboratories similar media based on grains (e.g. rolled porridge oats) or pulses (e.g. frozen peas, lima beans, chick peas or V8 juice) are preferred. However, the latter tend to be more nutrient rich and thus more readily contaminated, although they do support higher levels of sporulation.

Isolation of *P. infestans* on ryeA medium supplemented with the RAN antibiotic cocktail, simply involves plating small fragments of diseased plant material (5–10 mm³). Outgrowth of the *P. infestans* hyphae occurs after incubation at 19°C for 4–8 days. An alternative method uses a wedge of agar, which is lightly brushed against the aerial sporangia of the fungus. The sporangia detach readily and will germinate directly when incubated on ryeA medium.

Reference

Phytophthora, (1991). British Mycological Society Symposium Volume No. 17. Edited by J.A. Lucas, R.C. Shattock, D.S. Shaw and L.R. Cooke. Cambridge University Press, Cambridge, UK.

The next issue of the *Mycologist* will be published in August 1995 and will include articles on:

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