Research Project Report

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Title: Determining the impact of registered and non-registered fungicides and disinfestants on *Uromyces transversalis*, the causal agent of gladiolus rust, and their use as mitigation tools in combination with host plant resistance
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Abstract

The project entitled "Determining the impact of registered and non-registered fungicides and disinfestants on *Uromyces transversalis*, the causal agent of gladiolus rust, and their use as mitigation tools in combination with host plant resistance," had eight objectives (Table 1) with the primary goal of developing strategies to locally eradicate gladiolus rust, caused by *Uromyces transversalis*. Fungicides individually and in programs were evaluated and recommendations were included for Mexican growers and two growers in California. Four experiments were conducted screening gladiolus cultivars with more tolerant cultivars identified. In addition to examining survivability of urediniospores in the field, survival of spores under controlled environmental conditions has been examined. Polyclonal antibodies were developed from germinating urediniospores and based on antigens identified through development of cDNA libraries.

Table 1. Project Objectives and Milestones

Objective	Milestone
Obj.1: Fungicide efficacy in the field	Evaluate efficacy of fungicide programs in commercial gladiolus fields in Mexico.
Obj. 2: Determine fungicide toxicity	Determine relative in vitro toxicity of products from Obj. 1 to urediniospores (Florida)
Obj. 3: Determine pre-shipment treatments	Evaluate fungicides and other tools under simulated shipping.
Obj. 4: Gladiolus cultivar resistance	Evaluate the resistance of gladiolus cultivars in replicated field trials in Mexico in fall 2012 and spring 2013.
Obj. 5. Urediniospore survival	Conduct field trials in Mexico to determine survival potential of spores on plant debris. Conduct laboratory studies at Fort Detrick.
Obj. 6: Systemic latent infections	Determining location of fungal tissue after inoculation
Obj. 7: Develop serological diagnostic tools	Develop polyclonal and monoclonal antibodies specific to *U. transversalis*.
Obj. 8: Develop genetic diagnostic tools	Verify PCRprimers for ITS region in *U. transversalis* are specific enough to be definitive genetic based assay tool.
Obj. 1-8: Data summary	Summarize data, prepare reports, and communicate results.

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Objective 1: Fungicide efficacy in the field

To determine best fungicide treatments to manage or locally eradicate gladiolus rust, Dr. Alberto Valencia Botin conducted four experiments from 2010 through 2012. The first three experiments examined preventative applications of several contact and systemic fungicides on gladiolus rust severity. The final experiment tested several fungicide programs. All experiments were duplicated in commercial production fields located in two Mexican states Morelos and Puebla with the exception of the spring 2011 experiment which was conducted solely in Morelos. Natural infections were augmented with inoculations of *U. transversalis* spores.

Fall 2010. Four applications of 11 fungicides plus two controls (non-inoculated and inoculated untreated plants) were made every 14 days using a hand-held CO_2 backpack sprayer. Disease severity in each plot was recorded each week for 7 weeks after first application. In the Cuautla, Morales location, inoculum pressure was high with disease severity starting at 9.8% and finishing at 76.8% in the non-inoculated, non-treated plots (Table 2). All fungicide treatments significantly reduced disease severity compared to the inoculated, non-treated plots. Disease development was least with three triazole fungicides: epoxiconazole, propiconazole and tebuconazole. The addition of boscalid to pyraclostrobin did not improve performance. In the Atlixco, Puebla, location, inoculum pressure was also high with disease severity starting at 16.8% and finishing at 75.3% in the non-inoculated, non-treated plots (Table 3). Starting Oct. 16, epoxiconazole and tebuconazole exhibited statistically less disease severity than the inoculated, untreated plots. As the season progressed, propiconazole and trifloxystrobin reduced disease, and by Nov. 13, 2010 all treatments significantly reduced disease severity, with epoxiconazole providing the best season-long efficacy.

Spring 2011. A similar experiment was conducted from Mar. to May, 2011 with an emphasis on tank mix combinations of triazole fungicides with either strobilurins or chlorothalonil. This experiment occurred solely in a commercial gladiolus field in Tlayacapan, Morelos, Mexico. Natural infections were augmented with inoculations of *U. transversalis* spores. Six applications of 14 fungicides (Table 4) plus two controls (non-inoculated and inoculated untreated plants) were made every 14 days using a hand-held CO_2 backpack sprayer. Disease severity in each plot was recorded each week for 10 weeks after first application. Disease pressure was low with the uninoculated, untreated plots starting with no disease and ending with 9.8% disease severity (Table 3). There were no differences in disease severity until the final data collection date, where all products reduced disease severity in comparison to the inoculated untreated plots. The best treatments were cyproconazole alone and combinations with strobilurins, with acibenzolar-s-methyl, chlorothalonil, chlorothalonil + propiconazole, flutolanil, and oxycarboxin + tebuconazole statistically equivalent to the best performing treatments.

Fall 2011. Because disease incidence was very low spring 2011, the same treatments were repeated during fall 2011 in Cuautla, Morelos and Santa Isabela Chalulu, Pueblo (Table 5). From early September 2011 through early November 2011, disease incidence was collected. In Cuautla, Morelos, disease pressure was moderate with disease severity starting at 1.3% and finishing at 42.5% in the plots with non-inoculated, non-treated plants. Starting October 22, 2011, all fungicide applications significantly reduced disease severity (Table 5) compared to the inoculated, untreated plots. By November 5, 2011, most single fungicide programs reduced disease incidence (acibenzolar-s-methyl, cyproconazole, difenconazole, epoxiconazole, flutalonil). However, tank mixes of fungicides from different mode of action classes exhibited exceptional disease management, in that disease did not occur on plants sprayed with six opf the fungicide combinations. A similar trend was observed in Isabel Cholulu, Puebla where the triazole fungicides, with and without tank-mix combinations with other fungicide classes, reduced disease incidence to close to or at zero (Table 6). The two products that did not reduce disease incidence to the same level were Actigard and Daconil.

Table 2. Gladiolus rust disease severity after fall fungicide applications in commercial fields in Cuautla, Morelos, Mexico, Fall 2010

Active Ingredient(s)	Product or Trade Name	Rate per liter (Rate per 100 gal)	Disease Severity							AUDPC
			10/1	10/10	10/16	10/23	10/30	11/13	11/7	
Azoxystrobin	Amistar	750 mg per L (10.3 oz per 100 gal)	8.6 a	10.8 a	13.8 b	19.8 b	27.9 cd	45.5 bc	45.1 bc	171 b
Boscalid + pyraclostrobin	Cabrio	2g/L per L (27.3 oz per 100 gal)	13.7 a	9.3 a	20.0 b	28.5 b	34.2 bcd	51.0 b	51.8 b	208 b
Epoxiconazole	Opus	1.875 ml per L (24.6 fl oz per 100 gal)	9.1 a	9.0 a	21.3 b	23.3 b	25.9 cd	23.3 d	23.4 d	135 c
Fluoxastrobin	Vigold	750 mg per L (10.3 oz per 100 gal)	8.5 a	11.0 a	19.5 b	29.8 b	35.0 bcd	58.5 b	56.9 b	219 b
Kresoxim-methyl	Stroby	625 mg per L (8.5 oz per 100 gal)	7.4 a	10.0 a	22.8 b	34.8 b	45.4 b	51.8 b	51.4 b	223 b
Mycloblutanil	Rally	200 mg per L (2.7 oz per 100 gal)	8.0 a	12.0 a	19.0 b	25.0 b	30.0 bcd	49.3 b	48.8 b	192 b
Oxycarboxin	Plant Vax	3750 mg per L (51 oz per 100 gal)	6.8 a	16.0 a	16.5 b	26.0 b	33.8 bcd	44.0 bc	43.2 bc	186 b
Propiconazole	Tilt	2.5 ml per L (32.8 fl oz per 100 gal)	10.5 a	6.8 a	17.0 b	20.5 b	22.3 d	29.8 cd	30.8 cd	138 c
Pyraclostrobin	Headline	1 ml per L (13.1 fl oz per 100 gal)	10.5 a	15.3 a	26.8 b	34.8 b	41.1 bc	53.8 b	52.2 b	234 b
Tebuconazole	Tebucur	1.5 ml per L (19.7 fl oz per 100 gal)	8.3 a	10.8 a	17.8 b	22.5 b	27.6 cd	28.8 cd	24.3 cd	140 c
Trifloxystrobin	Flint	625 mg per L (8.5 oz per 100 gal)	12.8 a	12.0 a	20.0 b	31.5 b	40.0 bc	51.3 b	52.4 b	220 b
Uninoculated Control			9.8 a	21.3 a	48.5 a	57.3 a	66.0 a	76.8 a	76.1 a	356 a
Inoculated Control			13.8 a	17.8 a	46.0 a	53.8 a	65.8 a	79.8 a	77.0 a	354 a

Table 3. Gladiolus rust disease severity after fall fungicide applications in commercial fields in Atlixco, Puebla, Mexico, Fall 2010

Active Ingredient(s)	Product or Trade Name	Rate per liter (Rate per 100 gal)	Disease Severity							AUDPC
			10/1	10/10	10/16	10/23	10/30	11/13	11/7	
Azoxystrobin	Amistar	750 mg per L (10.3 oz per 100 gal)	16.1 a	24.0 a	29.3 abc	34.5 ab	39.5 abc	48.3 b	46.2 bc	238 abc
Boscalid + pyraclostrobin	Cabrio	2g/L per L (27.3 oz per 100 gal)	14.9 a	31.5 a	32.8 abc	35.8 ab	40.7 ab	41.3 b	43.3 bc	240 abc
Epoxiconazole	Opus	1.875 ml per L (24.6 fl oz per 100 gal)	13.6 a	13.0 a	17.3 c	18.0 b	21.8 c	22.5 c	23.5 d	129 d
Fluoxastrobin	Vigold	750 mg per L (10.3 oz per 100 gal)	15.1 a	20.3 a	26.8 abc	35.5 ab	42.7 ab	43.5 b	42.7 bc	226 abcd
Kresoxim-methyl	Stroby	625 mg per L (8.5 oz per 100 gal)	11.8 a	27.0 a	28.5 abc	37.8 a	47.4 ab	41.3 b	37.3 bc	222 bcd
Myclobutanil	Rally	200 mg per L (2.7 oz per 100 gal)	17.5 a	24.3 a	28.0 abc	29.5 ab	32.8 abc	48.8 b	47.1 b	228 abcd
Oxycarboxin	Plant Vax	3750 mg per L (51 oz per 100 gal)	18.7 a	22.5 a	27.5 abc	34.3 ab	39.7 abc	37.8 b	37.1 bcd	187 cd
Propiconazole	Tilt	2.5 ml per L (32.8 fl oz per 100 gal)	20.3 a	13.0 a	24.0 bc	25.5 b	30.1 bc	34.8 b	32.4 bcd	180 cd
Pyraclostrobin	Headline	1 ml per L (13.1 fl oz per 100 gal)	13.1 a	30.3 a	31.8 abc	32.0 ab	33.1 abc	39.0 b	40.4 bcd	220 bcd
Tebuconazole	Tebucur	1.5 ml per L (19.7 fl oz per 100 gal)	12.9 a	25.5 a	23.0 c	28.0 ab	31.0 bc	31.3 b	26.4 c	178 cd
Trifloxystrobin	Flint	625 mg per L (8.5 oz per 100 gal)	16.7 a	19.0 a	24.8 bc	28.5 ab	31.0 bc	32.5 b	30.6 bcd	214 cd
Non inoculated Control			16.8 a	30.3 a	43.8 a	46.3 a	50.4 a	75.3 a	68.6 a	319 ab
Inoculated Control			16.5 a	30.0 a	42.8 ab	45.8 a	51.2 a	72.0 a	67.1 a	325 a

Table 4. Gladiolus rust disease severity after spring fungicide applications in commercial fields in Tlayacapan, Morelos, Mexico, Spring 2011

Active Ingredient(s)	Product or Trade Name	Rate	Disease Severity					AUDPC
			3/30 - 4/30	5/7	5/14	5/21	5/28	
Acibenzolar-s-Methyl	Actigard 50 GS	75 mg per L (1 oz per 100 gal)	0.0 a	0.5 a	0.5 a	1.0 b	2.0 cdef	4.0 b
Azoxystrobin + difenoconazole	Amistar + Score	0.75 g + 1.25 ml per L (10.3 oz + 16.4 fl oz per 100 gal)	0.0 a	0.0 a	0.5 a	0.5 b	1.25 ef	3.3 b
Azoxystrobin + epoxiconazole	Amstar + Opus 125	1.875 ml + 0.75 g per L (24.6 fl oz + 10.3 oz per 100 gal)	0.0 a	0.0 a	0.75 a	0.0 b	0.25 f	11.0 b
Azoxystrobin + propiconazole	Amistar + Tilt	0.75 g + 2.25 ml per L (10.3 oz + 29.5 fl oz per 100 gal)	0.0 a	0.25 a	0.75 a	0.0 b	0.25 f	1.0 b
Clorothalonil	Daconil 2787	3.75 g per L (51 oz per 100 gal)	0.0 a	0.0 a	0.5 a	1.25 b	2.75 cdef	1.3 b
Epoxiconzole + clorothalonil	Daconil 2787 + Opus 125	3.75 g per L + 1.875 ml (51 oz per 100 gal + 24.6 fl oz)	0.0 a	0.5 a	0.5 a	1.5 b	2.75 cdef	5.3 b
Clorothalonil + propiconazole	Daconil 2787 + Tilt	3.75 g + 2.25 ml per L (51 oz + 29.5 fl oz per 100 gal)	0.0 a	0.5 a	1.25 a	3.25 b	5.0 bcdef	4.5 b
Cyproconazole	Alto 100 SL	1 ml per L (13.1 fl oz per 100 gal)	0.0 a	0.0 a	0.25 a	0.75 b	1.5 def	10.0 b
Difenconazole	Score	1.25 ml per L (16.4 fl oz per 100 gal)	0.0 a	0.25 a	0.75 a	1.75 b	6.75 bc	2.5 b
Epoxiconazole	Opus 125	1.875 ml per L (24.6 fl oz per 100 gal)	0.0 a	0.75 a	1.25 a	2.75 b	6.25 bcd	9.5 b
Fluoxastrobin + Myclobutanil	Disarm	1.928 g per L (26.4 oz per 100 gal)	0.0 a	0.75 a	1.0 a	2.75 b	5.5 bcde	10.0 b
Flutalonil	Contrast; Moncut 50WP	5 g per L (68 oz per 100 gal)	0.0 a	0.5 a	0.5 a	1.25 b	3.0 cdef	5.3 b
Oxycarboxin + Tebuconazole	Plant Vax + Folicur	3.75 g + 1.5 ml per L (51 oz + 20 fl oz per 100 gal)	0.0 a	0.25 a	1.0 a	1.0 b	2.0 cdef	4.3 b
Trifloxystrobin + Oxycarboxin	Flint + Plant Vax	0.625 g + 3.75 g per L (8.5 oz + 51 oz per 100 gal)	0.0 a	0.0 a	0.5 a	0.25 b	1.0 ef	1.8 b
Untreated Uninoculated			0.0 a	1.0 a	1.25 a	4.0 ab	9.75 b	16.0 ab
Untreated Inoculated			0.0 a	1.5 a	2.5 a	8.5 a	18.25 a	30.8 a

[z]Means followed by same letter do not differ significantly based on Fisher's LSD (p=0.05); shaded averages are significantly different from the untreated inoculated treatments.

[y] Area under the Disease Progress Curve was calculated on ratings from the Horsfall-Barrett scale.

Table 5. Gladiolus rust disease severity after fall fungicide applications in commercial fields in Cuautla, Morelos, Mexico, Fall 2011

Active Ingredients	Trade Name	Rate per Liter (Rate per 100 gal)	Disease Severity			AUDPC [y]
			10/8	10/22	11/5	
Acibenzolar-s-methyl	Actigard 50 GS	60 mg (0.8 oz)	0.0 a	0.0 a	7.5 a	10.3 a
Azoxystrobin + Difenoconazole	Amistar + Score	600 mg + 1,000 uL (8.0 oz + 12.8 fl oz)	0.0 a	0.0 a	0.0 a	7.5 a
Azoxystrobin + Epoxiconazole	Amistar + Opus 125	600 mg + 1,500 uL (8.0 oz + 19.2 fl oz)	0.0 a	0.0 a	0.0 a	7.5 a
Azoxystrobin + Propiconazole	Amistar + Tilt	600 mg + 2,000 uL (8.0 oz + 25.6 fl oz)	0.0 a	0.0 a	0.0 a	7.5 a
Chlorothalonil	Daconil 2787	3,000 mg (2.5 lb)	0.5 a	5.3 a	22.0 b	17.8 b
Chlorothalonil + Epoxiconazole	Daconil 2787 + Opus 125	3,000 mg + 1,500 uL (2.5 lb + 19.2 fl oz)	0.0 a	0.0 a	0.0 a	7.5 a
Chlorothalonil + Propiconazole	Daconil 2787 + Tilt	3,000 mg + 2,000 uL (2.5 lb + 25.6 fl oz)	0.0 a	0.0 a	0.0 a	7.5 a
Cyproconazole	Alto 100 SL	800 uL (10.2 fl oz)	0.0 a	0.0 a	0.5 a	8.5 a
Difenconazole	Score	1,000 uL (12.8 fl oz)	0.0 a	4.8 a	2.3 a	14.0 ab
Epoxiconazole	Opus 125	1,500 uL (19.2 fl oz)	0.0 a	0.0 a	0.3 a	8.3 a
Fluoxastrobin + Myclobutanil	Disarm	1.928 g (26.4 oz)	0.0 a	0.0 a	0.3 a	8.3 a
Flutolanil	Moncut 50 WP	4,000 mg (25.6 fl oz)	0.3 a	0.0 a	3.5 a	14.3 ab
Oxycarboxin + Tebuconazole	Plantvax + Folicur	3,000 mg + 1,200 uL (2.5 lb + 15.4 floz)	0.0 a	0.0 a	0.8 a	8.3 a
Oxycarboxin + Trifloxystrobin	Plantvax + Flint	3,000 mg + 500 mg (2.5 lb + 6.7 oz)	0.0 a	0.0 a	0.0 a	7.5 a
Non-treated, non-inoculated			1.3 a	23.5 b	42.5 c	23.5 b
Non-treated, inoculated			0.0 a	24.3 b	24.3 b	21.0 b

[z]Means followed by same letter do not differ significantly based on Fisher's LSD (p=0.05); shaded averages are significantly different from the untreated inoculated treatments.

[y] Area under the Disease Progress Curve was calculated on ratings from the Horsfall-Barrett scale.

Table 6. Gladiolus rust disease severity after fall fungicide applications in commercial fields in Santa Isabel Cholulu, Puebla, Mexico, Fall 2011

Active Ingredients	Trade Name	Rate per Liter (Rate per 100 gal)	Disease Severity			AUDPC
			10/8	10/22	11/5	
Acibenzolar-s-Methyl	Actigard 5O GS	60 mg (0.8 oz)	Cua	21.0 d	32.0 b	26.5 c
Azoxystrobin + Difenoconazole	Amistar + Score	600 mg + 1,000 uL (8.0 oz + 12.8 floz)	3.8 a	2.0 ab	0.0 a	11.8 ab
Azoxystrobin + Epoxiconazole	Amistar + Opus 125	600 mg + 1,500 uL (8.0 oz + 19.2 floz)	0.0 a	0.0 a	0.0 a	7.8 a
Azoxystrobin + Propiconazole	Amistar+Tilt	600 mg + 2,000 uL (8.0 oz + 25.6 floz)	0.0 a	0.0 a	0.5 a	7.8 a
Chlorothalonil	Daconil 2787	3,000 mg (2.5 lb)	7.3 a	15.8 cd	36.3 b	26.3 c
Chlorothalonil + Epoxiconazole	Daconil 2787 + Opus 125	3,000 mg + 1,500 uL (2.5 lb + 19.2 floz)	0.5 a	0.5 a	0.3 a	9.5 ab
Chlorothalonil + Propiconazole	Daconil 2787 + Tilt	3,000 mg + 2,000 uL (2.5 lb + 25.6 floz)	0.8 a	0.3 a	2.5 a	10.3 ab
Cyproconazole	Alto 100 SL	800 uL (10.2 floz)	6.5 a	14.0 bcd	0.0 a	18.8 b
Difenoconazole	Score	1,000 uL (12.8 floz)	0.5 a	0.0 a	0.0 a	9.0 a
Epoxiconazole	Opus 125	1,500 uL (19.2 floz)	0.8 a	5.0 abc	0.0 a	13.5 ab
Fluoxastrobin + Myclobutanil	Disarm	1.928 g (26.4 oz)	0.0 a	3.8 abc	1.0 a	13.8 ab
Flutolanil	Moncut 50 WP	4,000 mg (25.6 floz)	0.8 a	3.8 abc	1.0 a	13 ab
Oxycarboxin + Tebuconazole	Plantvax + Folicur	3,000 mg + 1,200 uL (2.5 lb + 15.4 floz)	0.8 a	0.0 a	0.0 a	8.0 a
Oxycarboxin + Trifloxystrobin	Plantvax + Flint	3,000 mg + 500 mg (2.5 lb + 6.7 oz)	0.0 a	0.8 a	1.8 a	10.0 ab
Non-treated, non-inoculated			7.0 a	35.8 e	58.8 c	30.8 c
Non-treated, inoculated			6.5 a	38.0 e	60.0 c	31.5 c

[z]Means followed by same letter do not differ significantly based on Fisher's LSD (p=0.05); shaded averages are significantly different from the untreated inoculated treatments.

[y] Area under the Disease Progress Curve was calculated on ratings from the Horsfall-Barrett scale.

Fall 2012. The goal of the Fall 2012 fungicide experiment was to determine whether rotations of different tank mixes could limit *U. transversalis* infections. Identical fungicide regimes were applied in two locations (Cuautla, Morelos and Santa Isabel, Cholulu, Puebla). In Cuautla, Morelos, disease pressure was moderate by the end of the experiment with 56.5% disease in the untreated inoculated plots. Ambient disease pressure was moderate in the untreated non-inoculated plots (35.3%). All treatment regimes provided excellent control including the rotation of flutalanil with different triazole fungicides. In Santa Isabel, Cholulu, Puebla, a similar pattern was observed. Both the inoculated and non-inoculated plots had high levels of disease (>80%), all treatments significantly lowered percent disease. While not statistically different, the rotation of flutalonil with triazole fungicides did not reduce the final disease percent to less than 1%, unlike the rotation of trifloxystrobin + oxycarboxin with either propiconazole + azoxystrobin or myclobutanil + fluoxastrobin.

Table 7. Rotational program of fungicides to manage rust gladiolus caused by *Uromyces transversalis*, in Cuautla, Morelos, Fall 2012

T	1st application	Percent Disease		2nd application	Percent Disease		3rd application	Percent Disease	
	9/14/2012	9/21/2012	9/28/2012	9/28/2012	10/5/2012	10/12/2012	10/12/2012	10/19/2012	10/26/2012
1	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.3 a	Epoxiconazole + Azoxystrobin 1.87ml+0.75 g	0.0 a	0.0 a	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.0 a	0.5 a
2	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a	Difenconazole + Azoxystrobin 1.25 ml +0.75g	0.0 a	0.0 a	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.0 a	1.0 a
3	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a	Epoxiconazole + Chlorothalonil 1.87 ml+3.75 g	0.0 a	0.0 a	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.0 a	1.8 a
4	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	1.8 a	4.3 a	Propiconazole + Azoxystrobin 2.25 ml+0.75g	0.0 a	0.3 a	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a
5	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.3 a	4 a	Myclobutanil + Fluoxastrobin 1.928 ml	0.0 a	0.3 a	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a
6	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.0 a	0.5 a	Propiconazole + Chlorothalonil 2.25 ml+3.75 g	0.0 a	0.3 a	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a
7	Flutalonil 5 g	0.3 a	1.0 a	Epoxiconazole 1.87 ml	0.0 a	0.0 a	Flutalonil 5 g	0.0 a	1.0 a
8	Flutalonil 5 g	0.5 a	1.8 a	Cyproconazole 1 ml	0.0 a	0.8 a	Flutalonil 5 g	0.0 a	0.8 a
9	Flutalonil 5 g	0.0 a	0.5 a	Difenconazole 1.25 ml	0.0 a	0.0 a	Flutalonil 5 g	0.0 a	0.5 a
10	Untreated inoculated	1.5 a	5.8 ab	Untreated inoculated	18.0 b	35.0 c	Untreated inoculated	48.0 c	56.5 c
11	Untreated non-inoculated	1.5 a	10.5 b	Untreated non-inoculated	18.5 b	22.8 b	Untreated non-inoculated	26.8 b	35.3 b

T = Number of treatment

Table 8. Rotational program of fungicides to manage rust gladiolus caused by *Uromyces transversalis*, in Santa Isabel, Cholula, Puebla, Fall 2012

T	1st application	Percent Disease		2nd application	Percent Disease		3rd application	Percent Disease	
	9/14/2012	9/21/2012	9/28/2012	9/28/2012	10/5/2012	10/12/2012	10/12/2012	10/19/2012	10/26/2012
1	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.3 a	Epoxiconazole + Azoxystrobin 1.87ml+0.75 g	0.0 a	0.0 a	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.3 a	2.5 a
2	Tebuconazole + Oxycarboxin 1.5ml+3.75g	1.5 a	3.0 a	Difenconazole + Azoxystrobin 1.25 ml +0.75g	0.0 a	0,0 a	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	0.5 a	2.3 a
3	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a	Epoxiconazole + Chlorothalonil 1.87 ml+3.75 g	0.0 a	0.3 a	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	1.5 a	3.5 a
4	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	2.25 a	5.8 a	Propiconazole + Azoxystrobin 2.25 ml+0.75g	0.0 a	0.0 a	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.0 a
5	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	1.0 a	4.0 a	Myclobutanil + Fluoxastrobin 1.928 ml	0.0 a	1.0 a	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	0.3 a
6	Trifloxystrobin + Oxycarboxin 0.625g+3.75g	1.0 a	3.8 a	Propiconazole + Chlorothalonil 2.25 ml+3.75 g	0.0 a	0.8 a	Tebuconazole + Oxycarboxin 1.5ml+3.75g	0.0 a	1.8 a
7	Flutalonil 5 g	2.5 a	8.0 ab	Epoxiconazole 1.87 ml	0.0 a	0.0 a	Flutalonil 5 g	0.8 a	3.0 a
8	Flutalonil 5 g	4.3 a	8.3 ab	Cyproconazole 1 ml	0.0 a	1.8 a	Flutalonil 5 g	0.8 a	2.8 a
9	Flutalonil 5 g	2.5 a	7.8 ab	Difenconazole 1.25 ml	0.0 a	0.8 a	Flutalonil 5 g	1.3 a	4.5 a
10	Untreated inoculated	8.0 a	20.5 b	Untreated inoculated	37.0 b	57.3 b	Untreated inoculated	74.0 b	88.5 b
11	Untreated non-inoculated	5.5 a	15.3 b	Untreated non-inoculated	33.5 b	51.5 b	Untreated non-inoculated	68.5 b	81.5 b

T = Number of treatment

Gladiolus rust management options in the United States

Based on results to date and what is currently registered in the US, the best approach for American growers is to utilize tank mix combinations of strobilurins, triazoles and chlorothalonil and rotate among these classes. The active ingredients tested in Mexico are not all registered in the US. The table below contains US registered products for the active ingredients in this research and the US labeled use rates for rust diseases. Please note that additional products with these active ingredients may be available.

Table 9. Products registered in the US for the active ingredients tested during this project

Active Ingredient	Product Trade Name	US Label Rate for Rust Diseases
Azoxystrobin	Heritage 50WDG	1 – 4 oz per 100 gal
Boscalid + pyraclostrobin	Pageant Intrinsic Brand Fungicide	6 – 12 oz per 100 gal
Chlorothalonil	Daconil	1.375 pints per 100 gal
Difenconazole + azoxystrobin	Alibi Flora	8 – 14 fl oz per 100 gal
Fluoxastrobin	DisArm 480SC	1 – 4 fl oz per 100 gal
Myclobutanil	Eagle 20EW	6 – 12 fl oz per 100 gal
	Eagle 40WP	3 – 6 oz per 100 gal
Propiconazole	Banner Maxx	5 – 8 fl oz per 100 gal
Tebuconazole	Torque 3.65SC	4 – 10 fl oz per 100 gal
Trifloxystrobin	Compass O 50WDG	2 – 4 oz per 100 gal

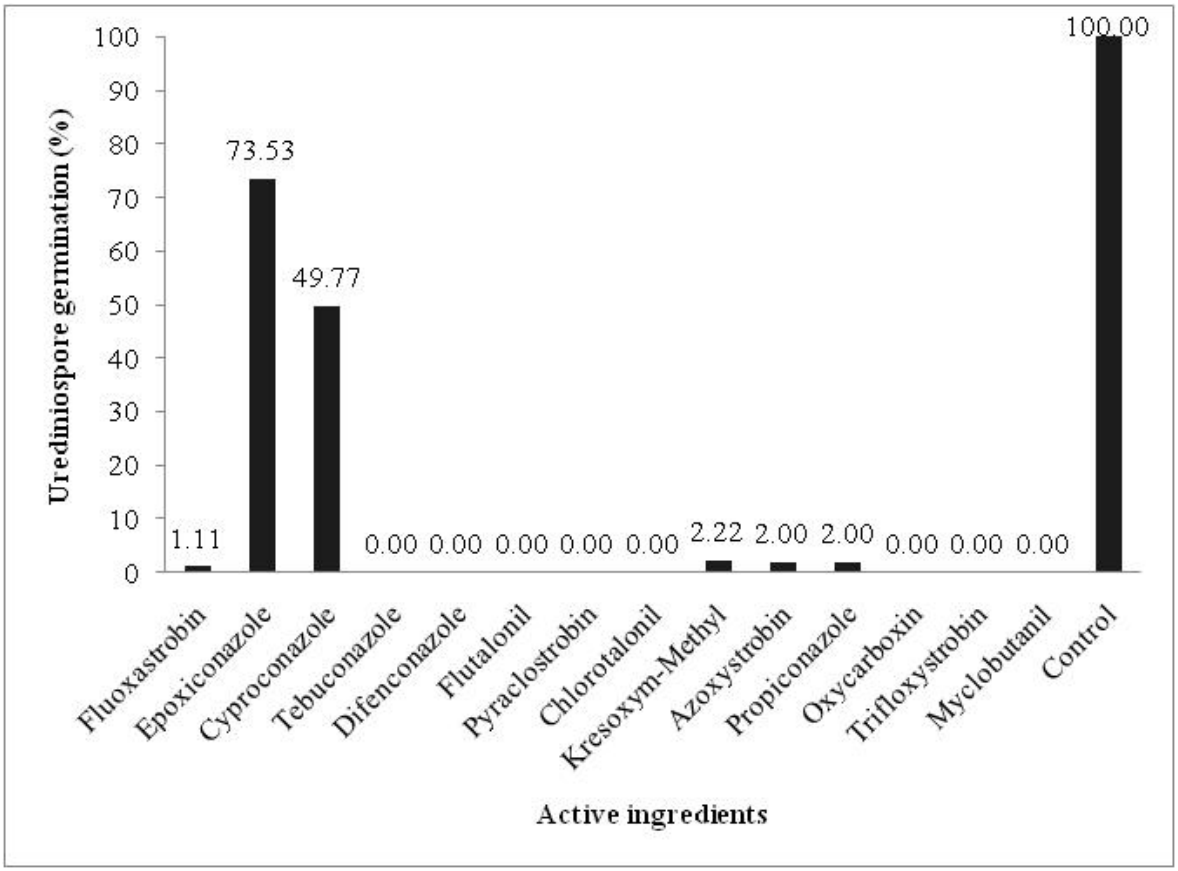
Objective 2: Determine fungicide toxicity

Dr. Botin examined urediniospore germination using a lab bioassay. Spores were transferred to fungicide amended potato dextrose agar. Fungicide concentrations were at label rates. Most fungicides prevented spore germination (Figure 1). All the strobilurins tested reduced germination to 2.2% or below. Flutalonil, myclobutanil, oxycarboxin and chlorothalonil completely inhibited germination. Triazoles were variable from some inhibition to complete inhibition.

Objective 3: Determine pre-shipment treatments

Dr. Valencia-Botin experienced difficulty in obtaining urediniospores from disease gladiolus leaves to test the ability of sanitizers to prevent germination on treated leaves.

Figure 1. Urediniospore germination on fungicide amended-potato dextrose agar



Objective 4: Gladiolus cultivar resistance

Six experiments were conducted from fall 2011 through spring 2013 in commercial gladiolus fields in Cuautla, Morelos and Santa Isabel Cholula, Puebla (Figure 2). Treatments were arranged in a completely randomized block design with four replications. All inoculum came from natural sources present in the commercial fields in which trials were conducted. Severity ratings were analyzed with one-way ANOVA and statistical differences among averages were determined to be different based on LSD test.

Fall 2011. In order to determine the resistance of 10 commercial varieties of gladiolus (*Gladiolus x hortelanus*) to gladiolus rust, two plots in commercial fields in Cuautla, Morelos and Santa Isabel Cholula, Puebla in Mexico were assessed for disease severity and rainfall during September to November 2011. Ten cultivars were established in both sites: 'Red Beauty', 'China', 'Cartago', 'Lupe', 'Primavera', 'Sanserri', 'Roja Borrega', 'Romulo Cantante', 'Portadel', and 'Ibadan'. Disease severity was collected in each plot as the percent foliar surface with symptoms each week for 7 weeks. Additionally, each location was recorded for monthly rainfall from September to November and this was correlated with disease severity.

By November 5th, disease incidence across cultivars was low in Cuautla, Morelos with disease severity ranging from 4.25% to 13.25% (Table 10, Figure 3). Statistically, 'Romulo Cantante' (fushia) exhibited the most disease, while 'Primavera' (orange) was statistically higher than 'Portadel' (dark pink) and 'Sanserri' with the rest of the cultivars statistically equivalent.

Figure 2. Panoramic view of gladiolus varieties planted in commercial fields

In Santa Isabel Cholula, Puebla, no significant differences ($p<0.05$) were detected among ten varieties throughout most of the experiment, but on Nov. 5, 2011 several cultivars had statistically less disease (Table 11, Figure 4). In this plot the severity ranged among 4.78% to 39.75%. In Cuautla, Morelos precipitation average was 87.73 mm, while in Santa Isabel Cholula, Puebla was 32.46 mm, with November being the driest month. The opinion of the growers of Cuautla, Morelos and Santa Isabel Cholula, Puebla, is that red and purple varieties are more resistant to rust infections. Two of the red cultivars, ‘’Cartago’ and ‘Roja Borrega’, exhibited less disease than most of the other cultivars Santa Isabel Cholula, Puebla, but ‘Red Beauty’ was the least tolerant in that location. High severity of *U. transversalis* in the growing season from August to November only occurs after the rainy season and the delay of the rainy season caused more rust severity.

Spring 2012. In this experiment, six of the cultivars in the fall 2011 experiment were planted in commercial fields in Cuautla, Morelos and Santa Isabel Cholulu, Puebla: ‘China’, ‘Primavera’, ‘Red Beauty’, ‘Roja Borrega’, ‘Romulo Cantante’ and ‘Sanserri’. Disease pressure was low throughout this experiment and no statistical difference among cultivars was observed (Table 12). No disease was observed at Santa Isabel Cholulu.

Spring 2013. In this experiment, seven gladiolus cultivars were planted in late fall to examine resistance under field inoculum pressure. Some cultivars included in the earlier experiments were able to be planted Spring 2013, but three new cultivars were planted: ‘Blanca Expuma’, ‘Borrega’, and ‘Extrema’. Disease severity in both Cuautla, Morelos and Santa Isabel Cholula, Puebla was low with no disease observed in the latter location, similar to the 2012 experiment (Table 13). Two cultivars, ‘Lupe’ and ‘Borrega’, exhibited the least infection while ‘Blanca Espuma’, ‘Extrema’, ‘Primavera’, and ‘Romulo Cantante’ exhibited more disease.

Table 10. Gladiolus rust disease severity in 10 cultivars grown in commercial fields in Cuautla, Morelos, Mexico, Fall 2011

Cultivar	Flower Color	9/24/2011	10/1/2011	10/8/2011	10/15/2011	10/22/2011	10/29/2011	11/5/2011	AUDPC	AUDPC (HB)
Cartago	Red	0.0 a	0.0 a	0.0 a	0.0 a	0.75 a	1.75 a	5.5 ab	5.6 a	9.8 ab
China	Yellow	0.0 a	0.0 a	0.0 a	0.0 a	2.25 ab	3.5 a	4.5 ab	8.0 ab	10.4 ab
Ibadan	Orange	0.0 a	0.0 a	0.0 a	0.0 a	1.0 a	3.5 a	5.5 ab	7.3 ab	11.0 ab
Lupe	Pink	0.0 a	0.0 a	0.0 a	0.0 a	3.0 abc	4.25 a	6.0 ab	10.3 ab	11.6 ab
Portadel	Dark-Pink	0.0 a	0.0 a	0.0 a	0.0 a	0.5 a	1.75 a	4.25 a	4.4 a	9.4 a
Primavera	Orange	0.0 a	0.0 a	0.0 a	0.0 a	5.5 b	8.75 b	8.75 b	18.6 ab	13.9 ab
Red Beauty	Red	0.0 a	0.0 a	0.0 a	0.5 a	3.75 abc	5.5 ab	6.0 ab	12.8 ab	12.3 ab
Roja Borrega	Red	0.0 a	0.0 a	0.0 a	0.0 a	1.25 ab	5.75 ab	6.0 ab	10.0 ab	11.9 ab
Romulo Cantante	Fuchsia	0.0 a	0.0 a	0.0 a	0.0 a	6.75 c	9.75 b	13.25 c	23.1 b	14.9 b
Sanserri	White	0.0 a	0.0 a	0.0 a	0.0 a	3.0 abc	4.25 a	4.25 a	9.4 ab	11.1 ab

Figure 3. Gladiolus Cultivar Screen in Cuautla, Morelos, Mexico, 2011

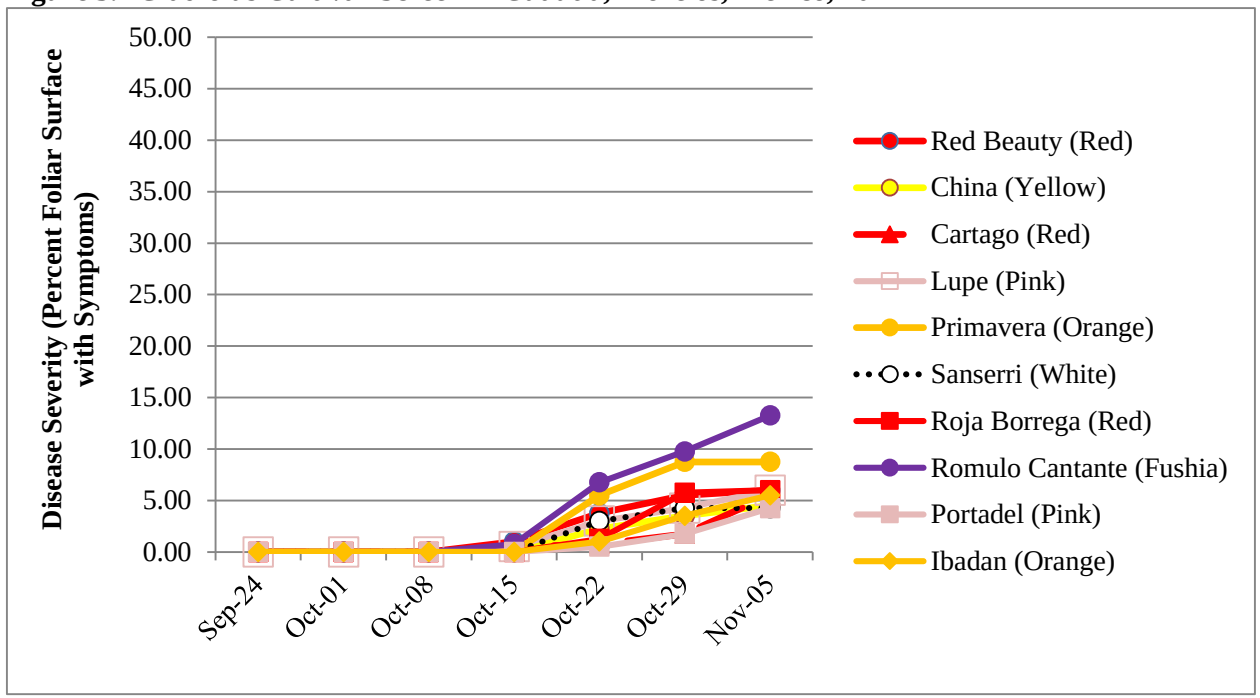


Table 11. Gladiolus rust disease severity in 10 cultivars grown in commercial fields in Santa Isabel Cholula, Puebla, Mexico, Fall 2011

Cultivar	Flower Color	9/24/2011	10/1/2011	10/8/2011	10/15/2011	10/22/2011	10/29/2011	11/5/2011	AUDPC	AUDPC (HB)
Cartago	Red	0.0 a	0.0 a	0.5 a	1.25 a	3.73 a	5.75 a	12.0 a	17.3 a	14.4 a
China	Yellow	0.0 a	0.0 a	3.0 a	3.25 a	9.75 a	12.5 a	39.75 b	48.4 a	19.1 a
Ibadan	Orange	0.0 a	0.0 a	0.75 a	2.0 a	6.5 a	9.0 a	21.5 ab	29.0 a	16.1 a
Lupe	Pink	0.0 a	0.0 a	1.5 a	3.75 a	10.25 a	12.75 a	37.0 b	46.8 a	18.9 a
Portadel	Dark-Pink	0.0	0.0	0.0	0.0	0.0	3.0	5.0	5.5 b	10.0 b
Primavera	Orange	0.0 a	0.0 a	1.0 a	6.25 a	17.25 a	19.5 a	36.75 b	62.4 a	20.8 a
Red Beauty	Red	0.0 a	0.0 a	2.5 a	5.75 a	17.75 a	20.5 a	35.0 b	64.0 a	19.9 a
Roja Borrega	Red	0.0 a	0.0 a	0.25 a	3.5 a	4.75 a	7.5 a	12.0 a	22.0 a	15.4 a
Romulo Cantante	Fuchsia	0.0 a	0.0 a	4.75 a	6.5 a	11.5 a	14.0 a	37.5 b	55.5 a	20.0 a
Sanserri	White	0.0 a	0.0 a	2.25 a	3.5 a	5.75 a	8.0 a	25.75 b	37.4 a	15.6 a

Figure 4. Gladiolus Cultivar Screen in Santa Isabel Cholula, Puebla, Mexico, 2011

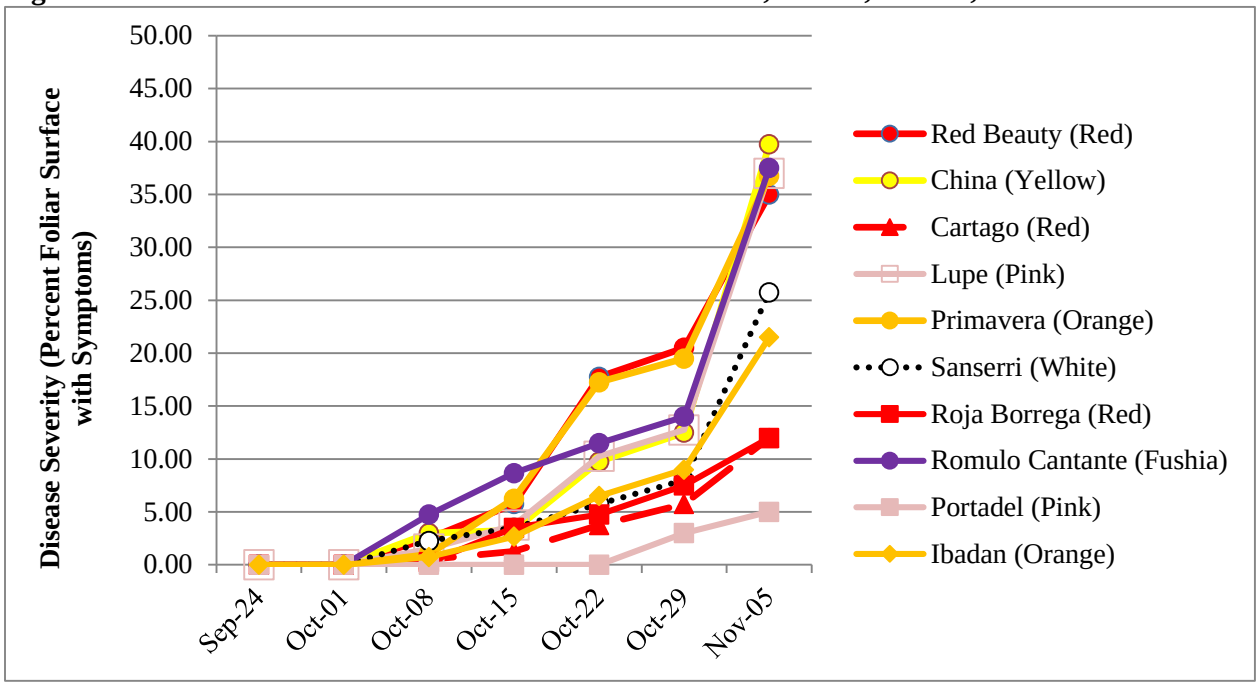


Table 12. Gladiolus rust disease severity among cultivars in commercial fields in Mexico, Spring 2012

Cultivar	Flower Color	Disease Severity [z]	
		Cuautla, Morelos	Santa Isabel Cholula, Puebla
		2012	2012
China	Yellow	5.5 a	0.0 a
Primavera	Orange	4.3 a	0.0 a
Red Beauty	Red	6.0 a	0.0 a
Roja Borrega	Red	10.8 a	0.0 a
Romulo cantante	Fuchsia	5.0 a	0.0 a
Sanserri	White	5.3 a	0.0 a
LSD (p = 0.05)		9.9	--

[z] Means within a column with a letter in common are not significantly different

Table 13. Cultivar tolerance to *U. transversalis* at two locations in Mexico, Spring 2013, Valencia-Botin.

Cuautla, Morelos									
Cultivar	Color	1/11	1/18	1/25	2/1	2/8	2/15	2/22	3/1 [z]
Blanca Espuma	White	0	0	0	0.3	2.8	6.5	7	7.8 a
Borrega	Red	0	0	0	0.3	0.5	1	1.3	1.5 c
China	Yellow	0	0	0	0	1.5	3	3.8	4.0 bc
Extrema	White	0	0	0.3	0.5	2.5	4.3	5	5.8 ab
Lupe	Pink	0	0	0	0.3	0.3	0.5	1	1.3 c
Primavera	Orange	0	0.3	0.5	1.8	3	5	5.5	6.0 ab
Romulo Cantante	Fushia	0	0	0	0.5	2.5	5.8	6.3	6.8 ab
Santa Isabel Cholula, Puebla									
Cultivar	Color	1/11	1/18	1/25	2/1	2/8	2/15	2/22	3/1
Blanca Espuma	White	0	0	0	0	0	0	0	0
Borrega	Red	0	0	0	0	0	0	0	0
China	Yellow	0	0	0	0	0	0	0	0
Extrema	White	0	0	0	0	0	0	0	0
Lupe	Pink	0	0	0	0	0	0	0	0
Primavera	Orange	0	0	0	0	0	0	0	0
Romulo Cantante	Fushia	0	0	0	0	0	0	0	0

[z] Statistical analysis were only performed for the last reading date at Cuautla, Morelos. Means within a column with a letter in common are not significantly different.

Fall 2013. In the final experiment conducted fall 2013, thirteen cultivars were planted at two sites (Cuautla Morales and Santa Isabel Cholula, Puebla), and disease severity evaluated weekly. The cultivars planted were 'Romulo Cantante', 'Primavera', 'Lupe', 'Red Beauty', 'Cartago', 'China', 'Borrega', 'Maravilla', 'Espuma', 'Escarla', 'Victoria', 'Sanserri', and Oasis. Corms were planted on August 16, 2013, and data on disease severity were recorded from September 20 to November 8, 2013 during the Fall growing period when infections have typically been heavy.

Under conditions with less inocula pressure, certain cultivars appear have some tolerance to *U. transversalis* infection as assessed by disease severity (Table 14). 'Victoria' and 'Escarla' exhibited no disease at the end of the experiment at Cuauatla, Morelos. However, under conditions with heavy disease pressure such as in Santa Isabel Cholula, Puebla in Fall 2013, both cultivars exhibited disease, albeit not as severe as most of the other cultivars.

Table 14. Disease severity of 13 gladiolus cultivars planted in commercial fields, Fall 2013, Valencia-Botin.

Cultivar	Average Disease Severity, Nov 8, 2013	
	Cuautla, Morelos	Santa Isabel Cholula, Puebla
Borrega	4.0	100.0
Cartago	10.5	100.0
China	21.0	100.0
Escarla	0.0	61.0
Espuma	13.5	100.0
Lupe	5.0	97.0
Maravilla	2.3	100.0
Oasis	21.8	100.0
Primavera	6.0	97.5
Red Beauty	9.5	100.0
Romulo Cantante	17.0	91.0
Sanserri	3.0	100.0
Victoria	0.0	80.0

Objective 5: Urediniospore survival

In the containment greenhouse in Florida, rust was never plentiful enough to initiate studies on urediniospore longevity or research on in vitro sensitivity to fungicides. Dr. Schubert initiated several methodologies to foster sporulating gladiolus rust lesions. However, while these measures were somewhat successful, a sufficient quantity of urediniospores was not available to undertake survival studies. Resources were shifted to the study of the phylogenetic relationships among *U. transversalis* populations from US, Mexico, South Africa, Australia, and New Zealand. See

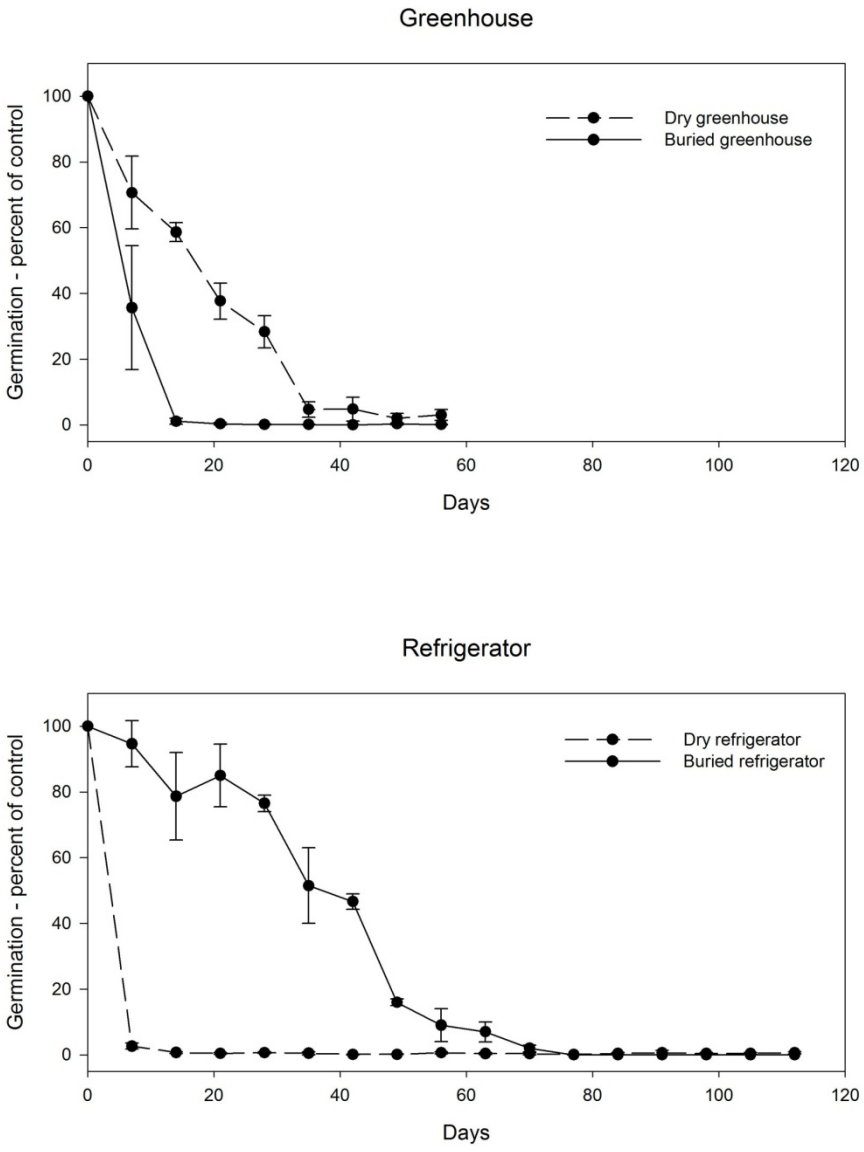
Field Studies in Mexico. In Mexico, Dr. Botin buried urediniospores in debris at depths of 5 and 10 cm. Spores were examined on PDA medium using a 200X stereomicroscope after 30 and 45 days. No germination occurred at either time point. This experiment was repeated to confirm results.

Laboratory Studies at Fort Detrick. At the USDA-ARS facility in Fort Detrick, green, infected leaves bearing gladiolus rust pustules were cut into sections and ½ of each section used to determine viability of the urediniospores at time zero. The first ½-leaf sections were placed in 50-ml centrifuge tubes, tubes placed in an atmosphere at 100% RH overnight, Tween 20 water added to each centrifuge tube, centrifuge tubes rocked 30 min to dislodge urediniospores from pustules, and urediniospores plated onto 1% water agar to determine urediniospore germination when incubated overnight at 17^{o}C in the dark.

The second ½-leaf sections were placed into 20-um pore size polyester mesh bags. One quarter of the mesh bags were placed into 1-quart plastic bags containing damp soil (Sunshine #1 Mix, with 250 ml water per 100 g oven dried Sunshine Mix), four polyester mesh bags per quart-plastic bag, and placed on a greenhouse bench at 22 to 26^{o}C (warm, damp). One quarter of the mesh bags were placed in a plastic beaker on the same greenhouse bench (warm, dry). One quarter of the mesh bags were placed into 1-quart plastic bags containing damp soil, four polyester mesh bags per quart plastic bag (as described above), and these placed in a 4^{o}C-refrigerator (cool, damp). One quarter of the polyester mesh bags were placed into a plastic beaker which was placed in the same refrigerator (cool, dry).

At weekly intervals, urediniospores were recovered from each environment, washed from the leaf pieces, and tested for their viability on 1.0 % water agar as described above. The experiment was conducted three times and results combined for analyses. After 14 days, 59% of urediniospores maintained under warm, dry conditions were viable compared to only 1% maintained under warm damp conditions (Figure 5). After 56 days, viability dropped to 3% and near 0%, respectively. Under cool conditions, viability exhibited a different pattern. After 14 days, 1% of urediniospores germinated under cool, dry conditions versus 79% under cool damp conditions. By 13 weeks, germination dropped to 0.7% and 0%, respectively. We concluded that under normal field conditions, in which urediniospores must pass through a warm period in the presence of moisture, the pathogen would not survive by means of urediniospores for more than a few weeks in either a greenhouse or field.

Figure 5. Percentage of urediniospores able to germinate on water agar after specific time durations, buried in soil or not buried (dry), in the greenhouse or refrigerator, up to 16 weeks.



Data are expressed as a proportion of the germination percentages of control urediniospores at time zero.

As part of a multiple pathogen survival study at Fort Detrick, urediniospores were buried in soil within a greenhouse bench or were placed on the soil surface in sachets. The bench was then steam sterilized and the urediniospore viability was assessed by spore germination. Preliminary reporting on the first experiment indicate that spores on the surface or buried in the soil were not viable.

A study was conducted to determine if *U. transversalis* urediniospores could survive on gladiolus corms through winter. Eighteen clay pots, each containing two inoculated gladiolus plants, were placed in a growth chamber programmed to simulate the average daily diurnal temperature conditions on November 10 (temperature high 26ºC, temperature low 15ºC) in Bradenton, Florida. This location was selected because of its closeness to a location where gladiolus rust was known to have survived through winter. At weekly intervals, the diurnal daily temperature high and low were each stepwise decreased until reaching temperature conditions characteristic for Bradenton, Florida at the coldest time of winter. At that point, the diurnal temperature high and temperature low

were stepwise increased weekly until reaching temperatures characteristic for Bradenton during spring. The specific temperatures selected were based on average daily temperature records from NOAA for each calendar day for that location from 2000 to 2012.

In spring, corms and newly formed cormlets attached to the bulbs were removed from the soil, cleaned to remove adhering soil, and placed in a 4ºC-refrigerator to simulate commercial storage. The soilless mix in pots was held in the growth chamber through the summer and into November, when two corms, variety "Border Mix", were planted in each pot. Plants were watered periodically to maintain moisture and allow germination of corms and plant growth. Plants that developed from the cv. Border Mix corms did not become infected by *U. transversalis*, and it was concluded that the pathogen did not survive in or on the soilless mix, and that corms, cormlets, and plants arising from corms and cormlets did not become infected from inoculum in the soil. This confirms that *U. transversalis* urediniospores are not capable of long-term survival in artificial soil.

The corms and cormlets, harvested in spring 2013 and refrigerated at 4ºC, were planted separately during April 2014 in pots and placed in a greenhouse. Once per week, plants arising from the corms and cormlets were placed in a dew chamber. Of a total of 15 plants, 12 developed a few pustules (e.g. average two per plant). When repeated, ½ receiving weekly dew periods and ½ receiving no dew, only those receiving dew developed sparse pustules. A set of plants developing from corms from non-infected plants, and on the same greenhouse bench, receiving weekly dew periods, developed a similar low number of pustules.

Objective 6: Systemic latent infections

To determine whether *U. transversalis* systemically infects gladiolus and if infected corms are a source of primary inoculum in the field, three *Gladiolus* plants, cv. Border Mix, about 1 month in age, were inoculated with a suspension of *U. transversalis* urediniospores (isolate Manatee Co., FL) and placed overnight in a dew chamber at 18ºC. The following day, the plants were transferred to a greenhouse at 22ºC to 26ºC. Rust pustules became evident on leaves after approximately 2 weeks. On March 14, after the plants had died back, the corms and cormlets that had developed from the infected parent plants were removed from the soil, cleaned, and refrigerated at 4ºC for 2 months. These were removed from refrigeration on May 10, and planted in clay pots in the greenhouse. Since dew is required for urediniospore germination and penetration, no dew period was provided. On October 4, plants that had arisen from the soil were examined for the presence of gladiolus rust pustules. The experiment was repeated exactly as before. No pustules were detected in either experiment, suggesting that *U. transversalis* had not systemically infected corms.

Objective 7: Develop serological diagnostic tools

Crude polyclonal antibodies (PAbs) have been raised against germinating urediniospores of *U. transversalis* and the liquid germination medium. The PAbs were found to be reactive against extracts of *U. transversalis* urediniospores and *U. transversalis*-infected gladiolus plants.

In addition, cDNA library was generated and sequenced using RNA isolated from germinating urediniospores (see below under Obj. 6), and the sequences were used as a database for identification of proteins isolated from germinating urediniospores as well. Over 10,000 cDNA sequences were filtered to 7 candidates with optimal characteristics to serve as immunogens for the production of polyclonal antibodies. Primers were designed for generation of recombinant proteins, using RNA isolated from infected, asymptomatic gladiolus plants. Two of five recombinant proteins were expressed and were sent to a commercial production firm for generation of polyclonal antibodies. One of these was highly reactive against antigen, extracts of U. transversalis urediniospores and U. transversalis-infected gladiolus plants.

These three PAbs are being further evaluated by Western and Dot blotting for sensitivity and to verify or identify their protein antigen targets.

Objective 8: Develop genetic diagnostic tools

Over 200 proteins were extracted from germinating *U. transversalis* urediniospores, separated on two-dimensional polyacrylamide gels and processed for mass spectrometry. RNA was isolated from *U. transversalis* germinating urediniospores, and Dr. Moraitis developed and sequenced cDNA libraries. These sequences were needed to construct a database of genes expressed in *U. transversalis* for proteome analysis. Details of the development and sequencing of cDNA libraries are below (Table 15).

Table 15. Summary of cDNA library development

Sequence data	
Total number of Reads	533,613
Total number of Bases	251,223,110
Assembly data (Newbler)	
Number of isotigs	10,995
Average contig count per isotig	3.7
Largest contig count per isotig	15
Number of isotigs with one contig	0
Bases assembled	12,428,483
Average isotig size	1130
N50 isotig size	1197
Largest isotig	3705
Number of singletons	20,404

Dr. Mockaitis prepared and sequenced cDNA libraries from total RNA of *U. transversalis*, and from these developed a predicted protein database. RNA was first prepared into a partially normalized double-stranded cDNA library optimized for sequencing on the Roche/454 GS FLX+ instrument. 533,613 reads totaling 251 Mb of nucleotides were assembled using Newbler software. Using the same original RNA, an additional library was generated for sequencing on the complementary Illumina technology platform. This was a strand-specific Illumina library for shorter, higher coverage reads (100 x 2 nts). 32 million read pairs were cleaned and assembled using Trinity software. To build a high confidence protein coding reference, all of the 454/Newbler and Illumina/Trinity assemblies were concatenated and analyzed for longest potential coding versus shorter alternative transcripts for a given gene product. Among all the *Uromyces transversalis* transcript assemblies, 13,812 were classified as non-redundant protein coding sequence. 25,201 transcripts were cleaned out due to potential redundancy with these 13,812 or lack of coding sequence. Protein coding transcripts were further classified into two categories: 1) the longest detected protein sequence among transcripts that align, called MAIN, and 2) smaller and/or slightly variable versions of the sequences that align to the MAINs, *i.e.*, putative alternate transcripts from the same gene. Finally, amino acid sequences were prepared by translating transcript references, and these were provided as fasta files to the group of Dr. Luster for use in searching protein sequence against protein mass spec data they have collected. See Objective 5 for details related to antibody development.

In addition, *U. transversalis* urediniospore cDNA library sequences were provided to Dr. Buck (University of Georgia) to aid in annotation of genes.

Supplemental Research: Phylogenetic analysis of populations

This research was undertaken to determine whether the *U. transversalis* populations in the US were related to populations in Mexico or other parts of the world where gladiolus rust is present.

Fungal specimens and plant materials. A total of 39 *Uromyces transversalis* isolates were used in this study. The majority of the isolates originating from infected leaves collected from different locations in Mexico. The samples were collected from gladiolus grown-fields during the 2010 growing season by Dr. Alberto Botin (University of Guadalajara, Mexico). Three isolates (2010-36361; P2006 1572; FL-new) had been collected

from samples collected in Florida in 2006 and 2010. Two isolates originated from samples found at a community garden located in San Francisco, California. Herbarium specimens of Gladiolus rust from three other geographic locations supplied by collaborators were also included: one specimen (PREM 47693) from South Africa was provided by Dr. Elna J. Van Der Linde (National Collection of Fungi Biosystematics Division ARC-PPRI, Pretoria, South Africa); two specimens originating from the Australian Herbarium VPRI were provided by Dr. Vyrna Beilharz (VPRI Plant Disease Herbarium, Australia); four herbarium specimens from New Zealand were obtained from Dr. Eric Mckenzie (Landcare Research, New Zealand).

DNA extraction. Genomic DNA was isolated from leaf tissue collected from infected plants. For each sample, DNA was extracted from one to three 1 cm^2 leaf disks that were frozen and ground to a fine powder in liquid nitrogen. Genomic DNA was extracted using a modified hexa-decyltrimethylammonium bromide (CTAB) protocol. Briefly, DNA extraction buffer (100 mM Tris, pH 7.5; 1% CTAB; 0.7 M NaCl; 10 mM EDTA; 1% 2-mercaptoethanol; 0.3 mg/ml proteinase K) was added to the ground tissue and incubated at 65°C for 30 min, followed by two rounds of chloroform: isoamyl alcohol (1:1) extraction, and precipitated with 2-propanol. DNA was resuspended in TE buffer containing 1 mg/ml RNase. The extracted DNA of each sample was quantified using a Nanodrop NO-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE).

ITS amplification, cloning, and sequencing. To amplify the rDNA-ITS regions from *U. transversalis* an initial amplification reaction was performed with the universal primer ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3’) and a rust-specific primer, Rust1 (5′-GCTTACTGCCTTCCTCAATC-3′). PCR was performed in 50-µl reaction mixtures containing 1× GoTaq® Flexi buffer (Promega, Madison, WI), 0.2 mM dNTP, 400 nM of each primer, 50 ng template DNA, and 5 units GoTaq® DNA Polymerase (Promega) using a GeneAmp® PCR System 9700 (PE Applied Biosystems Inc.), with initial denaturation at 95°C for 90 s, followed by 35 cycles of 94°C (1 min), 56°C (1min), 72°C (2 min), and a final extension at 72°C (5 min). A second PCR was then conducted to obtain the ITS region (including ITS1, 5.8SrDNA and ITS2) by using the universal primers ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) and ITS5 (5’-GGAAGTAAAAGTCGTAACAAGG-3’) (White et al.1990). PCR amplification was carried out under the following conditions: initial denaturation at 95ºC (90 s); followed by 30 cycles of 94ºC denaturation (30 s), 58ºC annealing (30 sec), and 72ºC extension (30 s); a final extension at 72ºC for 2 min. Amplified products were resolved on 1.2% agarose gel, and the bands were excised and purified using the QIAquick Gel Extraction kit (Qiagen). Purified PCR products were cloned into pGEM®-T Easy Vector kit (Promega) and transformed into *E. coli* JM109. Plasmid DNA was extracted from individual clones using a Nucleospin plasmid kit (Macherey-Nagel, Germany) following the manufacturer’s directions. Plasmids sequencing was conducted at the DNA Sequencing and Synthesis Facility (University of Georgia) using T7 and SP6 primers. All the sequences will be submitted to Genbank.

Data analysis. Nucleotide sequences of both strands were proofread and assembled. ITS5-Rust 1 and ITS5-ITS4 primers pairs were used to identify the beginnings and ends of the sequence regions, respectively. The primer sequences were excluded from further analyses. A NCBI Blast search was performed to identify similar sequences in Genbank. The resulting ITS sequences and those gathered from Genbank were aligned using the Clustal W algorithm and a neighbor-joining tree was constructed using the Molecular Evolutionary Genetics Analysis software MEGA 5.0 (Kumar et al., 2004) with statistical support for branch topology tested by bootstrap analysis derived from 1000 replicates.

Results and Conclusions. The ITS1-5·8S-ITS2 region was found to be ~ 630 bp long. Genbank nucleotide BLAST searches with our sequences returned several matches with *Puccinia* species. Preliminary phylogenetic analysis (neighbor-joining) analysis revealed no distribution of the isolates based on their geographic origin. For instance, all ITS-rDNA sequences from the U.S. (Florida and California) isolates analyzed here formed a common cluster together with many isolates from Mexico. Additional analyses of the sequence data are being conducted, and a manuscript is being prepared for submission to the European Journal of Plant Pathology.

Publications

Bonde, M.R., S. E. Nester, C. L. Palmer, J. M. Revell. 2014. Longevity of *Uromyces transversalis* Urediniospores under Various Environmental Conditions. Abstract. American Phytopathology Socitey Annual Meeting. Phytopathology. Phytopathology 104:S3.2

Valencia-Botin, A. J., S. N. Jeffers, C. L. Palmer, J. W. Buck. 2013. Fungicides Used Alone, in Combinations, and in Rotations for Managing Gladiolus Rust in Mexico. Plant Disease 97:1491-1496. http://cd.coi.org/10.1094/PDIS-03-13-0272-RE.

Valencia-Botin, A. J., J. W. Buck, S. N. Jeffers, C. L. Palmer. 2013. Resistance of gladiolus cultivars to *Uromyces transversalis* in field trials in Mexico: Preliminary results. Abstract. American Phytopathology Society Annual Meeting. Phytopathology 103(Suppl. 2):S2.151.

Valencia-Botin, A., J. W. Buck, S. N. Jeffers, C. L. Palmer. 2012. Efficacy of fungicides and mixtures of fungicides for management of gladiolus rust in Mexico. Poster Presentation at 2011 American Phytopathological Society Annual Meeting. August, 2011.

Valencia-Botín, A., Villegas-Elizalde, S., Buck, J.W., Jeffers, S.N., y Palmer, C. Evaluation of resistance of gladiolus cultivars in field trials in Mexico (Evaluación de la Resistencia de Variedades de Gladiolo en Experimentos de Campo en México), Poster num. 24, XIV Congreso Internacional /XXXIX Congreso Nacional de Fitopatología, 22 - 26 de Julio de 2012, Nuevo Vallarta, Nayarit, México

Valencia-Botín, A., Buck, J.W., Jeffers, S.N., Palmer, C. Managing gladiolus rust in Mexico with fungicides. Poster Number 167, American Phytopathological Society, August 5-9, 2010, Honolulu, HI.

Dr. Botin's undergraduate student, Karla Guadalupe Velaquez Rosiles**,** wrote an undergraduate thesis based on the fungicide screening data.