Distribution and Relative Glyphosate Resistance of Ryegrass in California

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Ryegrass (*Lolium* spp.) is a winter annual, common throughout California. In 1998, two orchard sites were identified as possibly having glyphosate resistant ryegrass populations. These populations were confirmed as being resistant to glyphosate (Simarmata et al. 2003). At least one orchard in the San Joaquin Valley has also been reported to contain glyphosate resistant ryegrass (R. Vargas, personal communication). The species of ryegrass, although reported to be rigid ryegrass (*Lolium rigidum*) (http://www.weedscience.org/in.asp), appears to be *L. multiflorum* or possibly a hybrid of *L. rigidum* and L. multiflorum.

Although it has only been confirmed in two or three orchards, many other non-confirmed reports have indicated that glyphosate resistant ryegrass may be more common in California than originally reported. If resistance to glyphosate is confirmed, alternative weed management programs would need to be developed. The objective of this study was to screen ryegrass populations collected throughout California for resistance to glyphosate and to determine the level of resistance among populations.

Materials and Methods

1. Seed Collection

Seed from mature annual ryegrass was collected in May, July, and August, 2004, from roadsides and agricultural fields and orchards throughout the Sacramento and San Joaquin Valleys (Table 1). A description of each collection site was noted, including collection date, and the GPS coordinates. At each site, seed from at least 15 plants was collected and combined into a single sample. Attempts were made to collect at least 1000 mature seed for each sample site. A total of 60 sites ryegrass sites were sampled in 2004. Seed was cleaned and dry seed stored at room temperature (70°F) until use. At least four months of dry storage was required to overcome dormancy and begin the experiments.

2. Preliminary sensitivity experiment

Seed from a known susceptible ryegrass population were planted into pots and grown in the greenhouse. Pots, 4-inch diameter X 4-inch deep, were filled with UC modified soil mix http://greenhouse.ucdavis.edu/materials/nutrients_soil.htm), and at least 10 seed planted 0.25 to 0.5 inches deep. Pots were placed in a temperature controlled greenhouse, 75°F day and 55°F night, with supplemental lighting (12 hr day length). Plants were thinned to 2 to 3 plants per pot at one week after emergence and allowed to grow until 4-6 inches in height. Once plants reached the desired height, they were treated with 8 glyphosate rates: 0, 0.031, 0.062, 0.125, 0.25, 0.50, 1.0, and 2.0 lbs acid equivalent (ae)/ac. Four replicate pots per treatment were used. Glyphosate applications were made using a track sprayer, with a final spray volume of 16 gal/acre. Distilled water was used for all applications, in order to avoid confounding effects of hard water. Twenty one to 25 days after treatment, plants were harvested and fresh weight determined. Percent control was calculated as the fresh weight of the treated plant divided by the fresh weight of the untreated plant. The rate that resulted in a 50% reduction in fresh weight (I_{50}) was calculated.

3. Screening experiment

Seed from each individual ryegrass population was planted into 12-inch X 18-inch flats to provide at least 20 plants in each flat. Application of glyphosate was made to each flat at 0.062 lbs ae/ac, the I_{50} rate determined in the preliminary sensitivity experiment. Plants were grown in the same greenhouse, under similar conditions as used in the preliminary sensitivity experiment. Ryegrass was cut at ground level and shoot fresh weight was measured for each individual flat at 21 days after treatment. Samples were then placed in a forced air dryer (120°F) and dried, and weighed. The distribution of responses within and between populations was compared.

4. Rate response experiment

In addition to comparing all populations at a low application rate, we also wanted to determine the rate of glyphosate necessary to kill all ryegrass from a population. Following cutting for biomass measurement, ryegrass was able to produce new shoot growth. Once plants reached 6 inches in height, they were once again treated with glyphosate, but at double the previous rate – 0.125 lbs ae/ac. At 21 days after treatment, plants were again cut at ground level for biomass measurement as done in the screening experiment. This was repeated with glyphosate rate doubling [0.25, 0.75 (triple rate), 1.5, 3.1, 6.1, and 12.2 lbs ae/ac] at each successive treatment, until all plants in a flat were killed.

Results and Discussion

Seed were collected by driving until a mature ryegrass was seen, stopping and collecting a sample. Thus, many samples were collected on the roadside, but in all cases adjacent to agricultural fields. Ryegrass was a common along roadsides and in fields in the Sacramento Valley. While driving in the San Joaquin Valley, ryegrass was less common on roadsides and fields, particularly further south in the Valley.

Treating the 60 ryegrass populations with glyphosate at 0.062 lbs/ac resulted in minimal growth reductions in all the populations (data not shown). The susceptible population was a population collected about 20 years ago from a location with no known glyphosate use. Thus, all the ryegrass populations appeared resistant relative to the susceptible ryegrass.

Of the 60 ryegrass populations evaluated, only five populations were killed by the recommended glyphosate rate of 0.75 lb/a (Table 1). At double the recommended rate of glyphosate (1.5 lbs/ac), less than 15% of the sampled ryegrass populations were killed. The observation that many populations are resistant to glyphosate is not surprising, since seed samples were collected from areas where it is likely they would have been treated with glyphosate, in many cases repeatedly, and thus only resistant plants would remain. Ten populations have not been completely killed by over 12 lbs of glyphosate per acre, and a few populations show less than 50% control from the 12.2 lb/ac rate, indicating a very high level of resistance.

The ryegrass populations in the northern Counties, near the locations of the original observations of resistance, required a high rate of glyphosate for control. It is likely that most populations in California contain some glyphosate resistant individuals and that once susceptible individuals are removed by treatment with glyphosate, resistant individuals breed with other resistant individuals, creating a highly resistant population.

The abundance of glyphosate resistant ryegrass along roadsides has likely allowed the rapid spread throughout the state, as mud picked up in tires could carry the seed. The high level

of glyphosate resistance in roadside ryegrass may be a reflection of repeated use of glyphosate by road crews, particularly in programs where residual herbicides are not used.

The data on glyphosate resistant ryegrass distribution and rate sensitivity will serve as a baseline, allowing other populations to be tested and compared in the future. Now that glyphosate resistant ryegrass has been confirmed in many areas of California, alternative management options will need to be implemented.

Table 1. Site description and coordinates for annual ryegrass seed collected in 2004 (60 sites), going from south to north, and the rate of glyphosate[1] (lbs/acre) needed to kill ryegrass.

Site description	County	Date collected	North Coordinate	West Coordinate	Glyphosate rate (lbs ae/acre) required for 100% ryegrass control
Roadside	Fresno	Aug-2004	36°35.277	119°29.693	1.5
Roadside - Aromas	Monterey	Aug-2004	36°52.669	121°38.234	3.1
Almonds Ave 18 1/2	Madera	1-Jul-04	37°01.106	120°16.165	6.1
Cotton Gin yard	Madera	9-Jul-04	37°02.531	120°35.504	12.2 +
Roadside Hwy 165	Merced	9-Jul-04	37°08.804	120°49.451	3.1
Roadside Hwy 59	Merced	9-Jul-04	37°14.296	120°29.275	3.1
Almond orchard	Merced	1-Jul-04	37°14.443	120°22.747	6.1
Almond orchard	Merced	1-Jul-04	37°15.503	120°22.787	3.1
Almond orchard	Merced	9-Jul-04	37°22.9726	120°53.176	3.1
Roadside	Stanislaus	9-Jul-04	37°25.193	120°59.100	0.75
Almond orchard	Stanislaus	1-Jul-04	37°32.198	121°14.313	3.1
Roadside near Keyes	Stanislaus	9-Jul-04	37°33.151	120°55.774	0.75
Next to RR tracks	San Joaquin	9-Jul-04	37°51.601	121°13.184	3.1
Abandoned orchard	San Joaquin	8-Jul-04	37°54.803	121°40.686	0.75
Tomato field	San Joaquin	8-Jul-04	37°55.743	121°23.844	1.5
Vacant lot near Rd 8 in Stockton	San Joaquin	9-Jul-04	38°04.102	121°14.715	3.1
Edge of corn field	San Joaquin	8-Jul-04	38°04.177	121°44.163	3.1
Vineyard near Stockton	San Joaquin	8-Jul-04	38°06.439	121°23.449	0.75
Roadside near Pear orchard	Solano	8-Jul-04	38°09.246	121°40.730	1.5
Hwy 113 & Jepson Prairie	Solano	7-Jul-04	38°16.991	121°49.431	3.1
Roadside	Solano	7-Jul-04	38°20.194	121°48.291	0.75
Hwy 99 and Grant Line rd	Sacramento	9-Jul-04	38°22.657	121°21.860	3.1
Roadside Pedrick rd.	Solano	11-Jul-04	38°24.545	121°48.276	3.1
Roadside near Pedrick road	Solano	8-Jul-04	38°28.904	121°48.326	3.1
Roadside Rd 98	Yolo	12-Jul-04	38°32.565	121°48.188	12.2+
Roadside north or Winters	Yolo	12-Jul-04	38°32.836	121°58.265	6.1
Roadside near Winters	Yolo	12-Jul-04	38°32.894	121°53.074	12.2+
Roadside	Yolo	2-Jul-04	38°33.722	121°57.023	6.1
Roadside near Turkovich Farm	Yolo	2-Jul-04	38°34.834	121°56.045	12.2+
Roadside	Yolo	2-Jul-04	38°36.518	121°58.275	3.1

Site description	County	Date collected	North Coordinate	West Coordinate	Glyphosate rate (lbs ae/acre) required for 100% ryegrass control
Roadside	Yolo	2-Jul-04	38°37.188	121°46.159	3.1
Roadside	Yolo	2-Jul-04	38°37.214	121°57.012	3.1
Roadside	Yolo	12-Jul-04	38°38.106	122°00.488	6.1
Roadside Capay Valley	Yolo	12-Jul-04	38°42.346	122°03.791	3.1
Almond orchard County Line rd	Yolo	12-Jul-04	38°55.550	122°00.706	12.2
Almond orchard	Colusa	14May04	38°55.934	122°03.641	3.1
Roadside	Colusa	12-Jul-04	38°57.095	122°22.874	3.1
Almond orchard	Colusa	14May04	38°57.296	122°03.643	3.1
Roadside	Colusa	12-Jul-04	39°04.969	122°04.737	12.2+
Roadside south of Willows	Colusa	12-Jul-04	39°06.356	122°09.111	3.1
Walnut orchard	Sutter	11-Jul-04	39°06.598	121°40.296	3.1
Roadside near Willows	Colusa	12-Jul-04	39°07.189	122°13.445	6.1
Roadside	Colusa	Aug.2004	39°11.944	122°00.487	6.1
Roadside	Colusa	Aug.2004	39°12.616	122°02.278	6.1
Abandoned orchard	Sutter	11-Jul-04	39°15.011	121°40.326	3.1
Roadside	Colusa	Aug.2004	39°15.32590	122°03.34777	12.2+
Roadside with rice all around	Butte	11-Jul-04	39°29.584	121°50.547	3.1
Roadside	Butte	20May04	39°34.844	121°56.044	3.1
Prune orchard	Butte	11-Jul-04	39°35.994	121°51.436	6.1
Almond orchard	Butte	11-Jul-04	39°36.804	121°51.463	3.1
Corn field	Glenn	Aug.2004	39°39.209	122°00.059	6.1
Roadside next to almond orchard	Glenn	11-Jul-04	39°41.081	121°52.745	12.2+
Young almond orchard	Glenn	11-Jul-04	39°42.541	122°04.460	6.1
Walnut orchard	Glenn	11-Jul-04	39°44.092	121°57.741	12.2+
Prune orchard	Glenn	11-Jul-04	39°47.382	122°04.009	12.2+
Roadside	Glenn	11-Jul-04	39°48.253	122°04.001	3.1
Prune orchard	Tehama	11-Jul-04	39°49.545	122°07.375	12.2+
Prune orchard near Corning	Glenn	11-Jul-04	39°49.555	122°08.028	6.1
Roadside	Glenn	11-Jul-04	39°51.435	122°09.185	3.1
Olive orchard near Corning	Tehama	11-Jul-04	39°54.850	122°07.530	3.1

[1] The glyphosate rate listed on the Roundup WeatherMax label for control of ryegrass is 0.75 lb ae/acre.

Literature Cited

Simarmata, M., J.E. Kaufmann, D. Penner. 2003. Potential basis of glyphosate resistance in California rigid ryegrass (*Lolium rigidum*). Weed Sci. 51: 678-682.