FINAL REPORT
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PROJECT: Evaluation of Biocontrol and Conventional Fungicides for the Control of Phytophthora and Masking of Root Infection Symptoms

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Goal: Evaluate the efficacy of commercially available biological and conventional fungicides for the management of soil-borne *Phytophthora* diseases in nursery crops. Test these fungicides as preventatives (applied before pathogen introduction to soil) and as curatives (applied after pathogen introduction to soil). Evaluate the potential for masking symptoms of *Phytophthora* infection.

SUMMARY

Eight fungicides were evaluated in repeated experiments over 6 months with a particularly tough and rigorous testing system. The relatively new conventional fungicides Segovis and Micora provided effective preventative control of crown rot on Gerbera caused by *Phytophthora cryptogea.* None of the other 6 tested fungicides proved to effectively prevent disease. Subdue Maxx, however, was the only fungicide to effectively control the disease when applied as a curative. *P. cryptogea* could be isolated from roots of some fungicide- treated and inoculated plants. The "masking" of disease symptoms was most notable with the curative Subdue Maxx treatment. Infected plants treated with Subdue Maxx could result in the inadvertent movement of the pathogen in the nursery trade.

Materials and Methods

Host plant and culture

Gerbera jamesonni 'Rose Dark Center' (Jaguar Series, Syngenta Flowers, Gilroy, CA) were seed- propagated and transplanted into 4" pots containing Premium Fern Mix (Berger, Watsonville CA). Plants were covered with 50% shade cloth, and greenhouse- grown at 80 F. maximum day temperature and 60F. night temperature. Plants were drip irrigated as needed. At each irrigation, Miracle Gro fertilizer (21-8-16) was injected at a 250 ppm nitrogen rate. After *Phytophthora* inoculation, described below, plants were irrigated twice per day to enhance infection.

Phytophthora inoculum and inoculation

P. cryptogea isolate (UCB FOR.63.B.EUGL.1) was isolated from *Eucalyptus globulus* in a urban forest landscape, and therefore it was not previously subjected to nursery fungicide applications and therefore less likely to be resistant to fungicides that are heavily applied in the nursery trade. The inoculum was made by placing approximately 25 autoclaved barley grains on each of 100 mm V-8 agar plates containing a culture of the isolate. The cultures were held at room temperature to colonize the grains for approximately 2 weeks. Test plants were grown as

described until root balls were established and at least the first flower buds had formed. A colonized grain was placed just under the soil surface at the middle of each of the four edges of the pot. The non-inoculated control treatments were as described above, except with non-inoculated autoclaved barley grains.

Fungicide Treatments

Treatments consisted of registered biological and conventional fungicides applied as soil drenches at labeled rates. (Table 1).

Table 1 Fungicide treatments, formulations, active ingredients, and rates applied

Treatment #	Formulation	Active Ingredient	Formulated Rate per 100 G
1	Non Inoc. Control	-	
2	Inoc. Control	-	
3	Segovis	oxathiapiprolin	3.2 fluid ounces
4	Subdue Maxx	mefenoxam	2.0 fluid ounces
5	Micora	mandipropamid	8.0 fluid ounces
6	Root Shield Plus WP	*Trichoderma harzianum* T22, *T. virens* G41	8.0 ounces
7	Segway O	cyazofamid	6.0 fluid ounces
8	Areca WDG	Al. phosphonate	12.8 ounces
9	Terrazole L	etridiazole	7.0 fluid ounces
10	Triathlon BA	Bacillus amyloliquefaciens D747	48.0 fluid ounces
Treatments applied as 90 ml soil drenches to 4 inch pots.			

Treatments were applied either approximately 7 days before inoculation (PRE) or 4 to 6 days after inoculation (POST) in 6 total experiments held over a 6 month period (Table 2). This was to demonstrate the effectiveness of the fungicide treatments applied as a preventative (prior to infection) or as curative (after infection) treatments. Generally, fungicides are considered preventatives because they prevent root infection process by some mechanism.

Table 2 Experiments, treatment type, treatments, and associated dates

Experiment #	Treatment Type	Treatment #	Treat. Application	Inoculation	Final Evaluation
1	PRE	1 to 6	1/26/2018	2/2/2018	4/4/2018
2	POST	1 to 6	3/16/2018	3/12/2018	4/13/2018
3	PRE	1 to 10	4/6/2018	4/13/2018	5/16/2018
4	POST	1 to 10	5/3/2018	4/27/2018	5/16/2018
5	PRE	1,2,6 to 10	4/26/2018	5/3/2018	5/23/2018
6	POST	1,2, 6 to 10	5/3/2018	4/27/2018	5/23/2018

Each fungicide treatment was tested in two separate experiments along with an inoculated and non-inoculated control. Treatments were applied to plants on greenhouse benches arranged in a randomized complete block statistical design. There were 6 to 10 single- plant replicates in each experiment.

Disease Assessment

Disease progression was monitored at least weekly and categorized into 3 basic disease states: % alive (not wilted or chlorotic), % wilted or chlorotic, and % dead. The effect of fungicide treatments was statistically analyzed at the final observation of each experiment based on the quality of the plants that were still categorized as alive (Table 3). This data was statistically analyzed by a one-way ANOVA and means were compared using a protected LSD test (P=0.05).

At the end of each experiment, any necrotic or potentially diseased roots were washed 3 times with distilled water and pieces were cultured on 100 mm Petri plates containing PARPH selective media to evaluate whether *P. cryptogea* could still be isolated and therefore a potentially viable pathogen (Table 3). Any resulting cultures from these root isolations with a morphology consistent with *Phytophthora* or *Pythium* were identified with molecular techniques.

Results

The first disease symptoms occurred in inoculated controls from 10 to 17 days after inoculation. Disease symptoms of some plants in ineffective treatments occurred at about the same time as these control treatments. Disease progressed relatively quickly, from alive to wilt to plant collapse (death) (Figs 1-6). The pathogen infected roots close to the inoculum source at the edge of the pot and progressed quickly to the root crown and the plant collapsed. Segovis was very effective in both preventative fungicide experiments. Micora was effective in one preventative experiment. Segway and Terrazole were only moderately effective in one experiment and resulted in plants that were not commercially acceptable. The other fungicides were not effective.

Disease progressed more quickly in the curative experiments. Subdue Maxx was highly effective as a curative. Most Subdue Maxx- treated plants could not be distinguished from the non-inoculated control at the end of the experiment (Fig 7). Other fungicides were not effective as curatives.

P. cryptogea was isolated from relatively high-quality plants in both preventative and curative treatments. Where plants were considered alive but of low quality (< 1.0), *P. cryptogea* generally could not be recovered from the roots probably because of the associated poor quality and suitability of the available roots or root crowns for isolation. Recovery was possible from some of the higher quality plants treated with Micora and Terrazole, yet generally these plants were not of commercial quality (< 2.0). Plants and roots were of high quality in the Segovis preventative treated plants and *P. cryptogea* was not recovered indicating this treatment may have completely prevented infection.

P. cryptogea could be recovered regularly from roots of plants treated after inoculation with Subdue Maxx. Since this treatment resulted in plants of high quality and infected roots, potted

Gerbera plants could easily be inadvertently harvested and shipped in the trade. This condition has been described as “masking symptoms”. It is thought to occur in the trade but until now has not been experimentally demonstrated for *Phytophthora* root infections. This masking would not necessarily be a problem for Gerbera cut flower crops where only the flower stem is harvested.

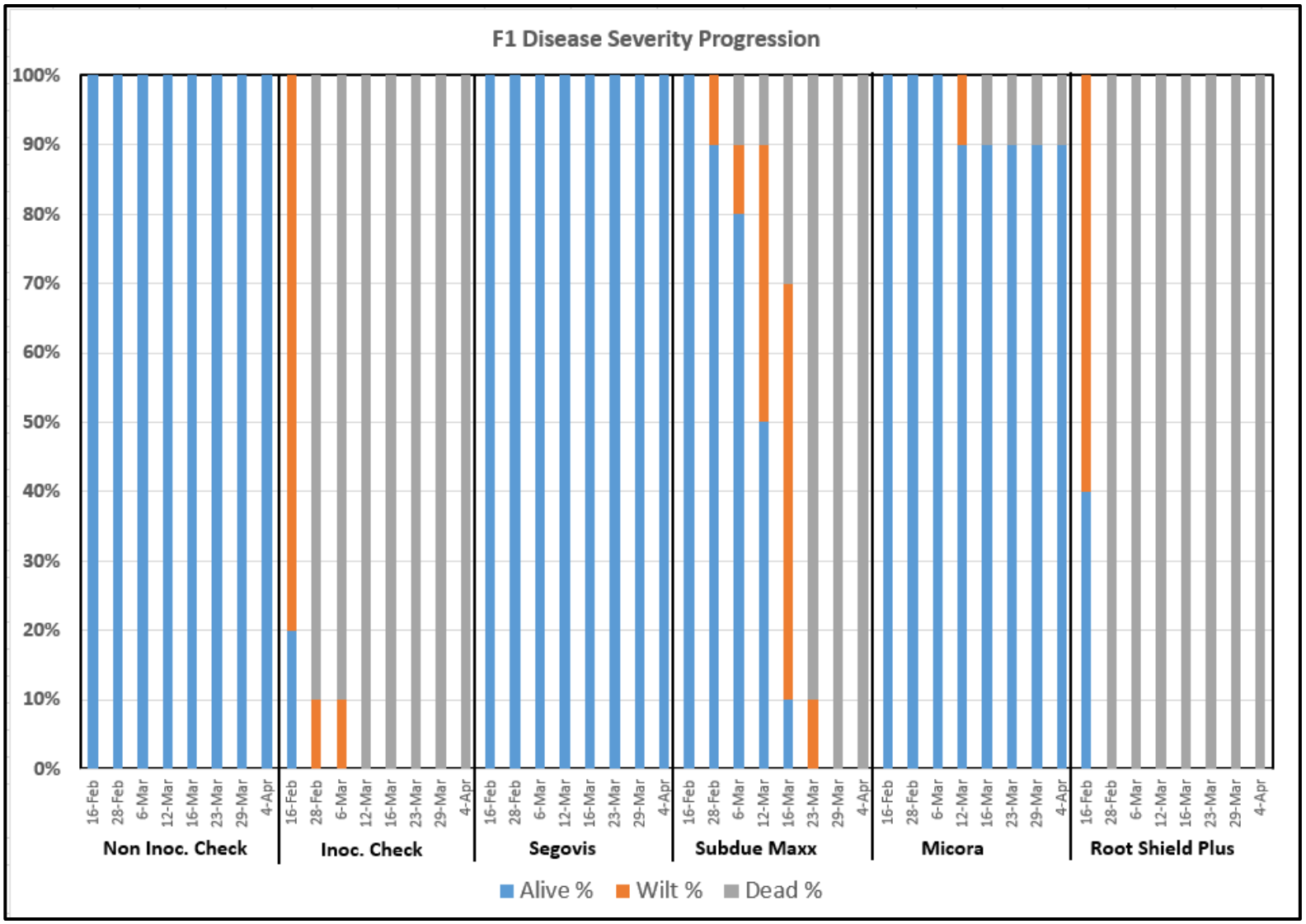

Figure 1. Experiment 1. Treatments applied before soil inoculation with P. cryptogea

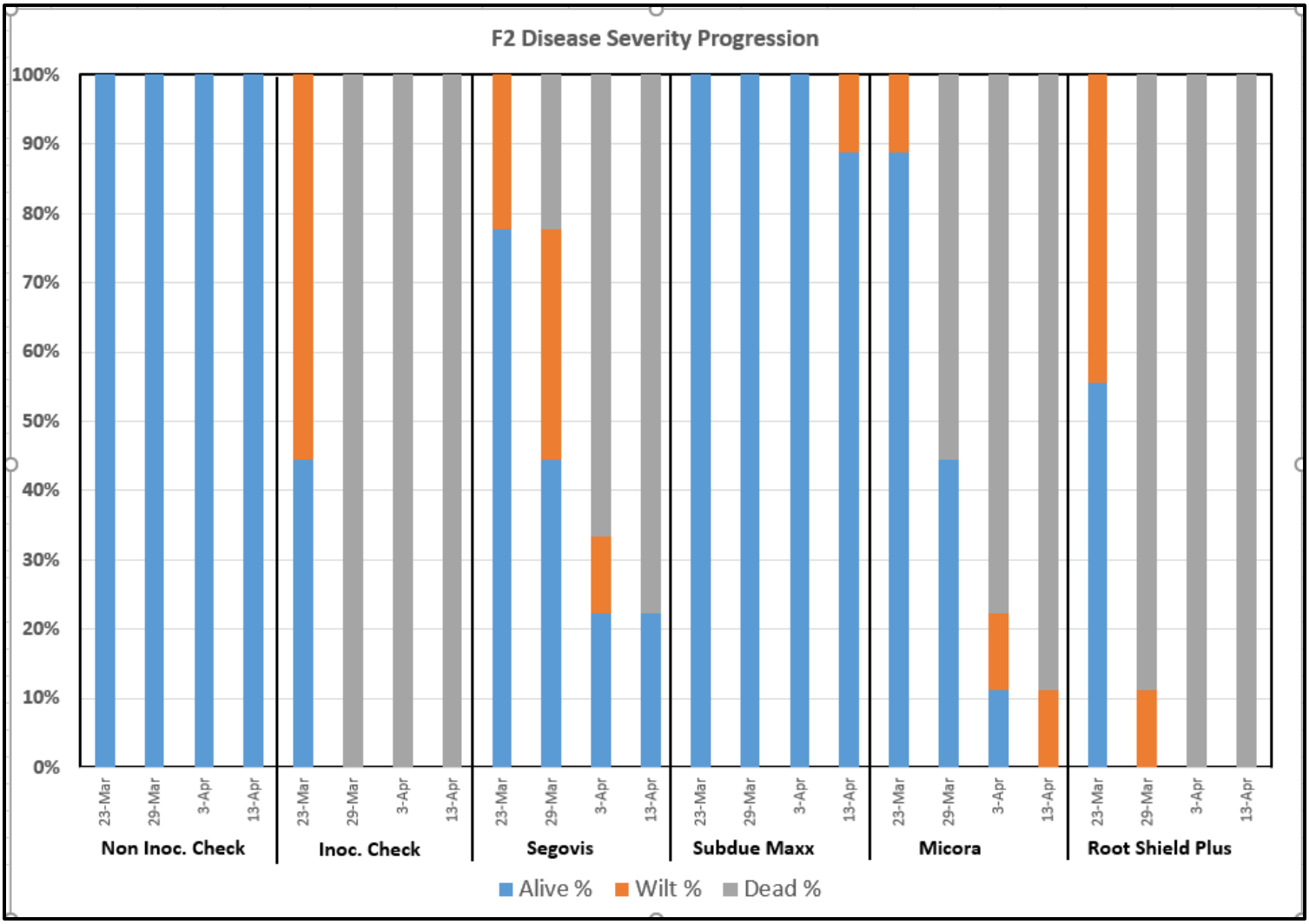

Figure 2. Experiment 2. Treatments applied after soil inoculation with P. cryptogea.

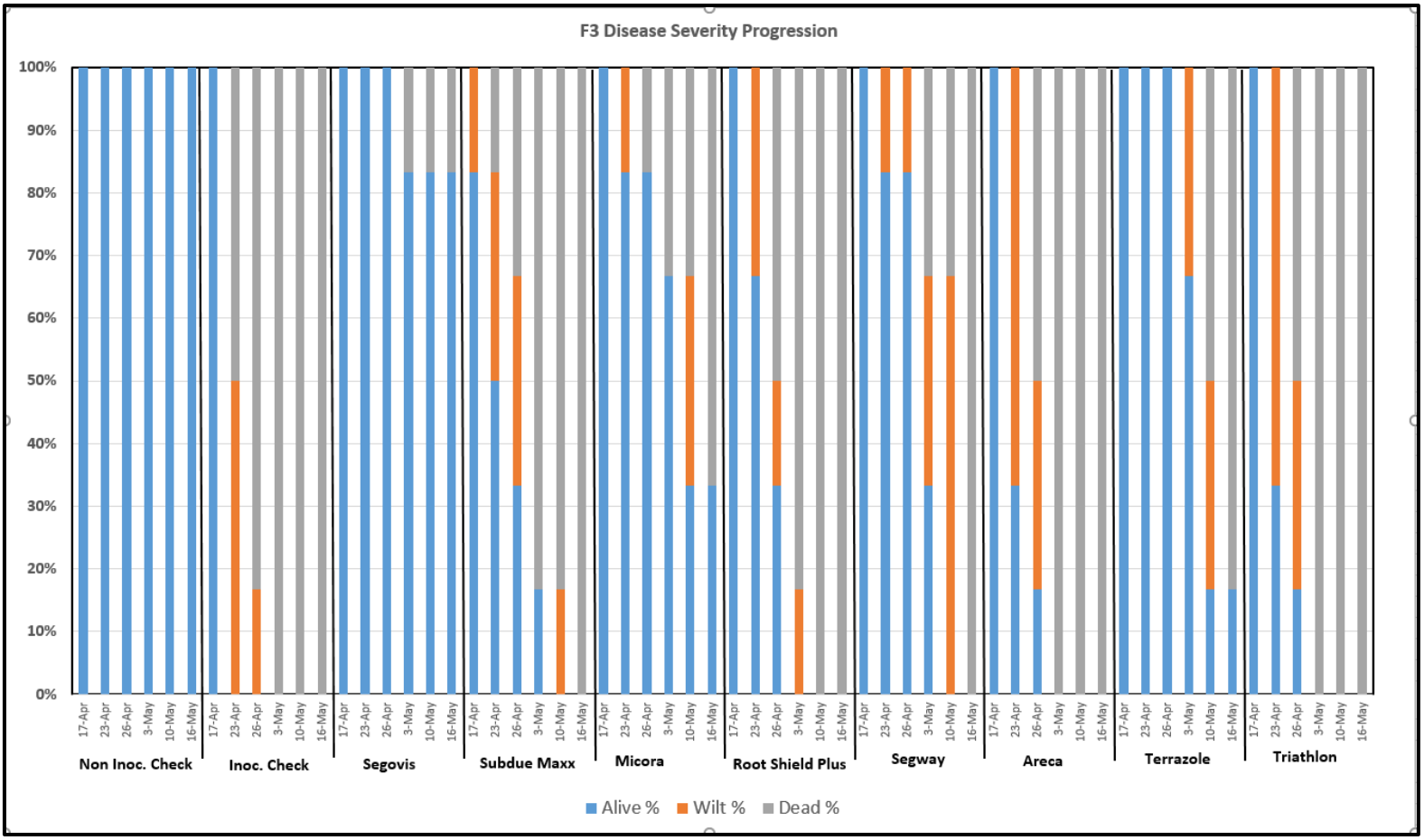

Figure 3. Experiment 3. Treatments applied before soil inoculation with P. cryptogea.

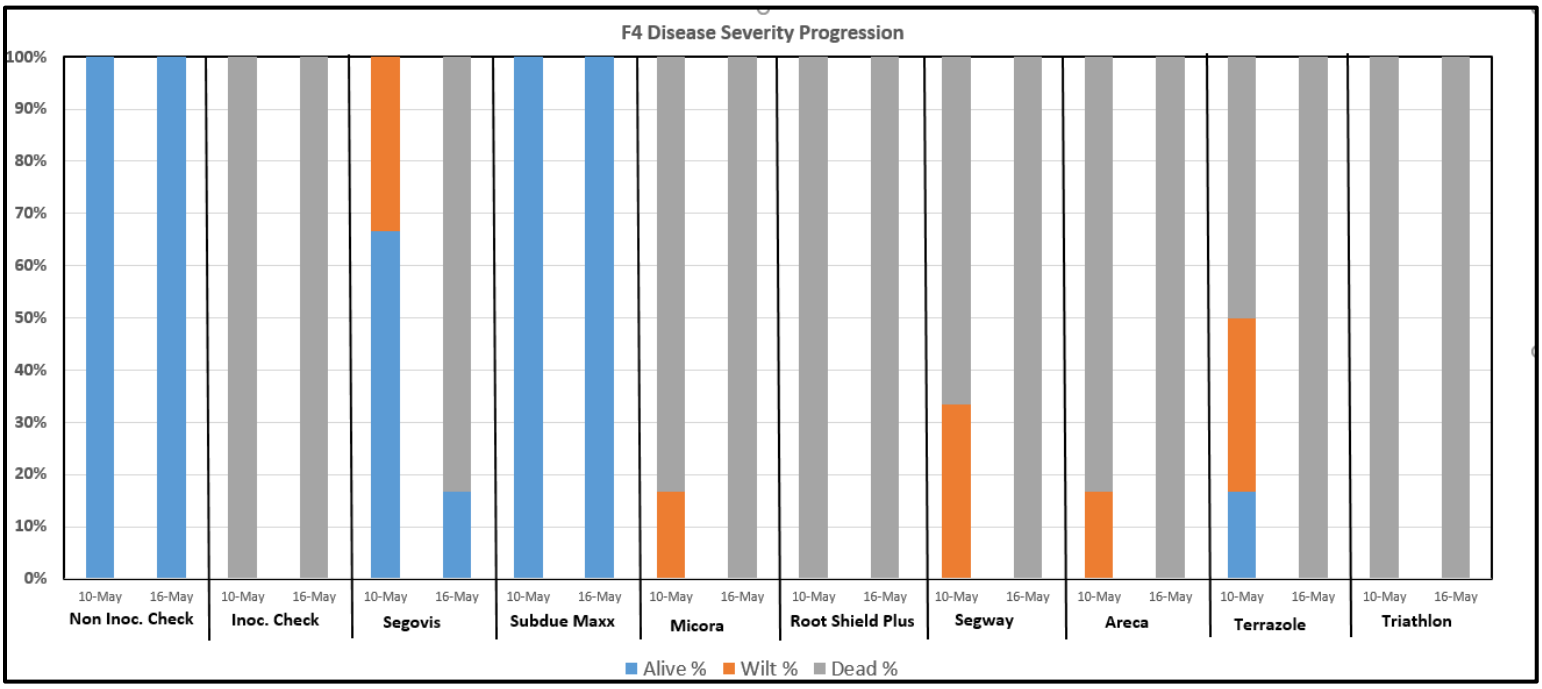

Figure 4. Experiment 4. Treatments applied after soil inoculation with P. cryptogea.

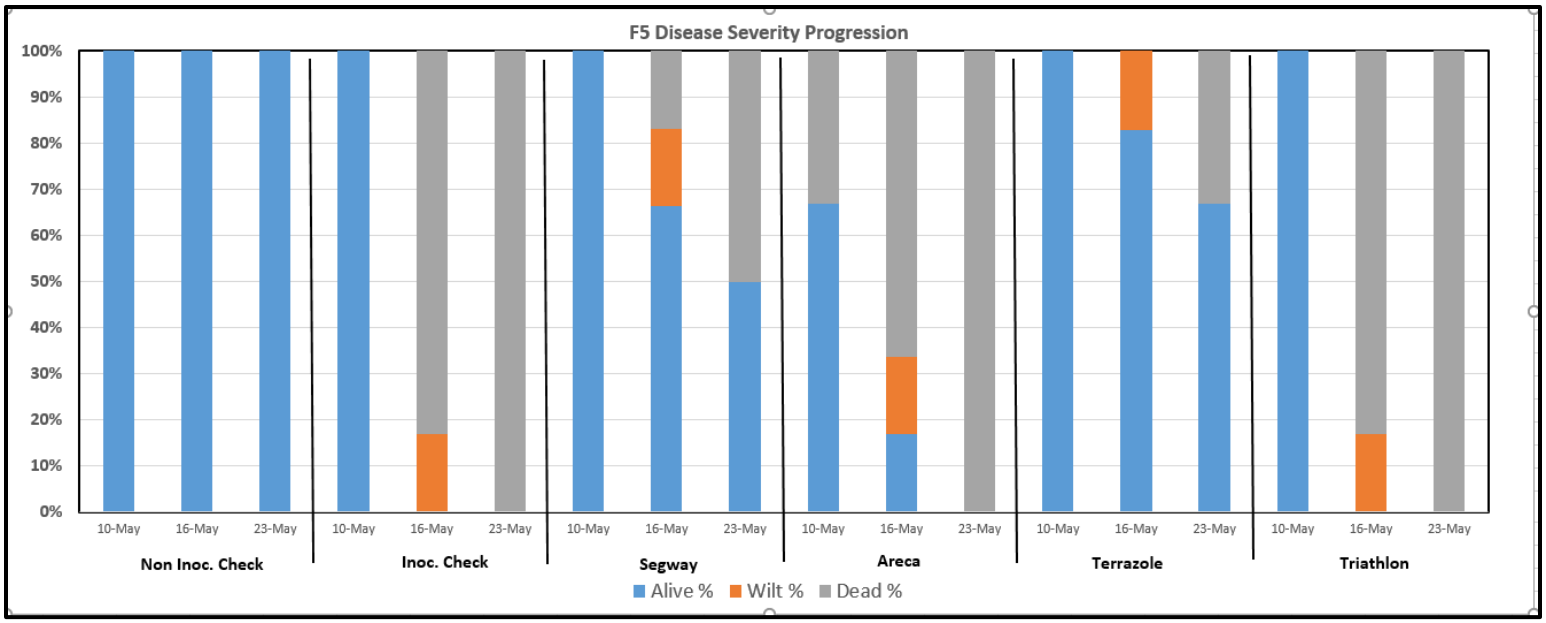

Figure 5. Experiment 5. Treatments applied before soil inoculation with P. cryptogea.

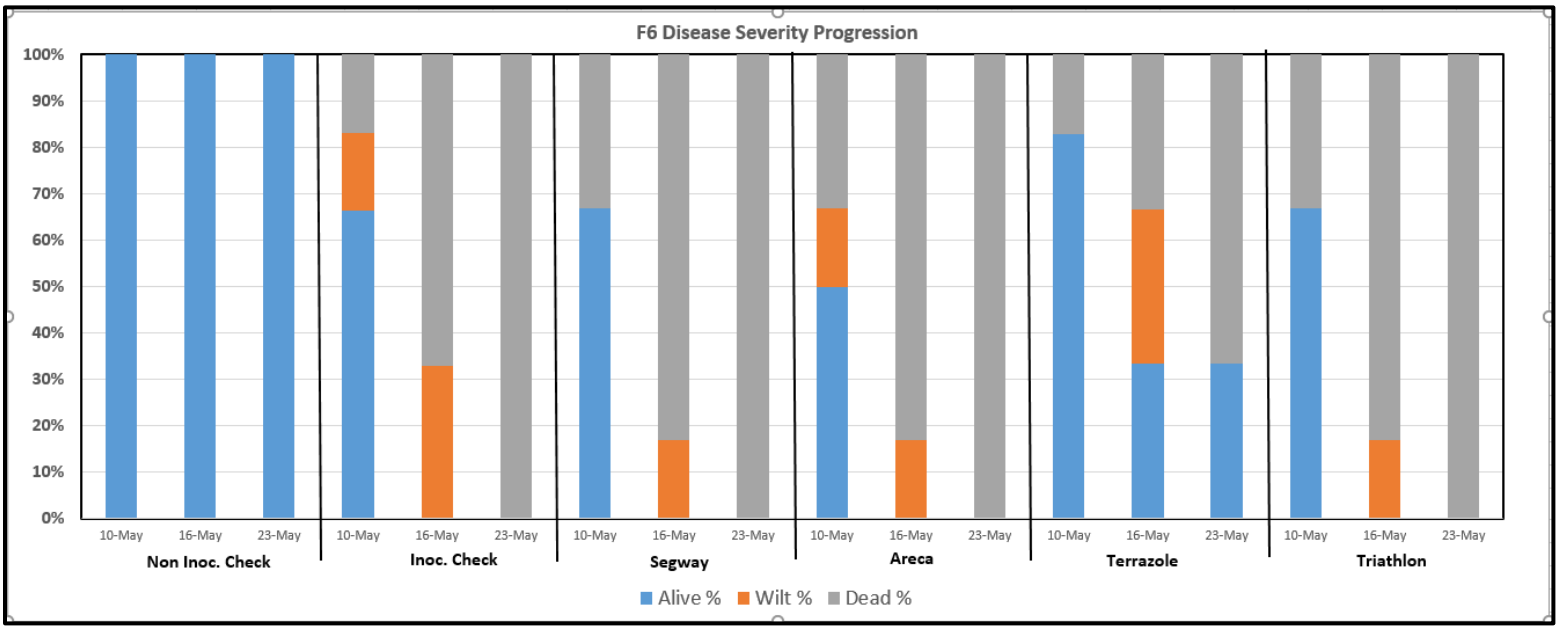

Figure 6. Experiment 6. Treatments applied after soil inoculation with P. cryptogea.

Table 3 Disease Severity and *P. cryptogea* Isolation Success from Roots of Alive Plants at the Conclusion of Each Experiment

		PRE-INOCULATION TREATMENT									POST-INOCULATION TREATMENT								
		EXPT. # 1			EXPT. # 3			EXPT. # 5			EXPT. # 2			EXPT. # 4			EXPT. # 6		
Treat. #	**Treatment**	**% Alive**	**Quality**	**P.c.**	**% Alive**	**Quality**	**P.c.**	**% Alive**	**Quality**	**P.c.**	**% Alive**	**Quality**	**P.c.**	**% Alive**	**Quality**	**P.c.**	**% Alive**	**Quality**	**P.c.**
1	Non Inoc. Control	100	2.9 NS	no	100	2.7 a	no	100	3.0 a	no	100	2.8 a	no	100	2.7 a	no	100	2.8	no
2	Inoc. Control	0			0			0			0			0			0		
3	Segovis	100	2.3 NS	no	83	2.0 a	no				22	0.7 b	no	17	0.5 b	no			
4	Subdue Maxx	0			0						100	2.4 a	yes	100	2.8 a	yes			
5	Micora	90	2.4 NS	yes	33	0.7 b	no				0			0					
6	Root Shield Plus	0			0						0			0					
7	Segway				0			50	0.8 b	no				0			0		
8	Areca				0			0						0			0		
9	Terrazole				17	0.5 b	no	83	1.0 b	yes				0			33	1.2	yes
10	Triathlon				0			0						0			0		

% Alive = alive and no wilting

Quality= only alive plants rated for quality

1= stunted and/or chlorotic leaves, not commercial quality, 2 = commercial quality , 3 = highest commercial quality

Quality significance: NS in colum= not statistically different quality; a or b following quality rating: numbers in colums with the same letters are not statistically different

P.c. = *Phtophthora cryptogea* isolated from roots of alive plants.

Figure 7 "Masking of symptoms" in Experiment 4. Treatments applied for curative control (after soil inoculation with P. cryptogea). Left to right: non-inoculated control, Segovis, Subdue Maxx, Segway, shown at the conclusion of the experiment. P. cryptogea was isolated from roots of this Subdue Maxx-treated plant.