Species:	*Ramularia* spp.
Product Class(es):	QoI fungicides
Method type described:	Microtiter plate
Date of protocol:	2026-06
Proven for	Metyltetraprole
Version	1
comments	Protocol adjustments may be needed for other fungicide classes depending on the individual compound characteristics.

Method:

1. Sampling: To obtain *Ramularia* spp. isolates, cotton leaves showing visible disease symptoms are collected. Leaves with low disease severity are preferable. The leaves should be dried thoroughly to remove any surface moisture, placed individually between dry paper towels, and sent to the laboratory.

2. Inoculum production: Conidia from these cotton leaves are transferred onto the surface of Petri dishes containing potato dextrose agar (PDA). The plates are incubated at 23 °C in the dark. A small piece of a mycelium is then transferred to potato dextrose broth (PDB) and incubated at 23 °C with shaking at 120 rpm in the dark until *Ramularia* spp. mycelia develop. The mycelia are then harvested and homogenized using a homogenizer. The mycelial suspension is adjusted to $1x10^3$ mycelial fragments / mL in PDB.

3. Sensitivity tests: The technical-grade active ingredient is dissolved in dimethyl sulfoxide (DMSO). A 100-fold dilution series of the active ingredient at the following final concentrations is prepared: 0, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 ppm. Aliquots (1 µL) of each dilution are mixed with the inoculum (99 µL) in 96-well microtiter plates. The microtiter plates are covered with plastic wrap to prevent evaporation and incubated at 23 °C in the dark.

4. Evaluation: Five to eight days after incubation, fungal growth is assessed by measuring the optical density at 600 nm using a microplate reader. The values are

corrected using the blank well without inoculum. The 50% effective concentration (EC_{50}) is calculated by logit regression analysis.

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