CALIFORNIA LEAFY GREENS RESEARCH PROGRAM RESEARCH REPORT, 2012-13 Season

Project title: Evaluation of relative susceptibility of lettuce varieties to *Tomato spotted wilt virus* and Fusarium wilt.

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Introduction

Lettuce is produced on approximately 20,000 acres in a relatively concentrated area in the Huron/Five Points area, within Fresno County, California. In this area, some lettuce fields were severely damaged due to *Tomato spotted wilt virus* as well as Fusarium wilt. In some fields, both pathogens were present at substantial levels.

Tomato spotted wilt virus, a thrips-transmitted virus with a wide host range, was known to be in this production area for decades. Approximately 7 years ago, severe damage was inflicted on several tomato fields and incidence and severity in fall lettuce has reached critical levels since 2010. While no work has been conducted to compare susceptibility of lettuce cultivars to this disease, antidotal field observations would suggest that incidence of symptomatic plants varies among varieties.

Fusarium wilt was first detected in this area more than 20 years ago, which was the first US report of this disease (Hubbard and Gerik, 1993). Since then, it has been reported in the Desert and the Central Coast production areas of California. Spread within Fresno County has been substantial.

Higher temperatures favor development of this disease. Disease severity was lower in susceptible varieties grown at $73^{\circ}/64^{\circ}$ F than at $83^{\circ}/68^{\circ}$ F and $91^{\circ}/73^{\circ}$ F (Scott et al., 2010). Under desert conditions, the disease is consistently more severe when planted earlier in the fall season while temperatures were higher (Matheron et al., 2005).

Varieties differ in terms of susceptibility to this disease and further breeding efforts are being made, but have not been tested recently in Fresno County. A few romaine varieties demonstrated a greater degree of tolerance under the high temperatures that favor the disease, a number of varieties expressed lower levels of the disease when planted under slightly cooler conditions (Matheron et al., 2005).

Management of Fusarium wilt relies in part on crop rotation to allow time for the pathogen population to decline during the interval between susceptible lettuce crops. This strategy assumes that crops grown in rotation with lettuce will not support significant development of the pathogen. However, the fact that long rotations have not always been sufficient to prevent the occurrence of Fusarium wilt implies that the pathogen can reproduce on other crops, and previous work has shown that cotton roots are colonized under greenhouse conditions. Thus it is reasonable to suspect that including cotton in rotation with lettuce may contribute to persistence of the pathogen.

Management studies have shown promise of some biofungicides for management of this disease in potted studies (Gilardi et al., 2007). However, there is limited information on these agents and even fewer studies have been reported on that compare these agents under field conditions. Transplant dips in which Trichoderma spp. were tested against very high inoculum levels at high soil temperatures did not appear to deliver a benefit (Gordon, 2011)

Development of a management plan against this pathogen would be very desirable for lettuce growers in Fresno County and it is likely that variety selection will be part of that plan. In this study, we investigated variety response to Fusarium and TSWV.

Long-range objectives:

Generate a list of varieties with varying levels of susceptibility to *TSWV* and Fusarium wilt to enable producers to better assess risk associated with planting that variety.

Immediate objectives

- Evaluate performance of varieties seeded in mid-Aug, late-Aug and early-Sep.
- o Evaluate performance of putative control agents against Fusarium wilt of lettuce

Methods (for each objective)

Lettuce was direct seeded into a Panoche clay loam soil in a naturally *Fusarium oxysporum* f. sp. *lactucum*-infested field west of Five Points. Fertility, pest management and irrigation and all other operations were as representative of common grower practice as possible, but due to the size of the irrigated area, it was necessary to use microsprinklers rather than high pressure sprinklers to germinate the seed. This study was conducted in a field in which drip irrigation buried at a depth of 6 inches was used, which is consistent with commercial practice of some growers in the area.

<u>Varietal influence on disease severity:</u> On 17 and 30 Aug, and on 13 Sep from 22 to 25 entries were seeded and irrigated. A four-replication randomized complete block design. Seventeen ft long, single-bed plots were used for these studies.

Entries for each study are presented in the following tables. Note that entries followed by "*" are included because of either extreme susceptibility or tolerance to Fusarium in other studies; entries followed by "**" are currently used in this production area and were provided by the grower.

17 Aug planting

lce	berg	<u>Romaine</u>
Autumn Gold*	Laguna Fresca	Conquistador*
Patriot*	Lighthouse	CR#4*
Salinas*	Raider	Del Sol*
Vanguard*	Sidewinder	Sawa Up*
Vanguard 75 *	Sharpshooter	
Beacon	Sniper	
Coyote	Heat Master**	
Diamondback	Sun Devil**	
Javolina	Sun Quest**	

30 Aug planting

Icel	<u>Iceberg</u>		
Autumn Gold*	Lighthouse	Conquistador*	
Patriot*	Raider	CR#4*	
Salinas*	Sidewinder	Del Sol*	
Vanguard*	Sharpshooter	Sawa Up*	
Vanguard 75 *	Sniper		
Beacon	El Guapo		
Coyote	Crusader**		
Diamondback	Heat Master**		
Javolina	Sun Devil**		
Laguna Fresca	Sun Quest**		

13 Sep planting

lce	<u>Romaine</u>	
Autumn Gold*	Raider	Conquistador*
Patriot*	Sidewinder	CR#4*
Salinas*	Sharpshooter	Darkland**
Vanguard*	Sniper	Del Sol*
Vanguard 75 *	El Guapo	Sawa Up*
Beacon	Crusader**	
Coyote	Deuce**	
Diamondback	Maxim**	
Javolina	Heat Master**	
Laguna Fresca	Sun Devil**	
Lighthouse	Sun Quest**	

* Standards for Fusarium wilt, which were obtained from Jim McCreight.

** Used commercially and obtained from grower

Plots were evaluated for disease symptoms of TSWV and Fusarium wilt at multiple times during the season for each planting. When the majority of the varieties were at maturity, 5 plants per plot were evaluated. Maturity levels were quantified on a 1-5 rating scale as follows: 0 = no head or open; 2 = cap leaves have closed but head is puffy; 3 = fully formed and filled head that yields under pressure; 4 = solid head that does not yield under pressure; 5 = head that is splitting. Five heads per plot were cut, trimmed and weighed. These 5 heads were sliced vertically. Head length and height, and core length were measured. The trial planted on 17 Aug was harvested on 29 Oct, the trial planted on 30 Aug was harvested on 15 Nov, and the trial planted on 13 Sep was not harvested.

<u>Assessment of biological agents for control:</u> Trials were conducted to evaluate the potential of mitigating Fusarium wilt of lettuce with applications of biological control agents. Iceberg lettuce varieties used were Heatmaster for the 17 Aug planting, Lighthouse for the 30 Aug planting and Crusader for the 13 Sep planting. All trials were sprinkler irrigated for 3 to 4 weeks and then, were irrigated by drip irrigation lines buried to a 6 inch depth.

Treatments are detailed below (tables 6-8). The SoilGard product used in the first trial was from 2010, but all other products were received shortly before applications, which include SoilGard in the second and third plant dates. In all plantings, the treatments were arranged in a randomized complete block design with 4 replications. Each plot was one 40 inch bed 50 ft long with two lettuce seed lines. The first treatments were applied in a 4 to 6 inch band over the seed and the second applications were injected into the buried drip irrigation system. Materials were applied immediately after planting with a CO₂-pressurized backpack sprayer at 30 psi and 20 gallons per acre. The sprinklers were turned on as soon as possible. However, there was a 4- and 2-hour delay between the time of application of materials and the sprinkle irrigation at the third plant dates. Forty-five minutes passed between applied over 45 mins and water was run for1 hour after the injections. The plants expressing Fusarium wilt symptoms were counted several times during the season and representative samples were taken and cultured to confirm the association of the pathogen with the symptoms.

Results

<u>Varietal influence on disease severity:</u> Differences in levels of disease among varieties were documented (Tables 1-3). Although most romaine entries had lower incidence of Fusarium wilt, there were also a few iceberg entries with relatively low levels of the disease. In addition, there were also differences in levels of TSWV, but some of the romaine varieties that appeared tolerant of Fusarium had among the highest levels of TSWV.

Consideration of the very high rates of decline in some varieties due to Fusarium wilt in the first two trials must be considered in interpretation of the TSWV-susceptibility data. Due to very high mortality at early stages of development in the first planting date, the percentage of TSWV incidence in varieties such as Coyote, Heatmaster, Laguna Fresca, Lighthouse, Patriot, Vanguard and Vanguard 75 could be understated (Table 1). However, some varieties, such as Sharpshooter had little mortality due to Fusarium and had very low levels of TSWV, so there are clearly differences in susceptibility to TSWV separate from the influence of Fusarium (Table 1). Furthermore, the

influence of mortality due to Fusarium wilt on TSWV incidence is less important in the second planting date when rates of plant death were lower, and differences among varieties in TSWV levels remained apparent (Table 2). In the last planting date, there was little mortality and lower levels of TSWV, but Laguna Fresca, Vanguard and Vanguard 75 had TSWV incidence that were among the highest levels so they are susceptible to the virus (Table 3).

As the lettuce approached maturity, many entries had suffered levels of mortality such that there were few or no heads present. In the first two trials, all entries with heads that might be marketable were harvested. The lettuce in the trial planted last did not reach maturity by the time that the season was over due to cold weather, so nothing was harvested. None of the iceberg entries were commercially acceptable due to small head size and in some, long cores at the first harvest date (Tables 4-5).

<u>Assessment of biological agents for control:</u> Disease pressure was very high in the first two trials, but even under lower pressure there was not a treatment that performed better than the untreated control. In the first trial the stand was poor due to the very high temperatures.

On the first plant date, there were no commercially acceptable treatments (Table 6). Throughout the majority of the season, treatments were not different (P=0.05). On the last evaluation date of the trial, Teagro 5.2 oz in 4 to 6" band over seed at planting and through drip post-thinning had lower disease levels than the control and some of the other treatment. However, under the conditions of this study, with nearly 42% mortality, it was not an acceptable treatment. If there is consistent evidence of benefit, it may be a component of a program in combination with varieties and planting dates, however that was not seen in the second and third trials (Tables 7 and 8).

Sources:

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- Matheron, M. E., McCreight, J. D., Tickes, B. R., and Porchas, M. 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.
- Scott, J. C., Gordon, T. R., Shaw, D. V., and Koike, S. T. 2010. Effect of temperature on severity of Fusarium wilt of lettuce caused by *Fusarium oxysporum* f. sp. *lactucae*. Plant Dis. 94:13-17.

V	Fusarium wilt incidence (%)				TSWV	/ (%)	Plants per	Healthy plants
cultivar	13-Sep	24-Sep	3-Oct	26-Oct	26-Sep	29-Oct	plot	29-Oct
Autumn Gold	18.7	44.9	48.5	54.7	3.6	3.6	24.0	16.1
Vanguard 75	19.0	74.2	74.2	92.6	0.0	0.0	27.5	0.0
Conquistador	7.3	20.1	20.1	24.1	7.8	20.6	19.0	57.0
CR#4	0.0	0.0	0.0	7.9	22.0	20.5	26.0	43.4
Salinas	3.8	25.1	25.1	28.4	5.2	6.9	29.3	35.2
Sawa Up	0.0	0.0	0.0	0.0	5.4	13.4	22.5	69.7
Heat Master*	11.6	73.9	73.9	76.4	1.0	3.6	23.5	6.3
Patriot	4.3	68.6	75.8	100.0	0.0	0.0	28.5	9.7
Del Sol	3.8	9.9	9.9	21.3	7.5	19.2	20.0	40.0
Sun Devil*	2.0	31.5	33.0	49.2	1.4	9.2	27.0	18.1
Beacon	9.6	75.6	78.7	85.0	0.0	0.0	15.3	0.0
Coyote	23.4	56.9	62.0	73.1	0.9	0.0	25.5	1.8
Diamond back	1.3	37.3	39.9	43.6	3.3	3.3	24.3	32.6
Javolina	8.3	23.4	24.5	32.1	3.4	7.6	26.8	30.3
Laguna Fresca	6.7	50.2	55.3	82.3	0.0	0.0	12.0	0.0
Lighthouse	18.6	64.0	68.4	95.0	0.0	0.0	25.0	0.0
Sidewinder	4.6	37.0	39.3	49.5	0.0	0.0	19.8	23.2
Sharpshooter	3.8	11.5	15.4	15.4	0.0	0.0	21.0	72.5
Sniper	9.8	19.7	21.1	25.2	1.2	1.2	19.8	44.6
Vanguard	27.9	84.0	84.7	96.6	0.0	0.0	31.0	0.0
Raider	4.3	22.0	33.5	55.9	1.1	1.1	27.0	15.3
Sun Quest*	0.0	38.6	44.3	66.9	0.0	0.0	23.3	6.0
LSD _{0.05}	11.96	21.45	21.27	20.51	5.20	6.72	7.96	20.05
CV (%)	97.05	38.45	35.70	21.18	127.48	96.62	23.92	59.66

Table 1. Response of lettuce cultivars to Fusarium wilt and *Tomato spotted wilt virus* in lettuce planted on 17 Aug in Fresno County.

	Fusariu	ım wilt iı	ncidence	e (%)		TSWV ((%)		Plants/	Health Y
cultivar	3-Oct	20- Oct	26- Oct	6-Nov	16- Nov	26- Oct	6-Nov	16- Nov	plot	(%)
Autumn Gold	0.0	7.7	8.6	10.4	13.1	0.0	6.0	7.6	27.0	8.9
Vanguard 75	19.0	43.2	70.1	76.8	80.7	1.0	1.0	5.8	25.5	0.0
Conquistador	0.0	2.8	2.8	6.0	8.2	0.0	0.0	0.0	20.8	40.6
CR#4	0.0	0.9	1.7	1.7	5.1	0.9	4.0	6.6	28.5	54.1
Salinas	1.7	4.2	6.8	6.8	7.6	0.0	3.5	3.5	29.3	17.3
Sawa Up	0.0	0.9	0.9	2.7	5.4	1.8	1.8	3.7	27.5	51.7
Heatmaster*	0.0	4.3	2.2	12.1	9.9	0.0	4.3	6.5	25.0	27.8
Patriot	4.4	45.9	47.7	66.8	68.6	0.0	0.0	2.9	28.3	0.0
Del Sol	0.0	0.0	0.0	0.0	6.2	1.2	7.5	11.3	26.7	61.1
Sun Devil*	0.0	4.1	4.1	5.1	8.4	0.0	4.3	9.8	24.3	21.7
Beacon	7.0	48.1	56.0	61.8	71.0	0.0	3.6	5.7	23.0	0.0
Coyote	5.3	10.8	12.8	18.2	29.5	0.0	2.0	3.1	24.0	4.3
Diamondback	1.0	6.8	6.8	10.0	10.0	0.0	0.0	1.6	25.3	22.9
Javolina	0.0	3.0	3.8	4.8	6.7	1.6	2.4	3.2	28.0	38.4
Laguna Fresca	2.2	31.9	43.7	42.7	43.9	0.0	0.0	0.0	23.8	0.0
Lighthouse	7.1	50.3	53.6	74.1	77.7	0.0	0.0	3.6	21.5	0.0
Sidewinder	1.0	3.1	2.2	2.2	5.2	0.0	1.0	4.0	24.5	25.4
Sharpshooter	0.0	0.0	1.7	1.7	1.7	0.0	0.0	0.0	26.8	37.4
Sniper	0.9	0.9	0.9	4.5	5.4	0.0	0.0	0.0	27.8	41.9
Vanguard	11.4	60.9	65.4	70.5	82.9	0.0	0.0	0.8	29.5	0.0
Raider	0.9	5.5	5.5	6.4	9.1	0.0	1.8	1.8	26.8	52.4
Sun Quest*	1.1	4.4	4.2	6.4	12.7	0.0	4.1	9.3	24.0	36.1
El Guapo	0.0	6.2	7.3	11.3	13.5	0.0	2.2	2.2	27.7	42.9
Crusader	0.0	6.1	7.1	10.2	19.4	2.1	3.9	6.0	22.0	47.4
LSD _{0.05}	6.42	18.06	13.49	15.15	15.04	1.982	5.410	7.091	3.94	26.12
CV (%)	172.9	85.0	55.05	50.14	42.44	291.4	145.4	115.4	10.67	55.37

Table 2. Response of lettuce cultivars to Fusarium wilt and *Tomato spotted wilt virus* in lettuce planted on 30 Aug in Fresno County.

	Fusarium wi	lt incidence (%)	TSWV (%)	Plants per
cultivar	20-Oct	14-Nov	14-Nov	plot
Autumn Gold	5.0	4.2	0.0	30.0
Vanguard 75	9.7	32.3	3.8	26.8
Conquistador	0.0	0.0	0.0	21.5
CR#4	0.0	0.0	0.0	29.5
Salinas	0.0	1.4	0.7	31.5
Sawa Up	0.0	1.6	0.0	30.5
Deuce*	0.0	10.7	0.0	13.3
Patriot	10.7	39.5	3.7	23.3
Del Sol	0.0	0.0	0.0	26.5
Sun Devil*	1.7	0.0	0.7	31.8
Beacon	8.2	23.2	0.8	29.5
Coyote	1.8	5.9	0.8	28.3
Diamondback	1.0	0.0	0.0	28.8
Javolina	2.3	2.4	0.8	31.8
Laguna Fresca	14.3	31.4	3.9	26.0
Lighthouse	23.5	55.1	0.8	32.8
Sidewinder	0.8	0.0	0.0	34.3
Sharpshooter	0.0	0.0	0.0	31.5
Sniper	2.8	0.0	0.0	26.3
Vanguard	9.5	46.7	3.5	28.0
Raider	2.2	2.2	0.0	30.7
Sun Quest*	1.4	0.0	0.0	32.8
Crusader	8.1	6.4	0.0	31.3
Maxim	5.3	6.1	0.0	28.3
Darkland	0.0	0.0	0.0	27.0
LSD _{0.05}	8.03	12.52	2.63	5.32
CV (%)	131.00	82.42	238.12	13.26

Table 3. Response of lettuce cultivars to Fusarium wilt and Tomato spotted wiltvirus in lettuce planted on 13 Sep in Fresno County.

	Maturity	Height	Width	Core length	Weight/head	
cultivar	(0-5)	(in)	(in)	(in)	(lbs)	
Salinas	4.3	4.8	4.4	3.8	1.3	
Sun Devil*	3.1	4.4	4.0	2.1	0.9	
Diamond back	3.7	4.4	4.1	1.6	1.0	
Javolina	3.5	4.4	4.5	1.5	1.0	
Sidewinder	3.8	4.1	4.1	1.3	0.9	
Sharpshooter	4.1	5.2	4.6	3.6	1.4	
Sniper	4.2	4.8	4.3	3.7	1.1	
Sun Quest*	3.6	4.3	3.8	1.4	0.9	
LSD _{0.05}	1.08	0.82	NS	1.10	NS	
CV (%)	19.72	9.94	23.72	44.01	22.50	

Table 4. Variety harvest quality of lettuce planted on 17 Aug and harvested on 29 Oct 2012 in Fresno County*

• Three replications were harvested.

Table 5. Variety harvest quality of lettuce planted on 30 Aug and on 15 Nov 2012 in Fresno County.

	Maturity	Height	Width	Core length	Weight/head
cultivar	(0-5)	(in)	(in)	(in)	(lbs)
Salinas	3.8	5.5	5.6	2.5	1.3
Sun Devil*	2.6	4.5	4.3	0.7	0.9
Diamondback	3.6	4.7	5.0	1.1	1.2
Javolina	3.6	4.6	5.5	1.3	1.3
Sidewinder	3.6	4.7	4.7	0.8	0.9
Sharpshooter	3.1	5.0	5.3	2.8	1.0
Sniper	3.1	5.3	4.0	2.2	1.1
Raider	3.6	5.0	5.0	1.1	1.3
Sun Quest*	3.3	4.5	4.5	1.7	1.0
El Guapo	2.0	4.1	4.3	0.7	0.8
Crusader	3.1	4.7	4.6	1.0	1.1
LSD _{0.05}	1.08	0.82	NS	1.10	NS
CV (%)	19.72	9.94	23.72	44.01	22.50

• Three replications were harvested.

Table 6. Influence of drip injected materials on incidence of Fusarium wilt in lettuce planted on 17 Aug 2012, Fresno County, CA

	Fusarium affected plants (%)			
Treatment	13-Sep	24-Sep	3-Oct	26-Oct
Serenade Soil 2 qts in 4 to 6 in. band over seed ² at and through drip at thinning ^y .	5.60	33.80	38.09	56.83
Serenade Soil 2 qts in 4 to 6" band over seed at planting and Optiva 16 oz through drip post-thinning.	6.05	35.72	43.49	55.58
Teagro 5.2 oz in 4 to 6" band over seed at planting and through drip post- thinning.	7.52	25.69	32.90	41.96
Teagro 5.2 oz broadcast at planting and through drip post-thinning.	4.45	39.23	48.65	55.73
SoilGard 5.0 lb in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-thinning.	5.50	39.44	58.19	63.52
Control	8.82	41.71	46.07	63.97
LSD _{0.05}	NS	NS	NS	12.85
Ρ	0.59	0.61	0.17	0.03
CV (%)	57.06	37.72	28.18	15.6

^z All applications at planting were made immediately after seeding and a volume of 20 gal/acre was used. Sprinklers were turned on within 4 hours following the application

^y All drip injections were accomplished with electric metering pumps and materials were directly injected into each drip line over 30 min and the system was run for an additional 1 hour after the injection was complete.

Table 7. Influence of drip injected materials on incidence of Fusarium wilt in lettuce planted on 30 Aug 2012, Fresno County, CA

	Fusarium affected plants (%)				
Treatment	3-Oct	20-Oct	26-Oct	6-Nov	16-Nov
Serenade Soil 2 qts in 4 to 6 in. band over seed ^z at and through drip at thinning ^y .	5 13	26.04	38 41	55 47	60 13
Serenade Soil 2 qts in 4 to 6" band over seed at planting and through drip post-thinning.	3.18	29.22	36.86	62.43	62.86
Teagro 5.2 oz in 4 to 6" band over seed at planting and through drip post- thinning.	5.13	26.70	37.43	60.60	62.78
Teagro 5.2 oz broadcast at planting and through drip post-thinning.	4.04	26.84	36.07	58.28	58.49
SoilGard 5.0 lb in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-thinning.	3.44	20.05	41.02	53.06	58.69
CX-9032 8.0 oz in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-thinning.	4.66	20.29	34.27	56.51	62.77
CX-9032 16.0 oz in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-thinning.	3.87	20.14	31.60	51.60	55.82
Control	2.20	26.46	29.01	52.92	56.10
LSD _{0.05}	NS	NS	NS	NS	NS
Р	0.93	0.68	0.95	0.96	0.95
CV (%)	89.96	36.28	40.40	25.99	18.52

^z All applications at planting were made immediately after seeding and a volume of 20 gal/acre was used. Sprinklers were turned on within 2 hours following the application.

^y All drip injections were accomplished with electric metering pumps and materials were directly injected into each drip line over 30 min and the system was run for an additional 1 hour after the injection was complete.

Table 8. Influence of drip injected materials on incidence of Fusarium wilt in lettuce planted on 13 Sep 2012 in Fresno County, CA

	Fusarium affected			
	plants (%)			
Treatment	13-Sep	24-Sep		
Serenade Soil 2 qts in 4 to 6 in. band over seed ^z at and through drip at thinning ^y .	7.87	7.87		
Serenade Soil 2 qts in 4 to 6" band over seed at planting and through drip post-thinning.	2.68	3.51		
Teagro 5.2 oz in 4 to 6" band over seed at planting and through drip post-thinning.	4.33	4.33		
Teagro 5.2 oz broadcast at planting and through drip post-thinning.	8.39	7.93		
SoilGard 5.0 lb in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-thinning.	3.92	4.82		
CX-9032 8.0 oz in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-thinning.	6.88	8.22		
CX-9032 16.0 oz in 4 to 6" band over seed at planting and CX-9032 16.0 oz through drip post-				
thinning.	6.27	5.40		
Control	4.67	5.51		
LSD _{0.05}	NS	NS		
Ρ	0.19	24.7		
CV (V)	57.42	51 17		
(%)	57.42	51.17		

All applications at planting were made immediately after seeding and a volume of 20 gal/acre was used. Sprinklers were turned on within 45 minutes following the application

^y All drip injections were accomplished with electric metering pumps and materials were directly injected into each drip line over 30 min and the system was run for an additional 1 hour after the injection was complete.