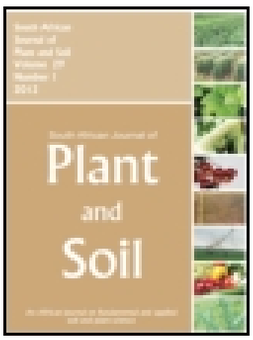

South African Journal of Plant and Soil

ISSN: 0257-1862 (Print) 2167-034X (Online) Journal homepage: http://www.tandfonline.com/loi/tjps20

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To cite this article: Tshekgene M Ramusi, Jacquie E van der Waals, Nico Labuschagne & Theresa AS Aveling (2016): Evaluation of mefenoxam and fludioxonil for control of Rhizoctonia solani, Pythium ultimum and Fusarium solani on cowpea, South African Journal of Plant and Soil, DOI: 10.1080/02571862.2016.1155764

To link to this article: http://dx.doi.org/10.1080/02571862.2016.1155764

Published online: 07 Jun 2016.

South African Journal of Plant and Soil 2016: 1–7
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This is the final version of the article that is published ahead of the print and online issue

SOUTH AFRICAN JOURNAL OF PLANT AND SOIL
ISSN 0257-1862 EISSN 2167-034X
http://dx.doi.org/10.1080/02571862.2016.1155764

Evaluation of mefenoxam and fludioxonil for control of *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium solani* on cowpea

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Cowpea (*Vigna unguiculata*) is susceptible to pathogens such as *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium solani*, which cause seedling diseases in cowpea and result in low yields. Three commercial synthetic fungicides containing mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1}, respectively, were evaluated against these pathogens on cowpea in the greenhouse following promising *in vitro* results. The fungicides were applied initially as a soil drench to seedling trays at planting and fortnightly as a drench according to the manufacturer's recommendations. All fungicides, except mefenoxam 350 g ai L^{-1} in one trial, were able to reduce diseases caused by *R. solani*. With the exception of mefenoxam 350 g ai L^{-1} applied to medium inoculated with *F. solani*, all fungicides increased seedling emergence, and dry shoot and root mass of plants, and all fungicide treatments reduced disease of seedlings grown in medium inoculated with *F. solani* and *P. ultimum*. Although all three fungicides reduced the percentage of diseased seedlings, none gave complete control of the diseases caused by the three pathogens under the trial conditions.

Keywords: damping-off, fludioxonil, mefenoxam, seedling diseases, *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is widely grown in Africa and is also one of the most economically significant traditional legume crops because of its high protein content and ability to tolerate drought and to improve soil fertility (Valenzuela and Smith 2002; Langyintuo et al. 2003). Cowpea is susceptible to many pests and pathogens that cause damage to the crop at all growth stages (Summerfield and Roberts 1985). Seedling diseases in cowpea result in low yields in rural areas where often no control measures are available against the diseases. Cowpea seedling diseases caused by *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp. can result in great losses, particularly in low-altitude rain forests in countries such as Nigeria due to seed decay and seedling damping-off (Singh and Rachie 1985).

Chloroneb, tebuconazole, fludioxonil plus metalaxyl, carboxin, thiram and pyraclostrobin are some of the fungicides presently registered for the control of *Rhizoctonia* diseases (McMullen and Bradley 2005; BASF 2014). Fludioxonil can reduce disease incidence caused by *Rhizoctonia* root rot on ornamental plants, as reported by Martinez-Espinoza et al. (2004). *Pythium* spp. can be controlled by treating the soil with fungicides such as propamocarb-hydrochloride, etridiazole, metalaxyl and mefenoxam, as well as fumigation with a methyl bromide–chloropicrin combination (King and Parke 1993). Mefenoxam is a fungicide that is generally known to be effective against oomycete pathogens such as *Pythium* spp. and *Phytophthora* spp. (Syngenta 2005). Fuchs and Hirnyck (2000), McMullen and Bradley (2005) and BASF (2014) reported control of *Fusarium* species using registered fungicides such as fludioxonil, captan, thiabendazole, tebuconazole, imazalil, thiophanate methyl and pyraclostrobin. Fludioxonil also controlled diseases caused by *Fusarium* spp. on maize (Munkvold and O'Mara 2002). McGovern et al. (2001) stated that mefenoxam could be used to control some *Fusarium* species on potted ornamentals. Kirk et al. (2013) also reported the effectiveness of mefenoxam against *Fusarium* pathogens, which caused dry rot disease of potatoes.

Little research has been done on control measures for seedling diseases of cowpea (Masangwa et al. 2013). Therefore, the current research was conducted to evaluate the efficacy of three unnamed commercial fungicides, containing mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1}, against *Rhizoctonia solani* Kühn, *Pythium ultimum* Trow and *Fusarium solani* (Mart.) Sacc. Although fludioxonil 100 g ai L^{-1} is mainly used as a seed treatment, the three fungicides were tested for effectivity as soil drenches at planting and at 14 and 28 d after planting.

Materials and methods

Fungi

Rhizoctonia solani (UPGH122), *Pythium ultimum* (UPGH050) and *Fusarium solani* (UPGH112), isolated

from cowpea, were obtained from the fungal collection of the Department of Microbiology and Plant Pathology at the University of Pretoria, Pretoria (25°45′6.94″ S, 28°15′34.69″ E, 1 380 m above sea level). To subculture the pathogens, a mycelial disc (5 mm diameter) from actively growing cultures of each fungus was placed in the centre of 90 mm potato dextrose agar (PDA; Merck, Johannesburg, South Africa) petri dishes. The cultures were incubated under fluorescent light at 25 °C for 7 d before use.

Fungicides

Three commercial synthetic fungicides were supplied by Syngenta South Africa (Pty) Ltd, namely mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1}.

***In vitro* study**

Potato dextrose agar was augmented with the various fungicides at the following rates: mefenoxam 350 g ai L^{-1} at 0.21 ml L^{-1} medium, mefenoxam 240 g ai L^{-1} at 0.27 ml L^{-1} medium and fludioxonil 100 g ai L^{-1} at 0.25 ml L^{-1} medium. The media were then poured into petri dishes (90 mm) and allowed to solidify. A mycelial disc (5 mm diameter) from a 7-day-old PDA culture of each of *R. solani*, *F. solani* and *P. ultimum* was placed in the centre of the amended or unamended (control) PDA petri dishes. Four replicates of 12 petri dishes per treatment were used for each pathogen. The petri dishes were incubated under fluorescent light at 25 °C for 9 d. Mycelial growth (colony diameter) was recorded in millimetres on the third, sixth and ninth day after inoculation. The experiment was repeated three times.

Greenhouse trials

Polystyrene seedling trays (128 cells; cell size 67 × 34 mm; 60 mm deep) were filled with steam-pasteurised growth medium (Braaks Lawn Dressing; Rietfontein Kleinhoewes, Pretoria, South Africa). One day before pathogen inoculation, the growth medium was drenched with tap water. Two mycelial discs (5 mm diameter) of each pathogen were prepared as described above and placed at a depth of 20 mm in each cell of the seedling trays 24 h before planting the cowpea seeds.

Cowpea 'Pietersburg Blue' seeds were obtained from the Dry Bean Seed Producer's Organisation, Pretoria. Seeds were planted in the seedling trays, which were then placed in a randomised block design in a greenhouse. Four replications were included per treatment, each replicate consisting of 56 plants. This also included two controls, which consisted of uninoculated and inoculated growth medium. Temperature in the greenhouse was maintained at between 22 and 25 °C with daylight of 13 h and seedling trays were watered daily with tap water. The experiment was repeated three times.

Fungicides were applied as drench treatments to the growth medium at the recommended concentrations: mefenoxam 350 g ai L^{-1} at 0.53 mL 1.5 L^{-1} water, mefenoxam 240 g ai L^{-1} at 0.77 mL 1.5 L^{-1} water and fludioxonil 100 g ai L^{-1} at 0.67 mL 1.5 L^{-1} water. At planting the fungicides were applied to each seedling tray cell by means of a handheld sprayer until the surplus leached through. The applications were repeated at 14 and 28 d after planting. Plants were not watered on the day that they received chemical treatment to avoid leaching of the chemicals.

Percentage emergence and disease incidence were recorded at harvest on the 35th day after planting. Shoot lengths were measured from seedling tip to the soil level a day before harvesting and averages calculated. The plants were harvested, roots were washed with tap water and disease symptoms on roots and shoots were recorded. Roots were then excised from the shoots using scissors, and roots and shoots were each placed separately into brown paper bags, dried for 48 h in a drying oven at 65 °C after which the dry mass of both roots and shoots were determined by weighing.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences ($P < 0.05$) were determined according to Student's *t*-test using MSTAT-C version 1.3 statistical software (Nissen 1983).

Results

***In vitro* study**

All three fungicides, viz. mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1}, applied at different concentrations, significantly reduced the mycelial growth of *R. solani* on the third and ninth day after the start of the experiment when compared with the control (Table 1). However, only fludioxonil 100 g ai L^{-1} was able to significantly inhibit mycelial growth of *R. solani* on the sixth day as opposed to the control. With the exception of fludioxonil 100 g ai L^{-1} on the ninth day, all fungicides significantly reduced mycelial growth of *P. ultimum* relative to the control (Table 1). All three fungicide treatments significantly reduced mycelial growth of *F. solani* throughout the experiment (Table 1).

Greenhouse trials

Rhizoctonia solani

In the first two trials application of mefenoxam 350 g ai L^{-1} increased percentage seedling emergence, and plant height, dry shoot mass and dry root mass significantly, relative to the inoculated control. However, in Trial 3 only percentage seedling emergence and plant height were increased significantly (Table 2). Mefenoxam 350 g ai L^{-1} was also found to have consistently reduced the percentage of diseased seedlings significantly in two of the trials when compared with the inoculated control (Table 2). Although *R. solani* symptoms were observed on some of the harvested seedlings, application of mefenoxam 240 g ai L^{-1} reduced percentage of diseased seedlings in all three trials when compared with the inoculated control (Table 2). Application of mefenoxam 240 g ai L^{-1} resulted in increased percentage seedling emergence, plant height, dry shoot mass and dry root mass compared with the inoculated control (Table 2).

Application of fludioxonil 100 g ai L^{-1} significantly reduced percentage of diseased seedlings caused by *R. solani* in all three trials when compared with the inoculated control (Table 2). Similar results were obtained from all three trials

Table 1: Effect of fungicides on *in vitro* mycelial growth of *Rhizoctonia solani*, *Fusarium solani* and *Pythium ultimum*. Values are the mean of three replicates. Values within the same column per pathogen followed by the same superscript letter are not significantly different ($p = 0.05$)

Pathogen/treatment	Colony diameter (mm)				Inhibition (%)			
	Third day	Sixth day	Ninth day	Average (%)	Third day	Sixth day	Ninth day	Average (%)
Rhizoctonia solani								
Control	2.2^{c}	5.7^{b}	8.3^{c}	5.4				
Mefenoxam 350 g ai L^{-1}	1.5^{b}	5.5^{b}	6.6^{b}	4.5	32.6	3.0	20.1	18.6
Mefenoxam 240 g ai L^{-1}	1.2^{b}	5.8^{b}	7.2^{b}	4.7	43.6	1.8	13.6	19.7
Fludioxonil 100 g ai L^{-1}	0.8^{a}	2.7^{a}	4.2^{a}	2.6	63.3	52.9	49.8	55.3
CV (%)	5.0	13.9	4.0					
P-value	<0.001	0.001	<0.001					
Pythium ultimum								
Control	6.7^{c}	8.5^{c}	8.5^{b}	7.9				
Mefenoxam 350 g ai L^{-1}	0.0^{a}	0.0^{a}	0.0^{a}	0.0	100	100	100	100
Mefenoxam 240 g ai L^{-1}	0.00^{a}	0.0^{a}	0.0^{a}	0.0	100	100	100	100
Fludioxonil 100 g ai L^{-1}	1.3^{b}	6.3^{b}	8.4^{b}	5.3	80.5	25.7	1.2	35.8
CV (%)	8.1	2.5	10.1					
P-value	<0.001	<0.001	<0.001					
Fusarium solani								
Control	2.2^{b}	5.0^{c}	6.9^{c}	4.7				
Mefenoxam 350 g ai L^{-1}	0.8^{a}	2.4^{b}	3.3^{b}	2.2	61.8	51.7	52.2	55.2
Mefenoxam 240 g ai L^{-1}	0.6^{a}	1.3^{a}	2.1^{a}	1.3	72.4	73.8	69.6	71.9
Fludioxonil 100 g ai L^{-1}	0.5^{a}	2.3^{b}	2.4^{a}	1.7	75.4	54.3	65.2	65.0
CV (%)	16.9	4.8	7.1					
P-value	<0.001	<0.001	<0.001					

Table 2: Effect of fungicides on disease incidence, seedling emergence, plant height, dry shoot and dry root mass of cowpea in *Rhizoctonia solani* inoculated growth medium in the greenhouse. Each value is the mean of four replicates of 56 seedlings. Values within a column followed by the same superscript letter are not significantly different ($p = 0.05$)

Treatment	Seedling emergence (%)	Diseased seedlings (%)	Reduction in diseased seedlings (%)	Plant height (cm)	Dry shoot mass (g)	Dry root mass (g)
Trial 1						
Inoculated control	21.5^{a}	66.9^{c}		4.9^{a}	5.3^{a}	1.2^{a}
Uninoculated control	64.0^{c}	0.0^{a}		11.8^{c}	12.9^{c}	4.9^{c}
Mefenoxam 350 g ai L^{-1}	41.8^{b}	62.1^{cb}	4.9	7.6^{b}	8.2^{b}	3.4^{b}
Mefenoxam 240 g ai L^{-1}	30.0^{ab}	55^{b}	12.0	5.6^{a}	6.5^{ab}	1.4^{a}
Fludioxonil 100 g ai L^{-1}	39.5^{b}	54.2^{b}	12.8	7.7^{b}	12.9^{c}	4.9^{c}
CV (%)	21.4	27.9		11.8	19.0	45.9
P-value	<0.001	<0.001		<0.0011	<0.001	0.002
Trial 2						
Inoculated control	49.3^{a}	46.5^{c}		8.3^{a}	7.6^{a}	3.1^{a}
Uninoculated control	81.8^{b}	0.0^{a}		17.0^{c}	19.8^{d}	9.2^{c}
Mefenoxam 350 g ai L^{-1}	79.3^{b}	18.8^{b}	37.8	14.5^{cb}	13.8^{b}	5.8^{b}
Mefenoxam 240 g ai L^{-1}	79.8^{b}	25.0^{b}	21.5	12.3^{cb}	14.4^{cb}	6.3^{b}
Fludioxonil 100 g ai L^{-1}	73.0^{b}	17.8^{b}	28.8	12.8^{b}	14.1^{b}	5.9^{b}
CV (%)	8.0	22.4		12.5	20.0	20.6
P-value	<0.001	<0.001		<0.001	<0.001	<0.001
Trial 3						
Inoculated control	33.0^{a}	79.7^{c}		4.1^{a}	3.9^{a}	0.6^{a}
Uninoculated control	78.8^{c}	0.0^{a}		11.5^{c}	14.4^{d}	2.7^{b}
Mefenoxam 350 g ai L^{-1}	42.8^{b}	31.3^{b}	48.5	6.0^{b}	4.2^{a}	0.5^{a}
Mefenoxam 240 g ai L^{-1}	42.0^{b}	39.6^{b}	40.1	6.1^{b}	6.4^{b}	0.6^{a}
Fludioxonil 100 g ai L^{-1}	40.8^{b}	40.8^{b}	38.9	4.5^{a}	6.9^{cb}	1.5^{a}
CV (%)	9.5	26.2		9.3	21.8	58.0
P-value	<0.001	<0.001		<0.001	<0.001	0.001

in which fludioxonil 100 g ai L^{-1} significantly increased the percentage seedling emergence, plant height, dry shoot mass and dry root mass consistently, relative to the inoculated control (Table 2).

During harvesting it was observed in all treatments that seeds which failed to germinate were brown and water-soaked. *Rhizoctonia solani* caused root rot and reddish brown sunken lesions on the stem below and above the soil line (Figure 1a).

Pythium ultimum

The percentage of diseased seedlings caused by *P. ultimum* was significantly reduced in all three trials following the application of mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} (Table 3). Likewise, mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} increased the percentage seedling emergence, plant height, and dry shoot and root mass in trials 1 and 2 but not always significantly in Trial 3.

Fludioxonil 100 g ai L^{-1} significantly reduced the percentage of diseased seedlings caused by *P. ultimum* in all three trials when compared with the inoculated control (Table 3). In all three trials fludioxonil application caused an increase in percentage seedling emergence, plant height, dry shoot mass and dry root mass, although not always significantly in Trial 3 (Table 3).

In the *P. ultimum* inoculated treatments, some seeds failed to germinate and they were brown and water-soaked, whereas some seedlings showed symptoms of root rot and stunting. The basal part of the stems of these seedlings was soft and reduced in diameter when compared with the upper part of the stem (Figure 1b).

Fusarium solani

Mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} significantly reduced the percentage of diseased seedlings in all three trials (Table 4). Similarly, in all three trials mefenoxam application at 240 g ai L^{-1} caused an increase in seedling emergence, plant height, dry shoot mass and dry root mass (except in Trial 3) compared with the inoculated control, but not always significantly in Trial 1 (Table 4). However, only results from the second and third trials indicated that

Figure 1: Disease symptoms on cowpea seedlings inoculated with (a) *Rhizoctonia solani*, (b) *Pythium ultimum* and (c) *Fusarium solani*, and (d) uninoculated seedlings, during greenhouse trials

Table 3: Effect of fungicides on disease incidence, seedling emergence, plant height, dry shoot and dry root mass of cowpea in *Pythium ultimum* inoculated growth medium in the greenhouse. Each value is the mean of four replicates of 56 seedlings. Values within a column followed by the same superscript letter are not significantly different ($p = 0.05$)

Treatment	Seedling emergence (%)	Diseased seedlings (%)	Reduction in diseased seedlings (%)	Plant height (cm)	Dry shoot mass (g)	Dry root mass (g)
Trial 1						
Inoculated control	41.1^{a*}	60.3^{c}		3.6^{a}	3.6^{a}	1.4^{a}
Uninoculated control	83.5^{d}	0.0^{a}		12.1^{d}	19.5^{d}	4.0^{d}
Mefenoxam 350 g ai L^{-1}	62.7^{cb}	14.7^{b}	45.5	9.8^{c}	12.6^{bc}	3.6^{cd}
Mefenoxam 240 g ai L^{-1}	56.0^{b}	13.9^{b}	46.4	7.1^{b}	9.4^{b}	2.7^{b}
Fludioxonil 100 g ai L^{-1}	71.0^{c}	10.7^{b}	49.5	9.7^{c}	13.8^{c}	3.6^{cd}
CV (%)	15.1	59.7		16.7	26.4	26.0
P-value	<0.001	0.014		<0.001	<0.001	0.002
Trial 2						
Inoculated control	52.0^{a}	46.8^{c}		9.5^{a}	5.7^{a}	1.5^{a}
Uninoculated control	86.3^{b}	0.0^{a}		16.5^{b}	16.8^{d}	8.0^{d}
Mefenoxam 350 g ai L^{-1}	75.0^{b}	19.0^{b}	27.8	15.3^{b}	12.8^{c}	4.0^{c}
Mefenoxam 240 g ai L^{-1}	82.3^{b}	16.8^{b}	30.1	13.8^{b}	10.8^{cb}	4.4^{c}
Fludioxonil 100 g ai L^{-1}	78.8^{b}	16.8^{b}	30.1	13.7^{b}	9.6^{b}	2.7^{b}
CV (%)	5.2	24.3		15.3	24.3	18.2
P-value	<0.001	<0.001		0.003	<0.001	<0.001
Trial 3						
Inoculated control	42.3^{a}	68.3^{d}		4.8^{a}	7.9^{a}	0.7^{a}
Uninoculated control	77.3^{c}	0.0^{a}		10.7^{d}	14.4^{b}	3.0^{bc}
Mefenoxam 350 g ai L^{-1}	53.0^{b}	27.1^{c}	41.2	8.7^{cb}	8.9^{a}	1.3^{ab}
Mefenoxam 240 g ai L^{-1}	50.3^{ab}	22.9^{c}	45.4	8.2^{b}	7.0^{a}	0.9^{a}
Fludioxonil 100 g ai L^{-1}	46.0^{ab}	18.8^{bc}	49.6	7.7^{b}	9.5^{a}	1.2^{a}
CV (%)	11.5	29.6		9.6	17.0	41.9
P-value	<0.001	<0.001		<0.001	<0.001	<0.001

Table 4: Effects of fungicides on disease incidence, seedling emergence, plant height, dry shoot and root mass of cowpea in *Fusarium solani* inoculated growth medium in the greenhouse. Each value is the mean of four replicates of 56 seedlings. Values within a column followed by the same superscript letter are not significantly different ($p = 0.05$)

Treatment	Seedling emergence (%)	Diseased seedlings (%)	Reduction in diseased seedlings (%)	Plant height (cm)	Dry shoot mass (g)	Dry root mass (g)
Trial 1						
Inoculated control	44.3^{a}	47.3^{d}		6.4^{a}	8.9^{a}	2.0^{a}
Uninoculated control	64.8^{b}	0.0^{a}		9.5^{c}	15.7^{bc}	6.6^{c}
Mefenoxam 350 g ai L^{-1}	59.3^{ab}	12.6^{b}	34.7	8.5^{bc}	12.6^{ab}	3.6^{b}
Mefenoxam 240 g ai L^{-1}	70.0^{b}	17.7^{bc}	29.6	8.0^{b}	17.7^{c}	3.4^{b}
Fludioxonil 100 g ai L^{-1}	68.8^{b}	17.4^{bc}	29.9	7.9^{b}	17.4^{c}	3.9^{b}
CV (%)	23.0	35.1		9.4	24.7	22.8
P-value	<0.001	<0.001		<0.001	0.021	<0.001
Trial 2						
Inoculated control	45.8^{a}	58.50^{d}		8.750^{a}	8.50^{a}	1.93^{a}
Uninoculated control	82.3^{b}	0.00^{a}		17.25^{c}	23.50^{c}	8.73^{c}
Mefenoxam 350 g ai L^{-1}	71.0^{b}	18.25^{cb}	40.25	15.25^{cb}	9.00^{cb}	8.28^{c}
Mefenoxam 240 g ai L^{-1}	78.8^{b}	12.00^{b}	46.50	14.25^{cb}	15.75^{b}	5.43^{b}
Fludioxonil 100 g ai L^{-1}	76.3^{b}	13.50^{b}	45.00	15.75^{cb}	16.00^{b}	4.75^{b}
CV (%)	14.0	27.7		13.0	21.7	17.8
P-value	<0.001	<0.001		<0.001	<0.001	<0.001
Trial 3						
Inoculated control	42.8^{a}	64.8^{c}		5.5^{a}	5.9^{ab}	0.6^{ab}
Uninoculated control	77.5^{c}	0.0^{a}		11.1^{d}	15.0^{e}	2.5^{c}
Mefenoxam 350 g ai L^{-1}	52.3^{b}	31.3^{b}	33.5	9.0^{c}	10.7^{dc}	2.6^{c}
Mefenoxam 240 g ai L^{-1}	53.0^{b}	39.6^{b}	25.2	8.6^{cb}	4.1^{a}	0.3^{a}
Fludioxonil 100 g ai L^{-1}	49.0^{ab}	33.3^{b}	31.5	7.6^{b}	8.2^{bc}	1.5^{abc}
CV (%)	9.6	37.0		10.1	26.2	60.2
P-value	<0.001	<0.001		<0.001	<0.001	<0.001

mefenoxam 350 g ai L^{-1} increased the percentage seedling emergence, plant height, dry shoot mass and dry root mass compared with the inoculated control.

Application of fludioxonil 100 g ai L^{-1} significantly reduced percentage of diseased seedlings caused by *F. solani* in all three trials (Table 4). Likewise, results from all three trials showed increased percentage seedling emergence, plant height, dry shoot mass and dry root mass following application of fludioxonil 100 g ai L^{-1} relative to the inoculated control (Table 4), although not always significantly in Trial 3.

Small brown lesions were observed at harvesting on the roots of plants grown in *F. solani* inoculated growth media. Infected seedlings also showed symptoms of root rot. There was a reddish discolouration over the entire below-ground stem and root system. Soft, dark brown or black cankers developed on the stem nodes and these often girdled the stem during disease development (Figure 1c).

Discussion

In vitro study

In this experiment we investigated the effect of mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1} at different concentrations for inhibition of mycelial growth of three pathogens, namely *Pythium ultimum*, *Fusarium solani* and *Rhizoctonia solani*. Mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} inhibited mycelial growth of all three pathogens. This fungicide is widely known to inhibit mycelial growth by interfering with the synthesis of ribosomal DNA (Syngenta 2014). Although mefenoxam gave better inhibition of *F. solani* when compared with fludioxonil, it is not widely known to be highly effective against the pathogen. However, Fravel et al. (2005) found mefenoxam to be effective in inhibiting the mycelial growth of *Fusarium oxysporum*, the causal fungus of wilt of tomatoes. Mefenoxam is known to be ineffective against *Rhizoctonia*, effective against *Pythium* and moderately effective against *Fusarium* (Syngenta 2014). The ability of fludioxonil 100 g ai L^{-1} to inhibit mycelial growth of *P. ultimum*, *F. solani* and *R. solani* was also investigated. Although the fungicide inhibited mycelial growth of all three pathogens *in vitro*, it is known to be ineffective against *Pythium* and intermediately effective against *Rhizoctonia* and *Fusarium* (Syngenta 2014). However, in similar studies, fludioxonil was reported to be effective in inhibiting mycelial growth of *R. solani* (Bucher and Pedersen 2004), *P. ultimum* (Errampalli 2004), and *Fusarium* spp. (Munkvold and O'Mara 2002; Wang et al. 2005; Solorzano and Malvick 2011). Based on our results the *in vivo* study was initiated to test the fungicides' ability to control seedling diseases of cowpea.

Greenhouse trials

This study showed that application of mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} as treatment fungicides against *R. solani*, *P. ultimum* and *F. solani* yielded positive results. The fungicides significantly reduced the percentage of diseased seedlings caused by all three pathogens in all three trials consistently. Of the seeds treated with mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1} and planted in inoculated growth medium, the percentage of diseased seedlings in *P. ultimum* inoculated growth medium was the lowest followed by that of *F. solani* and *R. solani*. This is because mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} contain a systemic fungicide that is known to be effective against seed and soil-borne pathogens such as *Pythium* and *Phytophthora* spp. (Syngenta 2014). Treatments with mefenoxam 350 g ai L^{-1} resulted in a reduction of 4.9%, 37.8% and 48.5% diseased cowpea seedlings infected by *R. solani* in trials 1, 2 and 3, respectively, 45.5%, 27.8% and 41.2% infected by *P. ultimum*, and 34.7%, 40.3% and 33.5% infected by *F. solani*, respectively. Mefenoxam 240 g ai L^{-1} reduced percentage diseased cowpea seedlings in trials 1, 2 and 3 by 12.0%, 21.4% and 40.1% in *R. solani* inoculated growth medium, 46.4%, 30.1% and 45.4% in *P. ultimum* inoculated growth medium, and 29.6%, 46.5% and 25.2% in *F. solani* inoculated growth medium, respectively. Augusto et al. (2010) reported positive results on the effectiveness of mefenoxam against *Pythium myriotylum*. Its effectiveness against pathogens such as *F. solani* is not well known. However, Chang et al. (2013) screened Apron Maxx (mefenoxam) against *F. avenaceum* on pea and found that the fungicide consistently increased seedling emergence, nodulation, seed yield and reduced root rot severity. Kirk et al. (2013) also reported the effectiveness of mefenoxam in reducing dry rot incidence caused by *Fusarium* pathogens. Martinez-Espinoza et al. (2004) reported that mefenoxam is effective against *Rhizoctonia solani* of ornamental plants.

Mefenoxam 350 g ai L^{-1} and mefenoxam 240 g ai L^{-1} not only significantly reduced the percentage of diseased seedlings caused by all three pathogens, but also increased the percentage seedling emergence, plant height, dry shoot mass and dry root mass in all three trials. The average percentage seedling emergence in both *P. ultimum* and *F. solani* inoculated growth medium for all three trials increased to 64% relative to that of the inoculated control (45%). For *R. solani* inoculated growth medium the percentage seedling emergence was higher (52%) when compared with the inoculated control (35%). When applied to the root zone, mefenoxam is absorbed by the roots and transported through the xylem upward in the plant (Greencast 2014). Although mefenoxam was applied at two concentrations (350 and 240 g ai L^{-1}), its effectiveness against all three pathogens showed little variation.

Fludioxonil 100 g ai L^{-1} reduced percentage diseased seedlings by 12.8%, 28.8% and 39.9% with *R. solani*, 49.5%, 30.1% and 49.6% with *P. ultimum* and 29.9%, 45.0% and 31.5% with *F. solani* in the greenhouse trials 1, 2 and 3, respectively. Likewise, application of fludioxonil 100 g ai L^{-1} also increased the percentage of seedling emergence, plant height, dry shoot mass and dry root mass in all three trials relative to those of the inoculated control. These results are in agreement with the findings by Aveling et al. (2012) who, when investigating the effect of Celest® XL (mefenoxam and fludioxonil) against *Fusarium graminearum*, reported that it reduced the percentage of diseased maize seedlings. This fungicide is registered in South Africa for the control of seed and soil-borne pathogens, including *Pythium* spp. and *Fusarium* spp. (Syngenta 2014). Fludioxonil has broad activity with a unique mode of action, which interferes with

the life cycle of the fungus/oomycete, i.e. spore germination and germ tube and mycelial growth (Syngenta 2014). Other studies have indicated that fludioxonil can also be effective when used in combination with mefenoxam against *R. solani* of soybean (Bucher and Pederson 2004; Lamprecht et al. 2011) and *Fusarium* spp. of maize (Munkvold and O'Mara 2002). In addition, fludioxonil was also found to be effective in reducing diseases caused by *Phytophthora infestans* on potatoes (Inglis et al. 1999), and against *Pythium* spp. and *R. solani* (Mazzola 1998).

Conclusion

The greenhouse experiment was carried out following positive promising results obtained during an *in vitro* experiment on the effectiveness of mefenoxam 350 g ai L^{-1}, mefenoxam 240 g ai L^{-1} and fludioxonil 100 g ai L^{-1} against *R. solani*, *P. ultimum* and *F. solani*. During the greenhouse experiment, although all three fungicides reduced the percentage of diseased seedlings and increased seedling emergence, plant height, dry shoot mass and dry root mass, none gave complete control of any of the diseases caused by the three pathogens under the trial conditions. Further research to test the effectiveness of these fungicides at different application times or their combination with other fungicides may provide improved results for the control of seed and soil-borne pathogens of cowpea.

Acknowledgements — We thank J Boshoff Fourie for providing cultures, Syngenta SA for supplying chemicals and the National Research Foundation for financing the research.

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Received 22 September 2015, revised 11 February 2016, accepted 13 February 2016
Associate Editor: Roy Neilson