



ISBN 978-85-66836-16-5

A HIGH-THROUGHPUT TEST TO ASSESS THE SENSITIVITY OF *Alternaria grandis* TO AZOXYSTROBIN, PYRACLOSTROBIN, BOSCALID AND CHLOROTHALONIL[1]/Um teste de alto rendimento para avaliar a sensibilidade de *Alternaria grandis* a azoxistrobina, piraclostrobina, boscalida e clorotalonil.

J.P.H. MACHADO[2]; M.L. SILVA[2]; E.S.G. MIZUBUTI[2]. [2]Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Brazil. E-mail: joao.honorato@ufv.br.

Monitoring fungicide sensitivity in populations of plant pathogenic fungi is crucial for the efficacy of chemical control. The objective of this work was to develop a high-throughput test to assess the sensitivity of the causal agent of potato early blight, *Alternaria grandis*, to Azoxystrobin (AZ), Pyraclostrobin (PC), Boscalid (BC), and Chlorothalonil (CT). Czapek dox broth medium with fungicide (45 µl) was added to wells of ELISA plate. The spore suspension (6 x $10^4$ conidia/mL) was amended with antibiotics and a 45 µl-drop added to each well. The final concentrations of AZ, PC, and BC were: 0.001; 0.01; 0.05; 0.1; 0.5; 1.0; and 10 µg/mL. For CT the concentrations were 1; 10; 25; 50; 100; 500; and 1000 µg/mL. A total of 32, 29, 18 and 27 isolates were evaluated for AZ, PC, BC and CT, respectively. Salicylhydroxamic acid (SHAM) was added to the suspensions of AZ and PC, solubilized with methanol. Dimethyl sulfoxide (DMSO) was used to solubilize CT and BC, water to AZ and acetone to PC. Control treatments varied according to the fungicide tested. The plates were kept at 300 rpm and 28ºC for 16h in the dark. After incubation, 10 µl of MTT at 4 mg/mL were added to the plates wells and kept at 300 rpm at 28 ºC for 3 h, lastly 150 µl of DMSO were added and incubated under the same conditions for 24h. A replicate of the assay was set in microscope slides with the same spore and fungicide suspensions, but maintained at 25 ºC. After 16h 10 µl of lactoglycerol was added to stop germination. The germination on slides was assessed counting 100 conidia/slide. Absorbance was read at 538 nm. All isolates were sensitive to CT, however 11, 14, and 3 isolates had reduced sensitivity to AZ, BC and PC, respectively. Absorbance and % germination were correlated: AZ ($r = 0.82$; $P < 0.01$), PC ($r = 0.79$; $P < 0.01$), BC ($r = 0.60$; $P < 0.01$), and CT ($r = 0.92$; $P < 0.01$). The assay allowed quick assessment of fungicide sensitivity in populations of *A. grandis*.

**Key words:** Resistance; Chemical control; Early blight; MTT.

[1]Grant information: CNPq.