Species:	*Alternaria solani*
Product Class(es):	QoI fungicides, and also suited for other fungicide classes
Method type described:	In vitro and whole plant
Date of protocol:	2006-03
Proven for	Azoxystrobin
Should be suitable for	other QoI and also SBI's. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results • validated routine method for labs with access to seed plants

Method:

Introduction

The *in-vitro* and *in-vivo* azoxystrobin sensitivity methods described here are used by Syngenta to determine the sensitivity of *Alternaria solani* isolates. The information obtained may be used to establish baseline sensitivity distributions, implement resistance management practices and to determine if resistance to azoxystrobin in *A. solani* isolates is the basis for unsatisfactory disease control.

Methodology

I. In-vitro sensitivity test

The sensitivity of each monoconidial isolate of *A. solani* to azoxystrobin is determined by comparing the conidial germination of each isolate on water agar (1000 ml of distilled H_2O and 20 g Bacto Agar) plates amended or not with azoxystrobin and salicylhydroxamic acid (SHAM). Technical grade azoxystrobin is dissolved in acetone and the final concentrations in the water agar media is adjusted accordingly. Azoxystrobin technical grade is used to amend the agar plates to the following concentrations: 0, 0.001, 0.01, 0.1, 1.0, and 10 mg/L. SHAM is dissolved in methanol at 100 mg/liter. The control is amended with acetone and SHAM without fungicide. It is recommended to have a control amended with acetone but without SHAM to determine if SHAM is inhibitory of the isolate being tested. SHAM is a known inhibitor of the alternative oxidase (AOX) pathway that has been suggested as a possible mode of QoI resistance *in vitro* in other fungi.

Conidia are produced by growing the isolates on clarified V-8 agar media. Inoculum is prepared by flooding the plates with 10-15 ml of sterile distilled water (or deionised water) and gently rubbing the surface of plates with a sterile glass rod. As soon as conidia are collected, store the conidial suspension in the refrigerator (about 4-5 °C) in order to delay germination. Adjust the conidial suspension to $1x10^4$ conidia per milliliter using a hemacytometer. A 50 µl sample of the *A. solani* conidial suspension is added to the control plates and plates containing azoxystrobin and spread with a glass rod.

Germination of 50 conidia is assessed at each concentration (2 replications) after incubation under continuous light for 4 h at 26 °C. A conidium is rated as germinated if a normally developing germ tube is at least the total length of a conidium (beak and tail), if an appressorium formed at the tip of the germ tube or if multiple germ tubes developed.

Isolate sensitivities are expressed as relative germination, which is defined as the ratio of conidia germinating in the presence of azoxystrobin to those germinating in the absence of azoxystrobin x 100. The relative germination data is usually transformed using arcsine transformation. The effective dose for 50% of conidial germination inhibition (ED_{50} values) is calculated by interpolation based on regression of relative germination on $\log_{10}$ transformed fungicide concentration.

2. In-vivo sensitivity test

The *in-vivo* sensitivity assay for *A. solani* isolates is conducted as a 24-h preventative test. Fungicides are applied 24 h prior to inoculation in the greenhouse using tomato plants of the cultivar Bonny Best. *A. solani* isolates collected from tomato or potato can be tested *in-vivo* using this tomato cultivar which is highly susceptible to early blight. Tomato plants are grown in the greenhouse for about 3 weeks. Plants are ready for inoculation when the first true leaves are fully expanded.

Tomato plants are treated with a commercial formulation of azoxystrobin (Quadris®) or technical grade azoxystrobin depending on the objective of the study. If plants are treated with azoxystrobin technical grade, prepare stock solutions in acetone as described above for studies *in-vitro*. When technical grade is used it is also recommended to add “Tween 20” to the azoxystrobin solution (0.05%).

Tenfold azoxystrobin concentrations are applied to plants (0, 0.01, 0.1, 1, 10, and 100 µg/ml) to obtain a dose response curve. Azoxystrobin doses are applied to runoff. Twenty-four hours after fungicide application, tomato plants are inoculated with a conidial suspension adjusted to 1×10^5 conidia per ml from 12 to 14 day-old cultures of an *A. solani* isolate grown on clarified V8 agar media. The inoculated plants are transferred to a dew chamber (22-24 °C) for 24 hours. Then, plants are taken to the greenhouse and placed on moistened capillary matting. The plants are misted twice a day with a fog nozzle to maintain high humidity. The average temperature is about 24 °C. Early blight symptoms develop after 7 to 14 days and disease severity is assessed by estimating the percent of infected leaf area of the first two true leaves. At least 3 replications (3 pots with 1 or 3 plants per pot) are tested for each fungicide treatment. ED_{50} values are calculated as described above for studies *in-vitro*.

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