Chemical Management of Fusarium Wilt in Iceberg Lettuce

Jiahuai "Alex" Hu

School of Plant Sciences, University of Arizona, Tucson, AZ

Introduction

Fusarium wilt of lettuce, caused by *Fusarium oxysporum* f.sp. *lactucae* (FOL), is the most important and severe soilborne disease of lettuce. As lettuce growers know, this soilborne pathogen, once established in a field, is virtually impossible to eradicate the FOL inoculum from the soil. This is because the fungus produces special overwintering structures that are resistant to environmental stress and persist in the soil for upwards of 10 years, regardless of rotation practices. FOL infects seedling roots, eventually moving into the vascular system. The activity of the fungus eventually disrupts the function of the vascular system, resulting in the wilting of lettuce plants. Since its first discovery in Yuma in 2001, Fusarium wilt continues to spread to non-infested fields, and annual economic losses due to FM are estimated to exceed 1 million dollars.

As FOL-infested acreage grows, so does the demand for effective management recommendations. Currently, there is no single silver-bullet solution to FOL problem in Yuma iceberg lettuce. Lettuce growers are recommended to use an IPM approach that includes the use of resistant cultivars, crop rotation, and cultural practices (Matheron et al. 2005; Matheron and Porchas 2010). There are several ongoing studies that evaluate resistant cultivars, biocontrol agents, the development of portable early detection tools, and the development of molecular tools to quantify soil inoculum potential. It is not clear when these new management tools will be available for Yuma lettuce growers. Therefore, growers have limited options for managing Fusarium wilt.

The goal of this project is to discover small doses of fungicide(s) that work well against Fusarium wilt in lettuce at very low, cost-effective rates. Specific objectives are to: (i) conduct *in vitro* screening of major fungicides and biofungicides against FOL in the laboratory; and (ii) conduct *in vivo* evaluation of selected fungicide and biofungicides for suppression of Fusarium wilt on lettuce in greenhouse experiments.

Materials and Methods

Isolation of *FOL*. Lettuce plants with typical symptoms of Fusarium wilt were collected from three iceberg lettuce field in September 2021. Necrotic tissues of root (5 × 5 mm) were cut from the margin between affected and healthy tissues. These small fragments were surface sterilized by soaking in 75% ethanol for 5 s, 1% sodium hypochlorite for 60 s, copiously rinsed with sterile distilled water, and dried on sterile filter paper in a laminar hood. Each fragment or piece was plated onto ADPA and water agar (WA) amended with 100 µg/mL streptomycin. Plates were incubated at 25ºC in the dark until typical fungal colonies were observed. Fungal colonies were subcultured onto PDA and incubated at 25° for 4 d. Hyphal tip subculture were obtained for each isolate by removing tips of hyphae (3-to-4 cells) from the colony margin and subculturing them onto fresh PDA.

***In vitro* fungicide sensitivity testing.** *In vitro* studies were conducted to evaluate efficacy of various fungicides against FOL isolates and develop baseline sensitivities to these fungicides. A total of thirty-four DMI, SDHI, and QoI fungicides with various modes of actions were evaluated with five FOL isolates. Tests were conducted on PDA agar amended with fungicide in a 10-fold dilution series ranging

from 0.01 to 1000 µg/ml and control plates not amended with fungicides. A 5-mm-diameter disk containing mycelium and agar from the margin of actively growing colonies of 4- to 6-day-old cultures were positioned in the center of a culture dish. Isolate growth will be determined by measuring colony diameters in two perpendicular directions after 6 days of incubation in the dark at room temperature. Measurements were averaged, the diameter of the mycelial plug were subtracted, and relative growth reduction for each rate of fungicide was calculated as follows: (100 – [growth with fungicide/growth in control plate] × 100). The experiment was repeated once.

***In vivo* evaluation of fungicides.** Best-performing fungicides were evaluated when applied at a single rate in the soil that was inoculated with FOL conidia suspensions. The experiments were conducted as a randomized complete block with 4 replications; An experimental unit is a pot filled with half a gallon of sand:peat:vermiculite (4:1:1). The susceptible iceberg lettuce cultivar "El Guapo" was seeded ten to a pot and thinned to three per pot 1 week later. Fungicides were applied 150 mL per pot at the first true leaf stage. Fungicide rates were calculated based on the product label rate. Three days after fungicide application, 150 ml of FOL conidia suspension (10^6 spores/mL) was applied to each pot by soil drench. FOL conidia inocula were harvested from 7-day-old cultures and concentration was adjusted using a haemacytometer under a compound microscope. Plants were watered and fertilized as needed. Wilt severity, the percent of the foliage showing symptoms of Fusarium wilt was rated starting 1 week after inoculation at 3-day intervals 5 times with the Horsfall–Barratt scale (Horsfall and Barratt, 1945). The Area Under the Disease Progress Curve (AUDPC) was calculated from wilt incidence data using trapezoidal integration (Shaner and Finney, 1977). At the end of the experiment, tap roots and stem tissues were taken to reisolated FOL and assess vascular damages by FOL on a scale of 3: 0 = healthy white without staining, 1 = yellowing, 2 = black staining with hollow stem. Yellowing and stunting due to fungicide phytotoxicity were rated on a scale of 4: 0 = no stunting and leaf yellowing; 1 = slight leaf yellowing without stunting; 2 = leaf yellowing with stunting, and 3 = dead leaves with plant death. The experiment was repeated once.

Data analysis. The EC_{50} and EC_{90} values relative to the control were estimated by plotting the percentage inhibition against the log-scale of fungicide concentration using drc package, R Project. ANOVA and multiple comparison tests were performed in R to determine whether there were any significant differences among fungicide treatments.

Results and Discussion

***In vitro* fungicide sensitivity testing.** A total of 34 commercial fungicides and chemical compounds were evaluated for their efficacy against FOL mycelial extension in PDA media. According to their EC_{50} values and EC_{90} values in Table 1, the efficacy of these fungicides can be divided into three groups (i.e. highly effective, intermediately effective, and ineffective): a) group 1 was highly effective and consisted of seventeen fungicides that exhibited strong inhibitory effect against mycelial growth of FOL, with EC_{50} values below 1 µg/ml (Table 1). For example, Spharex, Folicur, and Prosaro were the top three fungicides with the lowest EC_{90} values ranging from 0.93 to 2.70 µg/ml; b) group 2 were intermediately effective and had twelve fungicides with EC50 values ranging from 1.1 to 39.4 µg/ml; c) group 3 was ineffective and contained five fungicides with little inhibitory effect against FOL mycelial growth. Fungicides with EC_{90} values below 100 µg/ml were used as additional criteria for selecting a subset of highly efficacious fungicides for further evaluation using lettuce plants. Therefore, a total of fifteen fungicides in groups 1

and 2 were advanced to in vivo testing on plants in the greenhouse experiments and growth chamber experiments. In addition, Biofungicides Serifel (BASF) and Serenade (Bayer) were found highly inhibitory to FOL by reducing mycelial growth by more than 75% at concentrations as low as 0.1 µg/ml.

Table 1. EC_{50} values of 34 commercial fungicides against mycelial growth of 5 isolates of *Fusarium oxysporum* f.sp. *lactucae* collected from Yuma iceberg lettuce fields

Fungicide	Active ingredient	EC_{50} (ppm or µg/ml)			EC_{90} (ppm or µg/ml)		
		Mean	Min	Max	Mean	Min	Max
Spharex	Prothioconazole, Metconazole	0.07	0.06	0.08	0.93	0.66	1.20
Folicur	Tebuconazole	0.14	0.11	0.18	4.95	2.30	7.60
Prosaro	Prothioconazole, Tebuconazole	0.15	0.12	0.18	2.70	1.60	3.80
Tilt	Propiconazole	0.20	0.11	0.29	40.8	6.10	75.50
Trinity	Triticonazole	0.23	0.06	0.39	60.6	26.2	129.70
Luna experience	Tebuconazole, Fluopyram	0.26	0.21	0.27	4.04	2.71	4.68
Quadris	Azoxystrobin	0.28	0.15	0.41	352.0	243.0	573.30
Provysol	Mefentrifluconazole	0.30	0.03	0.57	>1000	>1000	>1000
Miravis	Pydiflumetofen	0.34	0.10	0.60	32.80	21.60	44.30
Cannonball	Fludioxonil	0.43	0.29	0.58	63.2	52.2	68.14
Indar	Fenbuconazole	0.64	0.31	1.11	>3000	>3000	>3000
Quadris Top	Azoxystrobin, Difenoconazole	0.65	0.23	0.90	142.69	134.80	232.65
Rally	Myclobutanil	0.72	0.48	0.97	41.30	20.90	51.70
Rhyme	Flutriafol	0.83	0.71	0.95	26.0	17.60	34.40
Stratego	Prothioconazole, Trifloxystrobin	0.91	0.57	1.26	92.9	18.10	167.70
Proline	Prothioconazole	0.94	0.81	1.07	5.30	3.40	7.30
Preemptor	Flutriafol, Fluoxastrobin	0.97	0.89	1.25	29.1	18.41	41.03
Mertect	Thiabendazole	1.10	1.0	1.20	2.60	1.40	3.60
Arbotect	Thiabendazole hypophosphite	1.20	0.20	2.10	2.80	1.20	4.40
Inspire	Difenoconazole	1.42	0.92	1.91	804	564.86	1044
Quilt Xcel	Propiconazole, Azoxystrobin	1.50	0.93	1.81	400.90	379.43	437.32
Luna sensation	Trifloxystrobin, Fluopyram	1.92	1.12	3.27	214.73	197.79	240.40
Elatus	Azoxystrobin, Benzovindiflupyr	3.12	1.86	5.61	>1000	>1000	>1000
Headline	Pyraclostrobin	4.28	1.10	7.20	>5000	>5000	>5000
Dowicide 1	2-Phenylphenol	8.90	8.10	9.90	37.70	24.20	46.10
Mettle	Tetraconazole	9.32	6.14	12.49	>1000	>1000	>1000
Approach	Picoxystrobin	14.91	11.64	18.61	>1000	>1000	>1000
Topsin-M	Thiophanate-methyl	25.70	13.30	37.90	109.00	74.50	177.60
Pristine	Pyraclostrobin, Boscalid	39.4	23.60	68.11	>1000	>1000	>1000
Fontelis	Penthiopyrad	>1000	>1000	>1000	>1000	>1000	>1000
Tanos	Famoxadone, Cymoxanil	>1000	>1000	>1000	>1000	>1000	>1000
Endura	Boscalid	>1000	>1000	>1000	>1000	>1000	>1000
Flint	Trifloxystrobin	>1000	>1000	>1000	>1000	>1000	>1000
Aliette	Aluminum tris	>1000	>1000	>1000	>2000	>2000	>2000

EC_{50} and EC_{90} values are the effective concentration of a fungicide at which mycelial growth is inhibited by 50% and 90%, respectively, when compared to growth on the control dish. Mean = mean of five isolates; Min = lowest EC_{50} value of a FOL isolate; Max = highest EC_{50} value of a FOL isolate

***In vivo* evaluation of fungicides.** Seven chemical treatments (tebuconazole, prothioconazole, propiconazole, myclobutanil, azoxystrobin, high rate of fludioxonil and 2-phenylphenol) significantly reduced the AUDPC and vascular damages in stem and taproots compared to the non-treated, inoculated controls. Treatments of prothioconazole, propiconazole and high rate of tebuconazole also significantly reduced the FOL recovery rate from root and stem tissues. However, Phytotoxicity such as stunting was observed on plants treated with high rate of tebuconazole, flutriafol, triticonazole, and thiabendazole. For example, tebuconazole caused stunting and yellow at concentrations above 50 µg/ml, however, no

phytotoxicity was noted on plants treated with tebuconazole at 30 µg/ml or below. These results indicated that tebuconazole, azoxystrobin, and prothioconazole were good candidate compounds to reduce the severity of Fusarium wilt of lettuce. A single application was not sufficient to protect lettuce plants from infections by FOL, as these chemicals appeared to be fungistatic against FOL. To achieve season-long reductions in disease and a yield increase, it may be necessary to apply these fungicides more than once. The chemical soil environment may pose potential problems for the management of Fusarium wilt with chemicals. Some fungicides may be strongly adsorbed to the soil, and the amount adsorbed varies between soils based on clay and organic matter content. In addition, once in the plant, they may be converted to the secondary fungitoxic compound and translocated to other plant tissues. Therefore, it is important to evaluate the efficacy of a fungicide under field conditions.

Table 2. Effect of 18 fungicides on vascular tissue damages, FOL recovery, and the Area Under the Disease Progress Curve (AUDPC) of Fusarium wilt and FOL recovery rate in greenhouse trials.

Fungicide formulation	Rate per liter [a]	Active ingredient	AUDPC [b]	Vascular damages [c]	FOL recovery [d] (%)	Toxicity [e]
Non-treated, non-inoculated control	N/A	Water	0	0	0	0
Non-treated, inoculated control	N/A	Water	880 a	2	100 a	0
Folicur 3.6 F	0.12 ml	Tebuconazole	180 c	0	20 b	2
	0.23 ml		120 c	0	20 b	2
	0.35 ml		80 c	0	0 b	2
	0.63 ml		80 c	0	0 b	2
Quadris 2.08 SC	1.17 ml	Azoxystrobin	440 b	1	75 a	0
Inspire 2.08 EC	0.55 ml	Difenoconazole	620 a	2	85 a	0
Inspire XT	0.55 ml	Difenoconazole, Tebuconazole	480 b	1	80 a	2
Proline 480 SC	0.54 ml	Prothioconazole	220 bc	1	35 b	1
Tilt 3.6 EC	0.63 ml	Propiconazole	240 bc	1	35 b	1
Rally 40 WSP	1.17 ml	Myclobutanil	380 b	1	100 a	1
Topguard Terra 4.16 SC	0.63 ml	Flutriafol	N/A	N/A	N/A	3
Sphaerex SC	0.57 ml	Prothioconazole, Metconazole	500 ab	1	50 ab	2
Prosaro 421 SC	0.51 ml	Prothioconazole, Tebuconazole	580 ab	1	35 b	2
Trinity 1.7 SC	1.70 ml	Triticonazole	720 a	2	90 a	2
Mertect 340F	0.45 ml	Thiabendazole	700 a	2	95 a	2
Cannonball WP	0.1 g	Fludioxonil	620 a	2	100 a	0
	0.2 g		560 ab	2	90 a	0
	0.5 g		520 ab	1	90 a	0
	1.0 g		400 b	1	95 a	0
2-phenylphenol	0.1 g	2-phenylphenol	740 a	2	95 a	1
	0.3 g		640 a	2	95 a	1
	0.5 g		320 b	2	95 a	1
	1.0 g		320 b	2	95 a	2
OxiDate 2.0	0.25 ml	Hydrogen peroxide	840 a	2	100 a	0
	0.50 ml		800 a	2	100 a	0
	1.00 ml		800 a	2	100 a	0
	2.0 ml		800 a	2	90 a	0
Serifel WP	1.17 g	*B. amyloliquefaciens*	760 a	2	100 a	0
Serenade ASO SL	0.42 ml	*B. subtilis*	820 a	2	100 a	0
Ecoswing SL	2.74 ml	Extract of *Swinglea glutinosa*	860 a	2	100 a	1

[a] Fungicide rates were determined based on the product label rates per hectare, N/A = not applied

[b] The percent of foliage on each plant showing symptoms of Fusarium wilt was assessed . Means within each column with a letter in common are not significantly different at P = 0.05 based on Fisher's protected LSD test.

[c] Rating scale of vascular damages: 0 = healthy white without staining, 1 = yellowing, 2 = black staining with hollow stem.

[d] Percentage of inoculated plants with the recovery of FOL from stem and root tissues.
[e] Rating scale of fungicide phytotoxicity: 0 = no stunting and leaf yellowing; 1 = slight leaf yellowing without stunting; 2 = leaf yellowing with stunting, and 3 = dead leaves with plant death

References

Horsfall, J.G., Barratt, R.W. 1945. An improved grading system for measuring plant diseases. Phytopathology, 35:655

Matheron, M.E. et al. 2005. Effect of planting date, cultivar, and stage of plant development on incidence of Fusarium wilt of lettuce in desert production fields. Plant Dis. 89:565-570.

Matheron, M. E., and Porchas, M. 2010. Evaluation of soil solarization and flooding as management tools for Fusarium wilt of lettuce. Plant Dis. 94:1323-1328.

Shaner, G., Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056