EUROBLIGHT Protocol - Version 1.2 - 4 October, 2004

Detached leaf test for foliage blight resistance

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Introduction

This protocol was produced in the framework of the EUCABLIGHT concerted action (2003-2006). The objective was to develop a harmonised method for testing host resistance to foliar late blight on detached leaves, therefore facilitating the comparison and compilation of data.

Facilities required

Use an isolated field where potatoes can be grown without late blight until leaflets are collected or, a greenhouse with good climate control suitable for growing potato plants. Use a climate room with illumination and temperature control for incubation of the inoculated leaflets.

Plants

It is important that plants are grown without heat or drought stress. Plants should be grown between early spring and mid-August, to ensure they are sturdy.

Replication

The minimum number of replicates is 2 replicates of 5-20 leaflets from 5-20 compound leaves.

Controls

SASA single R-gene differentials should be included in at least one replicate: r0 (Craig's Royal) and R1 – R11.

Inoculum

For inoculations single isolates of a complex race are better than race mixtures. A suitable inoculum density is 50000 zoospores/ml. This can be prepared by cooling (5-12 °C) a sporangial suspension of about 15000 sporangia/ml for 1.5-3 hours. Zoospores remain motile longer if you use 1% potato tuber extract (McKee, 1964: boil 300 g of sliced potatoes for 20 minutes in 1 litre water, remove the potatoes, allow debris to settle and use the clear liquid) in your suspension, especially if you have a clean suspension in tap water or distilled water. If no zoospores are released (this happens sometimes), you can use the sporangial suspension.

Inoculation

The plant stage in which leaves were collected is scored: 1 = no flower buds visible; 2 = onset of flowering; 3 = flowering and lower leaves yellowing. The youngest fully expanded compound leaves are collected before 10.00 AM, to avoid wilting. The leaves are to be kept cool and moist during transport to the lab. In the lab, leaflets are separated, the top (distal) leaflet is discarded, and one leaflet from each compound leaf is placed in moist containers, e.g. plastic trays with moist

filter paper wrapped in polythene bags. Leaflets are inoculated on the abaxial side, with a single inoculum droplet of about 10 – 50 µl. You can either inoculate leaf halves (2 droplets per leaflet) or entire leaflets (1 droplet per leaflet). The droplet should not dry out before the pathogen has penetrated (3 hours for zoospores, 24 hours for sporangia). The containers are incubated at 15°C in light, 16 h photoperiod (avoid direct sunlight).

Scoring

Scoring is done after 6 days. Both the leaf area affected (= sporulating or necrotized) by the pathogen and the intensity of sporulation are scored. Scores for the affected leaf area are given as percentage: 0.1%= small, separate necrotic lesions; 1%= inoculated area necrotic; 5-100% = percentage of leaflet necrotized. Scores for sporulation are given on a 1-3 scale, 1= no sporulation, 2 = slight to moderate sporulation, 3 = intense sporulation.

Infections 4 days after inoculation

Infection = 0,1% (centre of picture)

Infection = 15%, most of which is sporulating, but not yet necrotic

References

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