CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

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WEED MANAGEMENT SYSTEMS FOR LEAFY GREENS

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ABSTRACT

Projects reported here were undertaken to develop new herbicide options for lettuce and spinach. We have conventionally bred lettuce germplasm, IDBR1, from the University of Idaho that is resistant to sulfonyl urea herbicides like Express (tribenuron). The herbicide resistance gene has been backcrossed into commercial lines of butterhead, greenleaf, redleaf, Romaine, and crisphead lettuce. The tolerance of these lines to tribenuron applied postemergence to lettuce at 0.075, 0.17 and 0.33 oz product /A was tested at Salinas during May to September 2012. All resistant lettuce lines were tolerant to tribenuron and herbicide susceptible lines were injured or killed by the herbicide. We are trying to create a path for development of a new spinach herbicide by screening diverse spinach lines for tolerance to Lorox. From 390 spinach germplasm lines screened in 2009 for tolerance to Lorox, 21 lines were found to be partially to very tolerant to Lorox. In 2012 the spinach lines most tolerant to Lorox were screened again with Lorox. If this increased urea herbicide tolerance can be bred into commercial spinach lines, then it may be possible to develop higher levels of herbicide tolerance in spinach and to use herbicides which would otherwise be too injurious. Tank mixes of Ro-Neet + Dual Magnum were evaluated in spinach field trials. Tests were conducted to compare Dual Magnum applied at 0.17, 0.33, 0.63 and 0.84 pt/A and Dual Magnum + Ro-Neet at 0.31+2 pt/A 21 days before spinach planting compared to the same rates of Dual Magnum applied at spinach planting. Dual Magnum will need to be applied at time of spinach planting for best weed control results.

OBJECTIVES:

A. Develop weed control programs for sulfonyl urea (SU) herbicide resistant lettuce.
B. Screen for new herbicide-tolerant spinach germplasm.
C. Evaluate tank mixes of Ro-Neet and Dual Magnum for spinach

PROCEEDURES – OBJECTIVE A

A. Develop weed control programs for herbicide resistant lettuce.

IDBR1 lettuce germplasm was evaluated for tolerance to Express 75WG (tribenuron) applied post-emergence (2-3 true leaf stage) at 0.075, 0.17 and 0.33 ounces of product per acre (oz /A). The standard backcross procedure was used to transfer the resistance gene into different types of lettuce. The IDBR1 resistance gene was backcrossed into Buttercrunch, Lolla Rossa, Parris Island, and Salinas 88 and at each generation the progeny were screened for herbicide tolerance. Resistant butterhead, greenleaf, redleaf, Romaine and crisphead lettuce in addition to susceptible parents were seeded at Spence Research Farm near Salinas on May 30, 2012 (trial 1) and July 11, 2012 (trial 2). Tribenuron was applied postemergence (POST) to trial 1 on June 20 and to trial 2 on July 27, when the lettuce had 2-3 leaves. All treatments were applied at 40 GPA with a handheld, single-nozzle boom CO_2 backpack sprayer. Both trials included a nontreated control and were conducted on Chualar sandy loam soil. The trials used a randomized complete block design, with four replicates. The standard herbicide, Kerb, was applied PRE at 2.4 lbs. product per acre. Observations made were weed density counts, crop injury ratings (0 = no injury, 10 = dead) and lettuce yield. For each trial, following harvest of the herbicide tolerant and susceptible lettuce varieties, the treatment beds were lightly cultivated/reshaped and planted (August 16 - trial 1; September 21- trial 2) with rotational crops: broccoli, tomato, snap bean, spinach, head lettuce, carrot and green onion. All data were subjected to analysis of variance, and mean separation was performed using Fisher's Protected LSD (P=0.05).

RESULTS AND DISCUSSION – OBJECTIVE A

The visual injury estimates results show good tolerance to tribenuron in herbicide tolerant lettuce varieties (Tables 1 & 2). The susceptible lettuce lines were killed or severely stunted by tribenuron. The marketable yields of the resistant lettuce lines indicated that they were tolerant to tribenuron and Kerb, while the susceptible lines were tolerant to Kerb, but not tribenuron (Tables 3 and 4). The results also show no significant reduction in biomass (yield) from any of the seven rotational crops following application of tribenuron to the primary lettuce crop (not shown).

Conclusion. The level of lettuce tolerance to tribenuron justifies commercialization of this lettuce germplasm. The next steps are to seek a sponsor for the registration of tribenuron in tolerant-lettuce and to seek a food use tolerance required for tribenuron labeling in lettuce. We are currently talking to interested parties about commercialization of this lettuce germplasm.

Table 1. Treatment effect on early-season visual injury estimates in trial 1 (6/28/12) of sulfonylurea-resistant and susceptible lettuce lines at the Spence research farm at Salinas, CA.

Treatments	Nontreated Control	Kerb	Tribenuron			Treatment *P* –values
Rate	-	2.4 lbs./A	0.075 oz/A	0.17 oz/A	0.33 oz/A	
	-------------------------Visual injury estimates (Scale 0-10)[a, b]-------------------					
Resistant lettuce						
Butterhead	0.0	0.0	0.0	0.0	0.0	1.00
Redleaf	0.0	0.0	0.5	0.0	0.3	0.58
Romaine 1	0.0	0.0	0.0	0.0	0.0	1.00
Romaine 2	0.0	0.0	0.0	0.0	0.0	1.00
Romaine 3	0.0	0.0	0.0	0.0	0.0	1.00
Greenleaf	0.0	0.0	0.0	0.0	0.0	1.00
Crisphead 1	0.0	0.0	0.5	0.0	0.0	0.44
Crisphead 2	0.0	0.0	0.5	0.0	0.0	0.44
IDBR1	0.0	0.0	0.5	1.0	0.5	0.09
Susceptible lettuce						
Butterhead	0.0 b	0.0 b	7.5 a	7.5 a	8.0 a	0.0001
Redleaf	0.0 d	0.0 d	6.5 b	5.5 c	7.0 a	0.0001
Romaine	0.0 c	0.0 c	6.0 b	6.8 ab	7.5 a	0.0001
Greenleaf	0.0 c	0.0 c	8.0 b	8.0 b	8.8 a	0.0001
Crisphead	0.0 c	0.0 c	6.3 b	7.0 b	8.8 a	0.0001

[a] Visual injury estimates were on a scale of 0-10, with 0 = no injury and 10 = dead plants.

[b] Means with the same letter within a row are not significantly different from each other according to the least significant difference test at $P \leq 0.05$.

Table 2. Treatment effect on early-season visual injury estimates in Trial 2 (8/6/12) of sulfonylurea-resistant and susceptible lettuce lines at the Spence research farm at Salinas, CA.

Treatments	Nontreated Control	Kerb	Tribenuron			Treatment *P* –values
Rate	-	2.4 lbs./A	0.075 oz/A	0.17 oz/A	0.33 oz/A	
	-------------------------Visual injury estimates (Scale 0-10)[a, b]-------------------					
Resistant lettuce						
Butterhead	0.0 b	0.0 b	0.0 b	0.3 b	1.0 a	0.0188
Redleaf	0.0	0.0	0.0	0.0	0.5	0.0625
Romaine 1	0.0	0.0	0.0	0.0	0.1	0.44
Romaine 2	0.0	0.0	0.0	0.0	0.5	0.16
Romaine 3	0.0 c	0.0 c	0.1 c	0.6 b	2.0 a	0.0001
Greenleaf	0.0 b	0.0 b	0.0 b	0.9 b	3.8 a	0.0096
Crisphead 1	0.0	0.0	0.0	0.0	0.3	0.44
Crisphead 2	0.0	0.0	0.0	0.0	0.3	0.06
IDBR1	0.0	0.0	0.3	0.3	0.8	0.17
Susceptible lettuce						
Butterhead	0.0 c	0.1 c	8.0 b	9.1 a	9.6 a	0.0001
Redleaf	0.0 c	0.0 c	7.9 b	8.5 a	9.0 a	0.0001
Romaine	0.0 d	0.0 d	8.6 c	9.4 b	9.8 a	0.0001
Greenleaf	0.0 c	0.0 c	8.0 b	9.1 a	9.3 a	0.0001
Crisphead	0.0 c	0.0 c	8.3 b	9.1 a	9.5 a	0.0001

[a] Visual injury estimates were on a scale of 0-10, with 0 = no injury and 10 = dead plants.
[b] Means with the same letter within a row are not significantly different from each other according to the least significant difference test at $P \leq 0.05$.

Table 3. Treatment effect on marketable lettuce yield of sulfonylurea-resistant and susceptible lettuce lines in trial 1 at the Spence research farm at Salinas, CA.

Treatments	Nontreated Control	Kerb	Tribenuron			Treatment *P* –values
Rate	-	2.4 lbs./A	0.075 oz/A	0.17 oz/A	0.33 oz/A	
	----------------------------------- lbs. 1000s / A [a] ---------------------------------					
Resistant lettuce						
Butterhead	21.6 bc	24.3 ab	20.9 c	26.2 a	23.7 abc	0.0269
Redleaf	14.8	15.0	13.6	15.3	14.7	0.85
Romaine 1	32.6	41.0	38.3	33.2	35.8	0.83
Romaine 2	30.8	33.3	27.2	41.5	38.5	0.14
Romaine 3	44.0	49.9	49.6	53.2	53.3	0.63
Greenleaf	16.4	16.7	16.8	14.8	17.9	0.96
Crisphead 1	49.9	47.5	45.7	38.6	45.9	0.79
Crisphead 2	18.9	25.9	28.8	24.4	29.4	0.59
IDBR1	10.1	9.2	11.0	10.5	11.0	0.39
Susceptible lettuce						
Butterhead	13.7 a	13.6 a	0.0 b	0.0 b	0.0 b	0.0001
Redleaf	10.5 a	11.4 a	0.0 b	0.0 b	0.0 b	0.0001
Romaine	35.1 a	34.5 a	0.0 b	1.0 b	0.0 b	0.0001
Greenleaf	17.4 a	20.6 a	0.0 b	0.0 b	0.0 b	0.0001
Crisphead	71.3 a	70.5 a	4.5 b	1.1 b	0.1 b	0.0001

[a] Means with the same letter within a row are not significantly different from each other according to the least significant difference test at $P \leq 0.05$.

Table 4. Treatment effect on marketable lettuce yield of sulfonylurea-resistant and susceptible lettuce lines in trial 2 at the Spence research farm at Salinas, CA.

Treatments	Nontreated Control	Kerb	Tribenuron			Treatment *P* –values
Rate	-	2.4 lbs./A	0.075 oz/A	0.17 oz/A	0.33 oz/A	
	---------------------------------- lbs. 1000s / A [a]					--------------------------------
Resistant lettuce						
Butterhead	11.1	13.7	12.3	11.9	9.3	0.07
Redleaf	7.4	7.7	8.0	6.4	6.0	0.10
Romaine 1	43.4	48.0	44.3	43.1	44.9	0.84
Romaine 2	31.0	35.8	32.9	33.6	29.8	0.45
Romaine 3	37.0	45.8	46.4	40.2	41.9	0.52
Greenleaf	15.1 a	15.0 a	14.7 a	13.6 a	8.5 b	0.0156
Crisphead 1	56.0	54.4	55.1	53.1	54.9	0.95
Crisphead 2	44.2	51.0	38.3	41.1	37.1	0.40
IDBR1	5.5	5.2	4.7	5.1	5.9	0.83
Susceptible lettuce						
Butterhead	7.5 a	9.3 a	0.2 b	0.0 b	0.0 b	0.0246
Redleaf	5.3 a	5.1 a	0.1 b	0.0 b	0.0 b	0.0001
Romaine	36.3 a	30.3 a	0.4 b	0.1 b	0.3 b	0.0001
Greenleaf	8.4 a	9.0 a	0.5 b	0.0 b	0.0 b	0.0001
Crisphead	60.6 a	57.5 a	0.4 b	0.0 b	0.0 b	0.0001

[a] Means with the same letter within a row are not significantly different from each other according to the least significant difference test at $P \leq 0.05$.

PROCEEDURES – OBJECTIVE B

B. Screen for new herbicide-tolerant spinach germplasm.

<u>Screening for herbicide tolerant spinach germplasm.</u> Our objective is to identify spinach germplasm that has a higher level of tolerance to Lorox (linuron), using conventional breeding. We screened 20 populations (progenies of resistant accessions from our screening of the USDA germplasm collection in 2009) and 16 control cultivars. After the post-planting/pre-emergence application (August 8, 2012) of linuron at 1.0 lb ai/A, the survival rate for the breeding populations ranged 0.0 – 28.2 %, average 10.0 %. Survival rate for control cultivars ranged 0.0 – 20.0 %, average 7.5 %. Two breeding populations had many plants little affected by the herbicide with survival rates of 27 and 28%, while plants of all cultivars were stunted to a certain extent compared to unsprayed controls. We selected most surviving plants from resistant populations and cultivars, and transplanted them into isolators to produce seeds for further screening and selection in 2013.

PROCEEDURES – OBJECTIVE C

C. Evaluate tank mixes of Ro-Neet and Dual Magnum for spinach

<u>Trial 1.</u> The purpose of this work was to determine if Ro-Neet (cycloate) and Dual Magnum (*S*-metolachlor) can be safely used in combination on spinach at low rates, and if weed control is improved by the combination compared to either herbicide alone. We also tested Dual Magnum applied 21 days before spinach planting. A field trial was conducted at the USDA Hartnell farm at Salinas, CA on a Chualar loam soil. The trial was arranged in a randomized complete block design,

with four replicates. The spinach variety was 'Whale'. Dual Magnum was applied 21 days before planting (DBP) and preemergence (PRE) at 0.31, 0.42 and 0.63 pt/A. Dual Magnum + Ro-Neet were applied 21 DBP on June 25, 2012 and PRE on July 17, 2012 at 0.31 + 2 pt/A and Ro-Neet was applied PRE at 2 pt/A. All treatments were spray applied using a handheld single-nozzle CO_2 backpack sprayer. A control was included in the trial. Weed density counts, spinach injury estimates and yield were measured.

Trial 2. The trial was conducted at the Hartnell Research farm as for trial 1 above. At listing 300 lbs. of 6-20-20 was applied (18 lbs. N/A). Preplant treatments were applied to shaped beds on September 20 and were watered into the soil with 0.50 inch of water on September 21. Three weeks later on October 11, the beds were lightly worked by running a non-powered bed shaper over the beds to loosen the crust and then planted with the variety 'Racoon' at 1.2 million seeds/A. The at-planting treatments were applied on October on October 12 and watered with 0.75" of water (and followed by 0.50" applied on October 15; the stand was emerging on October 16). The soil type was Chualar loam (55% sand, 29% silt and 11% clay; 2.0 organic matter and pH 7.7). Each plot was two 40-inch beds wide by 30 feet long; all treatments were replicated four times in a randomized complete block design. Applications were made with a backpack CO_2 applicator with one pass of a one-nozzle wand with an 8008EVS tip pressurized at 30 psi applying the equivalent of 34 gallons of water per acre. Phytotoxicity ratings were made on two dates. Given the relatively uniform distribution and large population of weeds, weed counts were made in a 2 ft^2 sample area per plot on October 23. Yield evaluation was made on November 8 by harvesting a three foot long strip in each plot. Time of weeding evaluations were made on November 9 by hand weeding a 10 ft^2 section of each bed; the obvious weeds that would be problematic for a mechanical harvest were removed in this evaluation, and smaller weeds that would be below the level of the cutter bar were not removed.

RESULTS AND DISCUSSION – OBJECTIVE C

Trial 1. Dual Magnum controlled purslane better PRE than 21 DBP (Table 5). Burning nettle populations were very high and not well controlled by any treatment (not shown). Dual Magnum + Ro-Neet caused some visual injury, but did not reduce spinach yield. The Dual Magnum 0.42 pt/A PRE treatment provides better purslane control and good spinach yields compared to Ro-Neet PRE at 2 pt/A and should be further evaluated. The Dual Magnum + Ro-Neet 0.31 + 2 pt/A PRE treatment provided good purslane control, but did cause visual injury.

Trial 2. The phytotoxicity ratings made in this study were primarily based on evidence of stunting and reduced vigor of the spinach. On the October 23 evaluations date, phytotoxicity ratings increased with the rate of Dual Magnum in both the 21 DBP and PRE Dual Magnum treatments (Table 6). We consider phytotoxicity ratings of 0 to 2.0 to be the range of "acceptable" crop injury; ratings above 2.0 are considered unacceptable. For both 21 DBP and PRE applications of Dual Magnum 0.31 to 0.42 pint/A had acceptable phytotoxicity ratings on October 23 and 0.31 to 0.63 pint/A had acceptable phytotoxicity ratings on October 29. The combination of Dual Magnum + Ro-Neet at 0.31 + 2.0 pint/A and Ro-Neet alone at 2.0 pint/A had unacceptably high phytotoxicity ratings on both dates. Nettle was the dominant weed at this site and all rates of all materials reduced the nettle population compared to the control. There was a trend indicating that higher rates of Dual Magnum increased control of weeds. The combination of Dual Magnum and Ro-Neet provided the best weed control. The highest yielding treatment in the trial was the control, in spite of heavy weed pressure. A general trend indicating lower yields with increased rate of Dual Magnum is evident, but the yield dropped abruptly at 0.84 pint/A (Table 6). The combination of Dual Magnum + Ro-Neet had the lowest yield (we observed depression of yield with the combination of these two materials in the 2011 trials). Ro-Neet by itself also had relatively low yield. The most interesting observation was that 21 DBP applications of Dual Magnum at 0.31 to 0.63 pint/A took longer to weed than PRE applications at the same rate. This may have been due to greater stunting of the nettle plants in the PRE application treatments. Weeds that were below the level of the cutting bar (1.5 – 2.0 inches) were not removed. This observation needs to be confirmed by further study.

Conclusion. Dual Magnum has a 50 day preharvest interval for spinach which is too long to be practical most of the year on the central coast of California. The 21 DBP treatments were an attempt to find a way to use Dual Magnum in spinach. However, the poor weed control results from 21 DBP treatments in trial 1 indicate that the early application strategy did not control weeds as well as the PRE application. In trial 2 the 21 DBP treatments had weeding times that were higher than for the PRE applications. Dual Magnum is not an easy fit for California fresh market spinach, the preharvest interval of 50 days is long and there is a tendency for this herbicide to injure spinach.

Table 5. Evaluation of Dual Magnum and Ro-Neet combinations on weed control, injury estimates (0 = safe, 10 = dead) and spinach yields at the Salinas field station.

Treatments	Rate (pt/A)	Timing	Purslane	Spinach injury	Spinach yield
			1,000/A	0 to 10	Tons/A
Dual Magnum	0.31	21 DBP	264 b	0.0 b	10.2
Dual Magnum	0.42	21 DBP	174 bc	0.0 b	9.9
Dual Magnum	0.63	21 DBP	148 bcd	0.5 b	10.8
Dual Magnum + Ro-Neet	0.31 + 2	21 DBP	183 bc	0.2 b	12.0
Dual Magnum	0.31	PRE	76 cde	0.7 b	10.5
Dual Magnum	0.42	PRE	52 de	0.2 b	11.6
Dual Magnum	0.63	PRE	35 de	1.2 ab	9.7
Dual Magnum + Ro-Neet	0.31 + 2	PRE	28 e	2.5 a	11.1
Ro-Neet	2	PRE	205 b	0.7 b	9.7
Control	0		460 a	0.0 b	9.5

Table 6. Phytotoxicity ratings (0 = safe, 10 = dead) on two dates, weed densities, spinach yield evaluation and hand weeding times.

Treatments	Rate (pt/A)	Timing	Oct 23 Phyto[1]	Oct 29 Phyto[1]	Oct 23 Nettle	Oct 23 Total weeds	Nov 8 Yield	Nov 9 Weed Time
			0 to 10		1,000/A		Tons/A	Hrs/A
Dual Magnum	0.31	21 DBP	1.0	0.3	697.0	812.4	2.428 ab	67.4
Dual Magnum	0.42	21 DBP	1.8	0.0	355.0	463.9	2.347 ab	49.7
Dual Magnum	0.63	21 DBP	2.3	0.8	409.5	463.9	2.155 ab	40.7
Dual Magnum	0.84	21 DBP	3.5	2.8	355.0	402.9	1.722 bc	13.7
Dual Magnum Ro-Neet	0.31 2.00	21 DBP PRE	4.3	2.5	130.7	169.9	1.194 c	13.3
Dual Magnum	0.31	PRE	1.3	0.5	681.7	762.3	2.372 ab	32.3
Dual Magnum	0.42	PRE	1.5	1.3	616.4	664.3	2.275 ab	16.5
Dual Magnum	0.63	PRE	2.5	1.5	442.1	463.9	2.436 ab	19.4
Dual Magnum	0.84	PRE	3.8	1.8	463.9	500.9	1.843 bc	14.5
Dual Magnum Ro-Neet	0.31 2.00	PRE	4.0	2.5	392.0	413.8	1.682 bc	10.9
Ro-Neet	2.00	PRE	2.8	2.3	496.6	594.6	1.979 abc	47.6
Control	---	---	0.0	0.0	2,058.2	2,199.8	2.764 a	296.7
		Pr>treatment	0.0002	0.0005	0.0043	0.0037	0.1030	0.0001
		LSD (0.05)	0.2	1.4	777.5	814.6	0.900	34.6