Industry-driven field and landscape research on transgene dispersal in genetically engineered alfalfa

Sandya Kesoju, Washington State University
Stephanie L Greene, USDA ARS
Ruth Martin, USDA ARS

USDA Agricultural Research Service

Washington State University
World Class. Face to Face.

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

Time Line of Genetically Engineered (GE) alfalfa

June
GR alfalfa deregulated

March
Injunction preventing further planting. Seed fields removed but hay remained in production

October
GE low-lignin alfalfa deregulated

2005 **2006** **2007** **2008** **2009** **2010** **2011** **2012** **2013** **2014**

GR alfalfa planted on 80,000 ha, approximately 5% of total acreage planted that year

February
Second deregulation

Coexistence is important in alfalfa since it has GE sensitive markets

Export market for conventional seed and hay

- Up to 95 percent of the 3.85 million tons (2013) of hay is from California, Washington, and Oregon

- US largest producer/exporter of alfalfa seed ($84.2 million 2009-2013)

- Organic hay ~ 1% (2005) and 2% (2011) of U.S. harvested alfalfa acres

Project Objectives

1. Has transgene dispersed? Determine Glyphosate Resistant (GR) transgene presence in roadside(feral) alfalfa

2. Determine extent of GR transgene movement **from** GR seed fields, GR feral plants and GR hay fields **to** conventional seed fields to better understand how transgene flow impacts the occurrence of adventitious presence (AP) of the transgene in conventional seed lots

Objective 1. *Determine Glyphosate Resistant (GR) transgene presence in roadside(feral) alfalfa*

Method

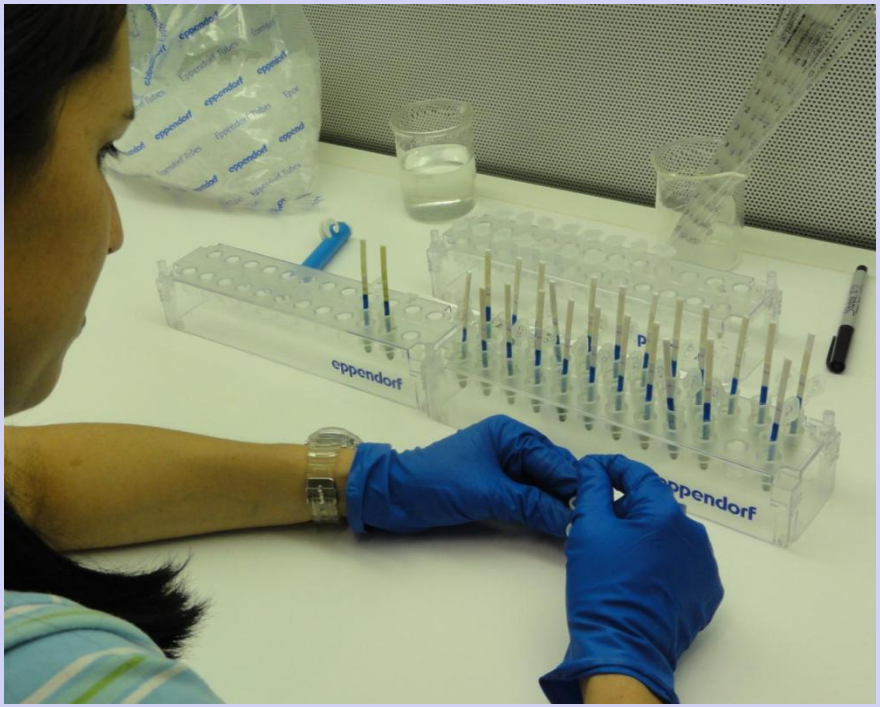
- Surveyed for feral plants within 600 km^2 area in Fresno Co, CA, Canyon Co., Idaho, Walla Walla Co., WA- major seed production areas

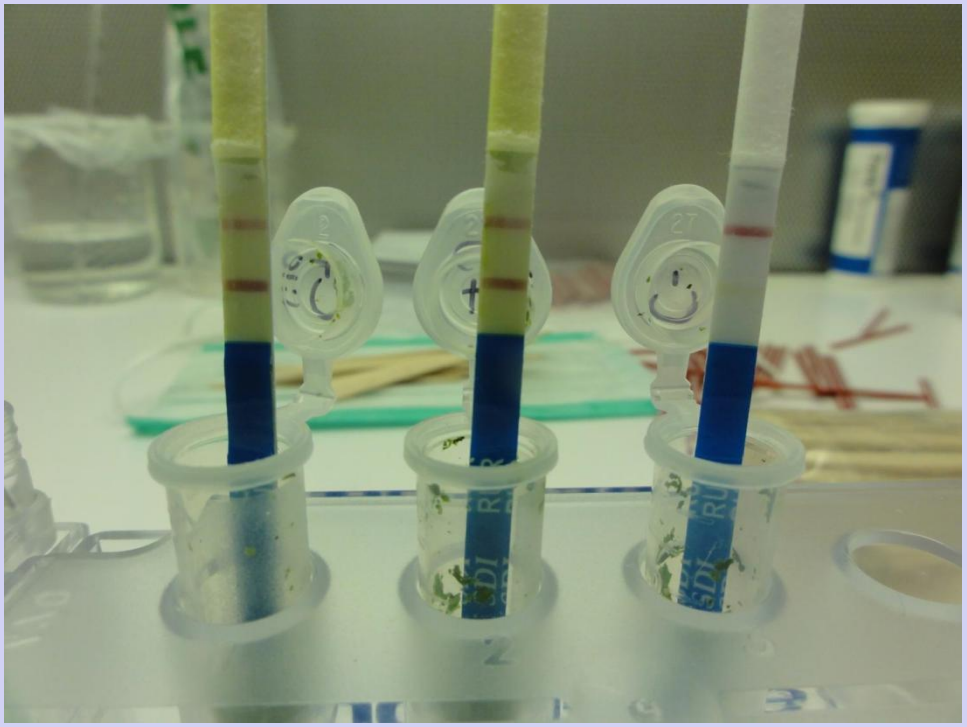
- 600-800 random survey sites were identified along rural roads in each of three counties. Due to low frequency of feral populations, we also sampled feral plants encountered while driving between random sites.

- Data were collected on both sides of the road.

Method

- Individual plant leaves were sampled, and seeds were sampled if present.
- In populations with less then 10 plants, individual plants were sampled separately; > 10 plants, population was sampled by bulking 10 plants.
- Leaf and seed were tested with AgraStrip test strips.
- Positive samples were confirmed with PCR using event-specific primers.





Method

Site specific	
Cropping pattern	Crop adjacent, behind, ahead (Wild/ruderal, Orchard, Forage, Row Crop, Other)
Roadside habitat	Weed management (burned/grades/mowed, sprayed, tilled), species diversity (high, low, medium), vegetation cover (bare, continuous, patchy), vegetation height (short, medium, high), digital image
Topographical	
Elevation, slope, aspect	USGS National Elevation Dataset at a 30 x 30 m spatial resolution.
Climatic	2005-2012 PRISM dataset
Ave seasonal precipitation	mm
Seasonal min, max temp	° C
Proximity	NASS Cropland data layer
Proximity to alfalfa production area	1= < 3000 m to alfalfa; 2= >3000 m, but within production area; 3= bordering production area (5000 m) ;4=outside of production area > 5000 m)
Seed Spillage	
Potential for seed spillage during production and transport	1= High likelihood (adjacent to historic seed field or along route to seed conditioning plant); 2= Medium (i.e. secondary road in seed production area feeding into main road to plant), 3= Low (tertiary road in seed production area, mainly local traffic) , 4= Very Low; outside of production area or gravel road, only local traffic

Method

Historic Seed Fields	
Distance to closest historic seed field	geographic coordinates provided by industry of historic seed fields in study areas. Fresno (1); Canyon (51); Walla Walla (14)
Historic Hay Fields	
Distance to closest historic hay field	Industry provided distance classes for 192 points where we observed either GR feral or feral plants. Distance classes: < 1 mile, 1-5 miles; 5-10 miles, >10 miles. We then classified our remaining feral locations according to where they fell into buffer zones surrounding 192 points, starting at <1 mile and working outward. 90% of data fell into the 1-5 mile class
Hay and Seed Producer Survey	
55 question survey was sent to 530 alfalfa hay and seed producers in three counties (growing > 25 acres)	Survey questions focused on production practices and perceptions of avenues of transgene dispersal. Response rate was 32%

Method

Data Analysis

Generalized linear models were used

1. To determine spatial relationship between historic GR fields and location of GR feral plants

2. Identify variables that explain the occurrence of feral plants, in general

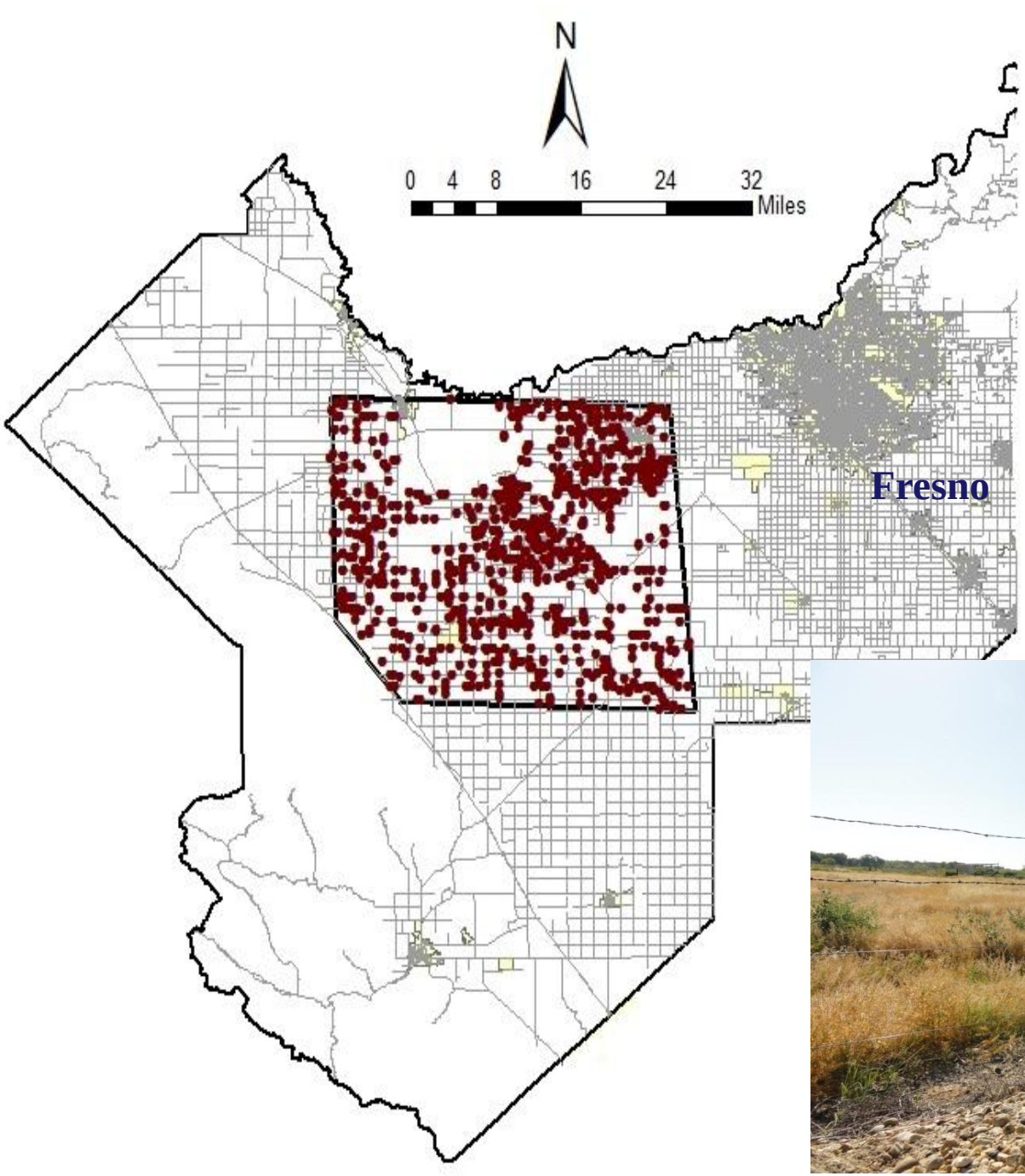
3. Identify variables that explain the occurrence of GR feral plants

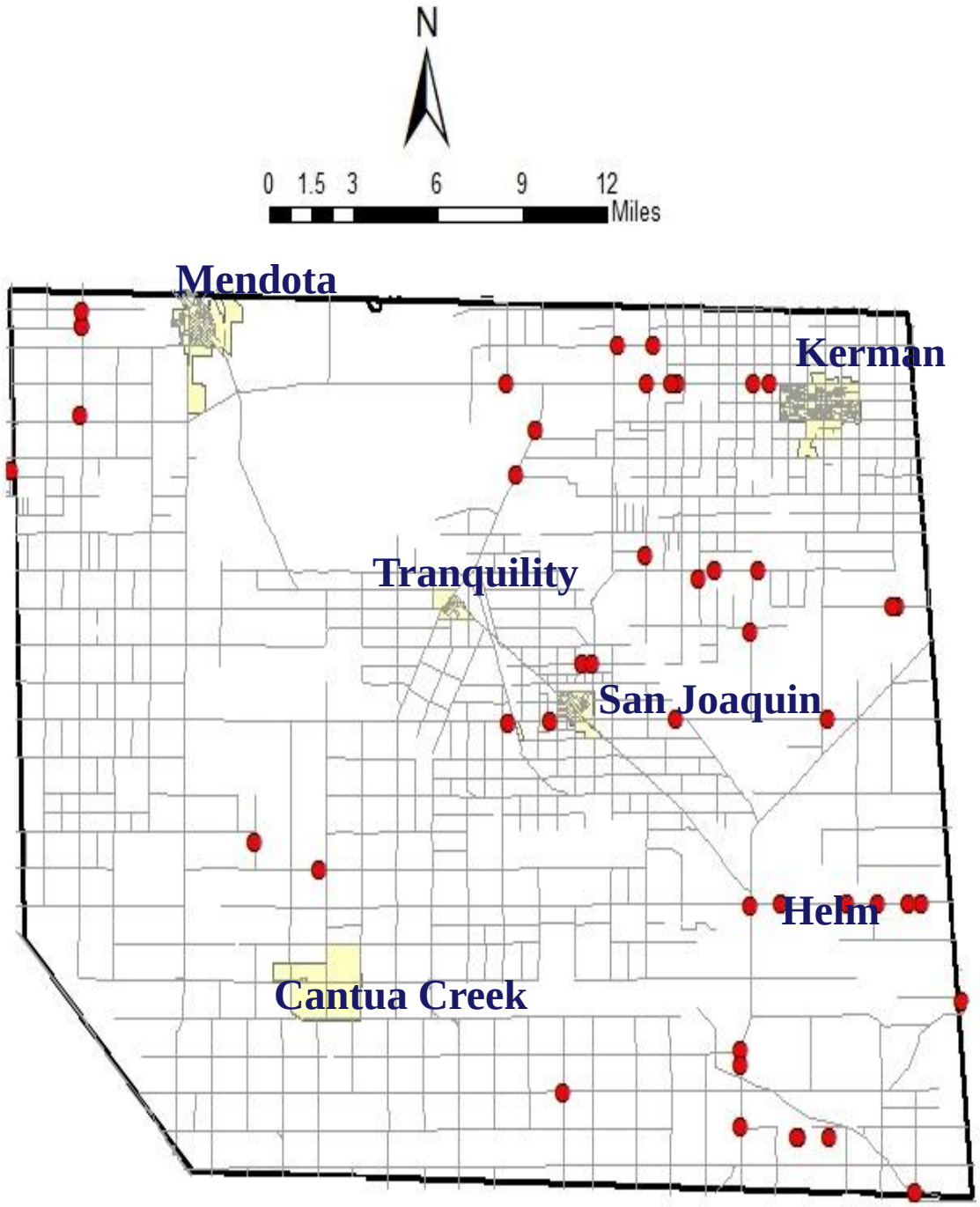
Spatial autocorrelation by fitting variogram was used

1. To determine spatial relationship between feral plants and neighboring feral plants

Fresno Co, CA

1668 roadside locations were surveyed in August 2011, May 2012



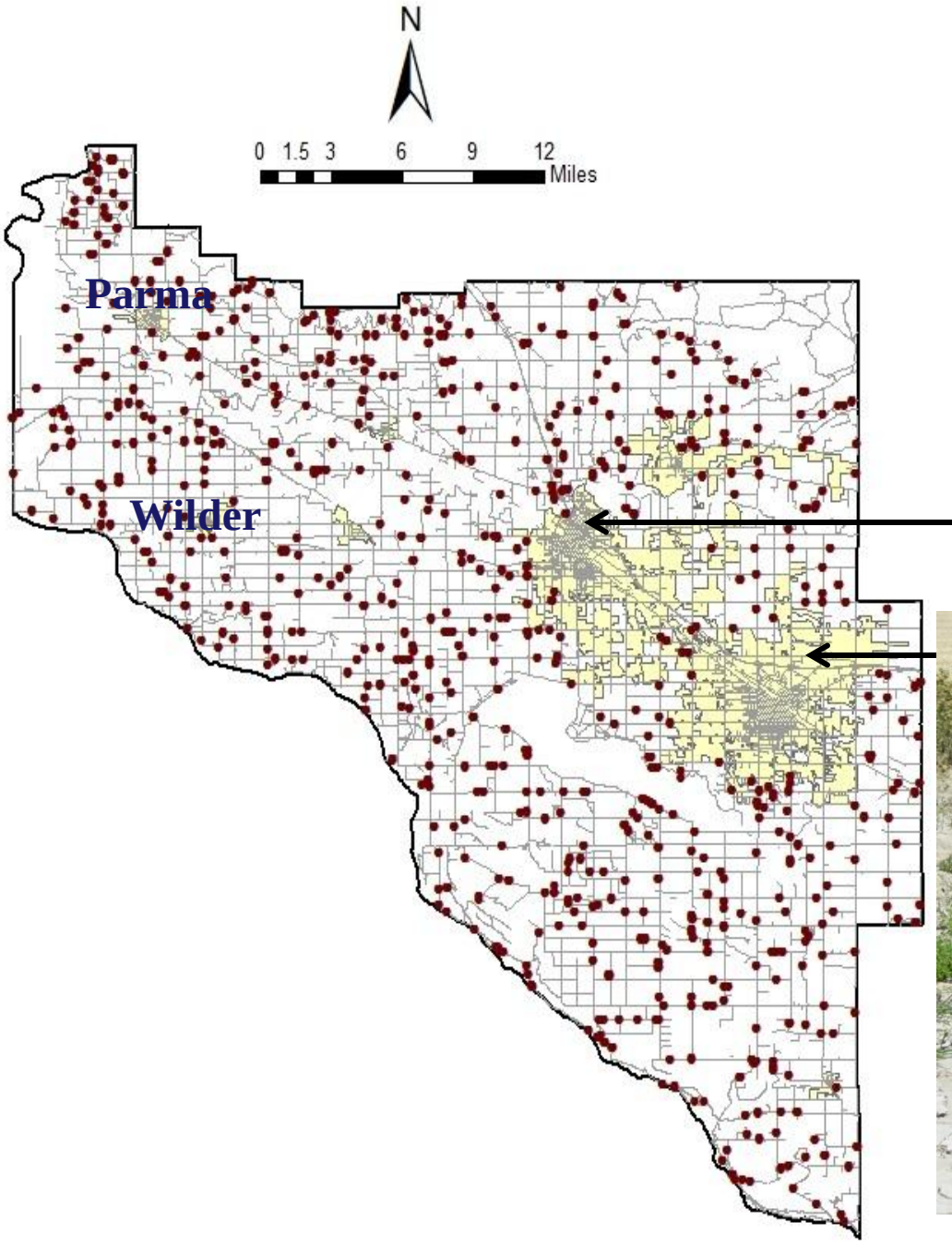


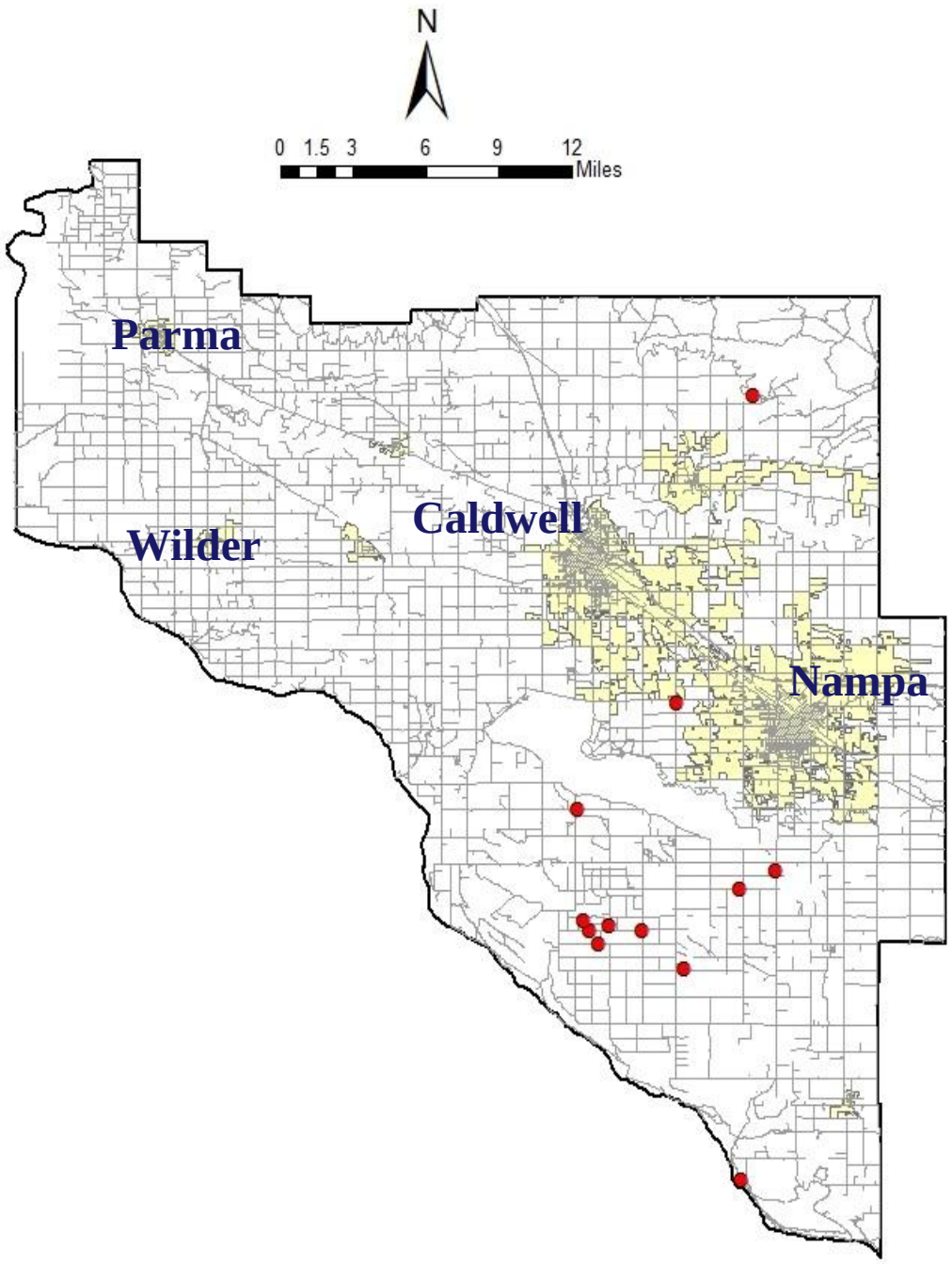
Fresno Co results

- 171 locations had feral plants
- 73 of these locations had GR feral plants

Canyon Co, ID

1398 locations were surveyed in July 2011



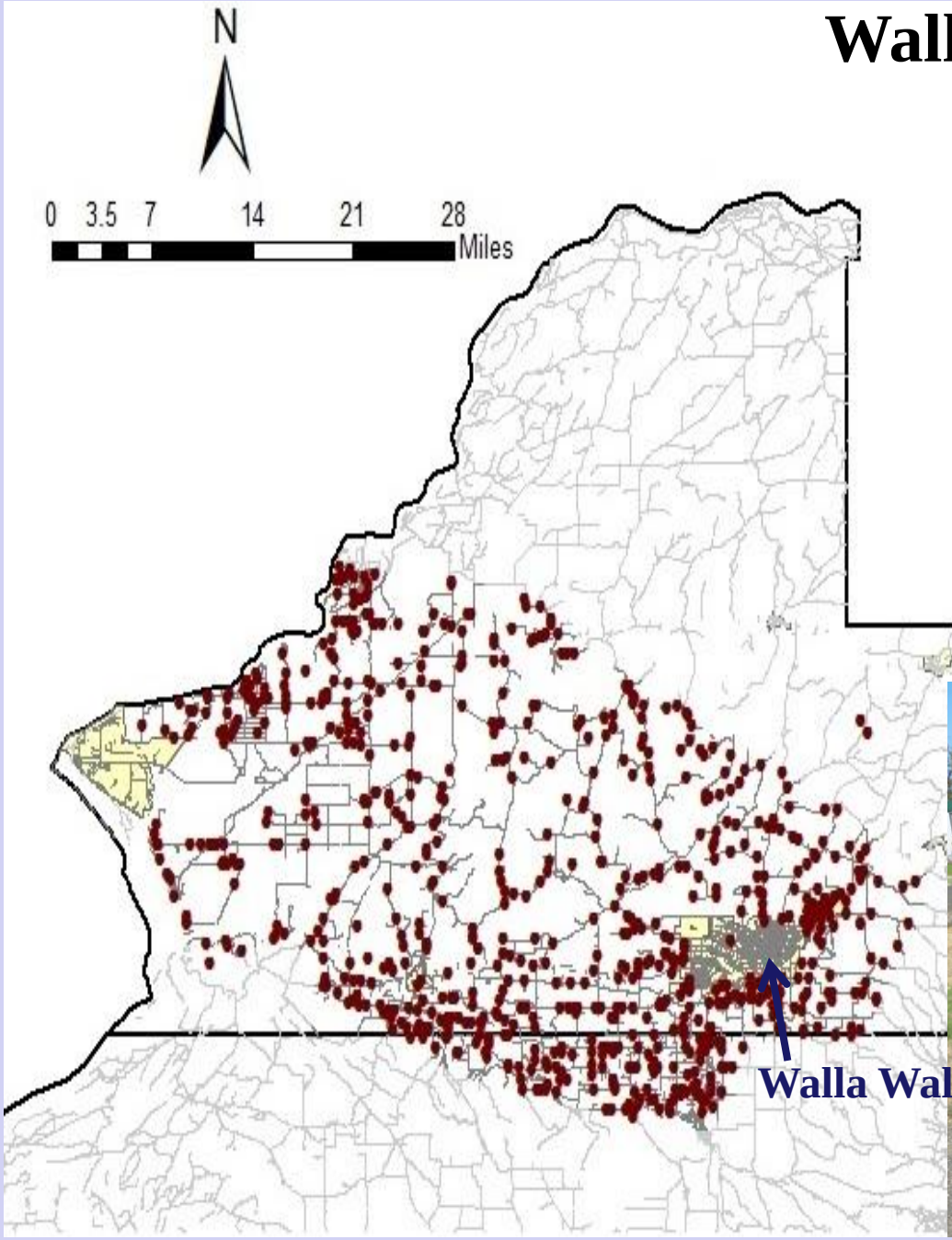


Canyon Co results

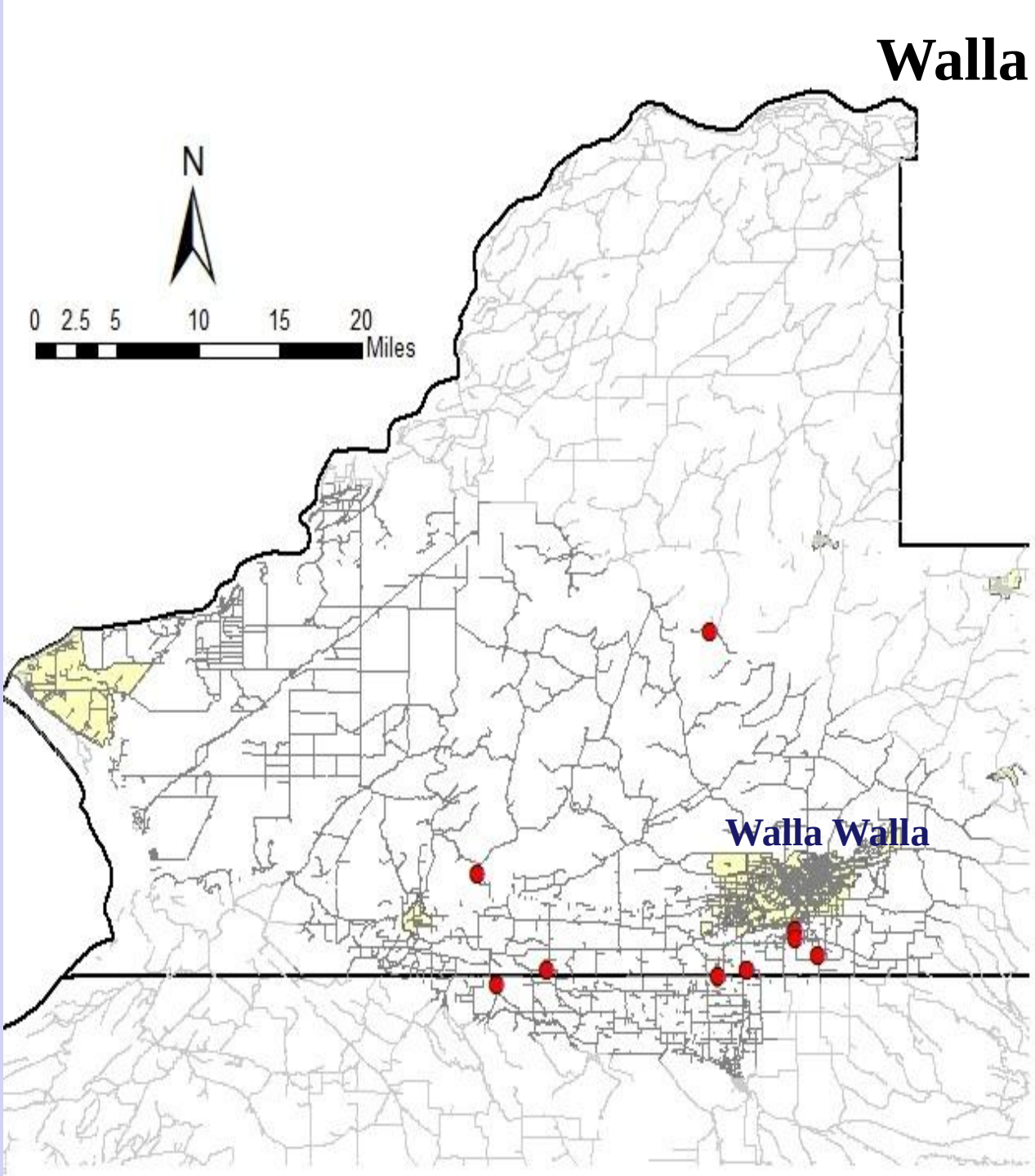
- 97 locations had feral plants
- 24 of these locations had GR feral plants

Walla Walla County, WA

1566 locations were surveyed, August 2011



Walla Walla Co results

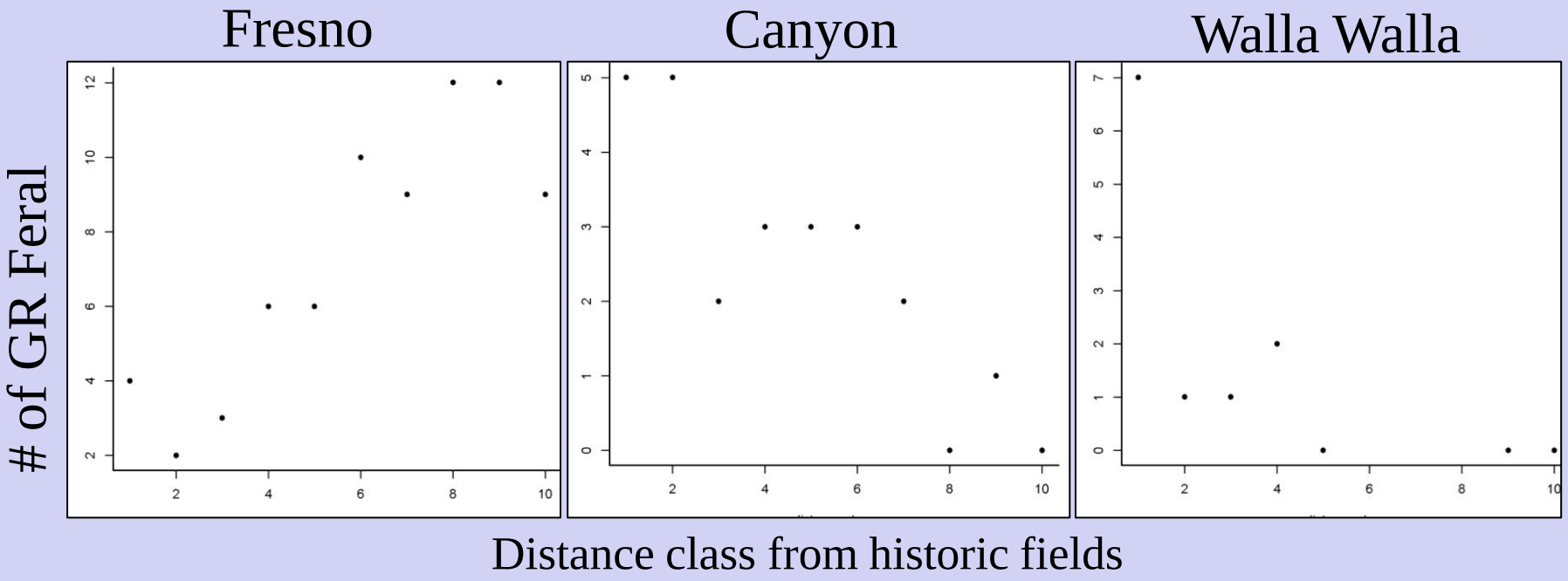


142 locations had feral plants

12 of these locations had GR feral plants

Results

To determine spatial relationship between historic GR fields and location of GR feral plants



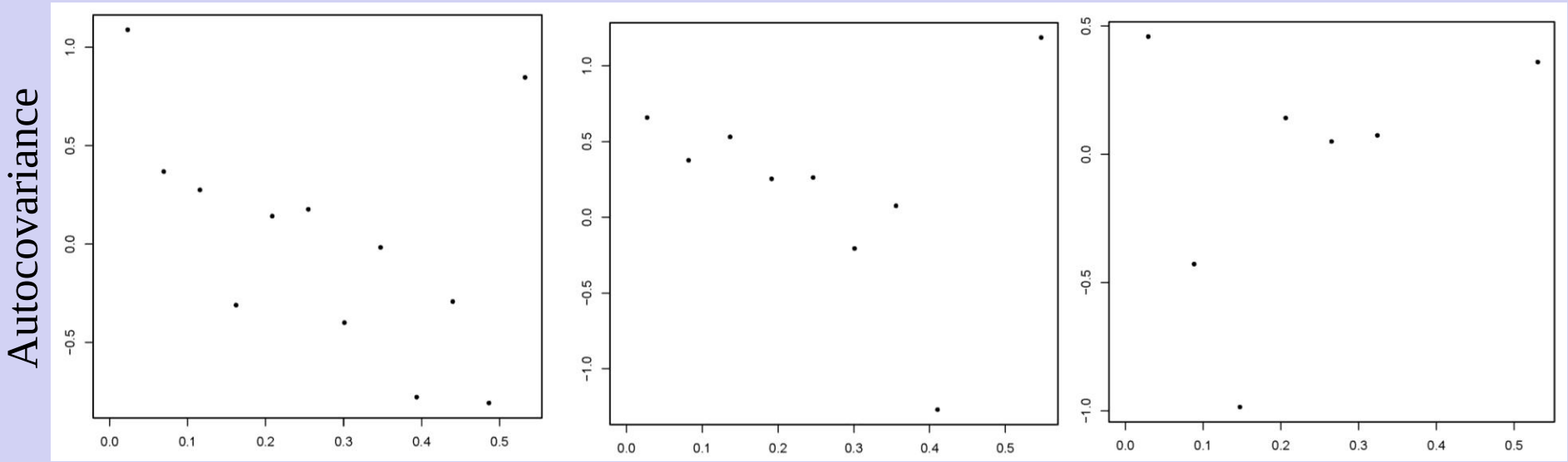
- Significant relationship was found between GR feral plants and proximity to historic GR seed fields but were not consistent across the counties.

- Using available data on historic hay field locations, we were unable to make a determination, however we found no relationship based on four known GR hay field locations in Fresno.

Results

To determine spatial relationship between feral plants and neighboring feral plants?

Fresno Canyon Walla Walla



- Fairly low spatial autocorrelation was observed suggesting that neighboring feral plant colonies influence each other. Influence between plant colonies was less than 200 m, and varied by location (Fresno -190 m, Canyon- 70 m, Walla Walla- 82 m).

Results

Identify variables that explain the occurrence of feral plants in general

County	Total Random Survey sites	Presence sites
Canyon	1350	72
Fresno	1416	57
WW	1424	60

Although we evaluated presence/absence of feral plants at a total of 4190 random locations, only 4.5% of locations had plants. When we examined data from each county, no variable we examined was strong enough to explain the occurrence of feral plants since we had few locations with feral plants to analyze.

Results

Identify variables that explain the occurrence of GR feral plants

Based on the analysis Spillage was found to be a significant and predictive variable that explained the occurrence of GR feral plants in Fresno and Canyon Co., but not Walla Walla. This suggested that GR feral plants tended to occur more often in locations where the likelihood of seed spillage was highest- either directly adjacent to historic seed fields or along the routes taken from seed fields to the conditioning plant.

Summary

County	Locations surveyed	Feral plants present	Feral plants with GR
Fresno	1668	171	73
Canyon	1398	97	24
Walla Walla	1566	142	12

GR feral plants were detected in all three counties, four years after the 2007 injunction, suggesting transgene can persist in the environment

Feral plants occurred rarely. Frequency of feral plants in general and GR feral plants differed in each county.

Fresno had the highest occurrence, and had three times as many GR feral plants as the next highest county, despite having only one historic GR seed field. Interestingly, this is the only county where glyphosate is used exclusively in roadside sprays.

Summary

GR feral plants were not associated with proximity to historic GR hay fields. Based on available hay data, we were unable to test the association with GR hay fields.

The occurrence of GR feral plants was associated with locations that had a high likelihood of seed spillage during production and transport of historic seed in Fresno and Canyon Co. However, the variable did not explain the occurrence of GR feral plants in Walla Walla.

Discussion and Conclusion- Objective 1

- In a survey of 530 hay and seed growers, out of 237 replies, only a small portion indicated they felt seed escape was highly likely or likely, and only about half of respondents indicated they controlled feral plants, and control was limited to their land.

- Our results show that GR feral plants were more likely to be found in proximity to historic GR seed fields and roads leading to conditioning plants.

Objective 2

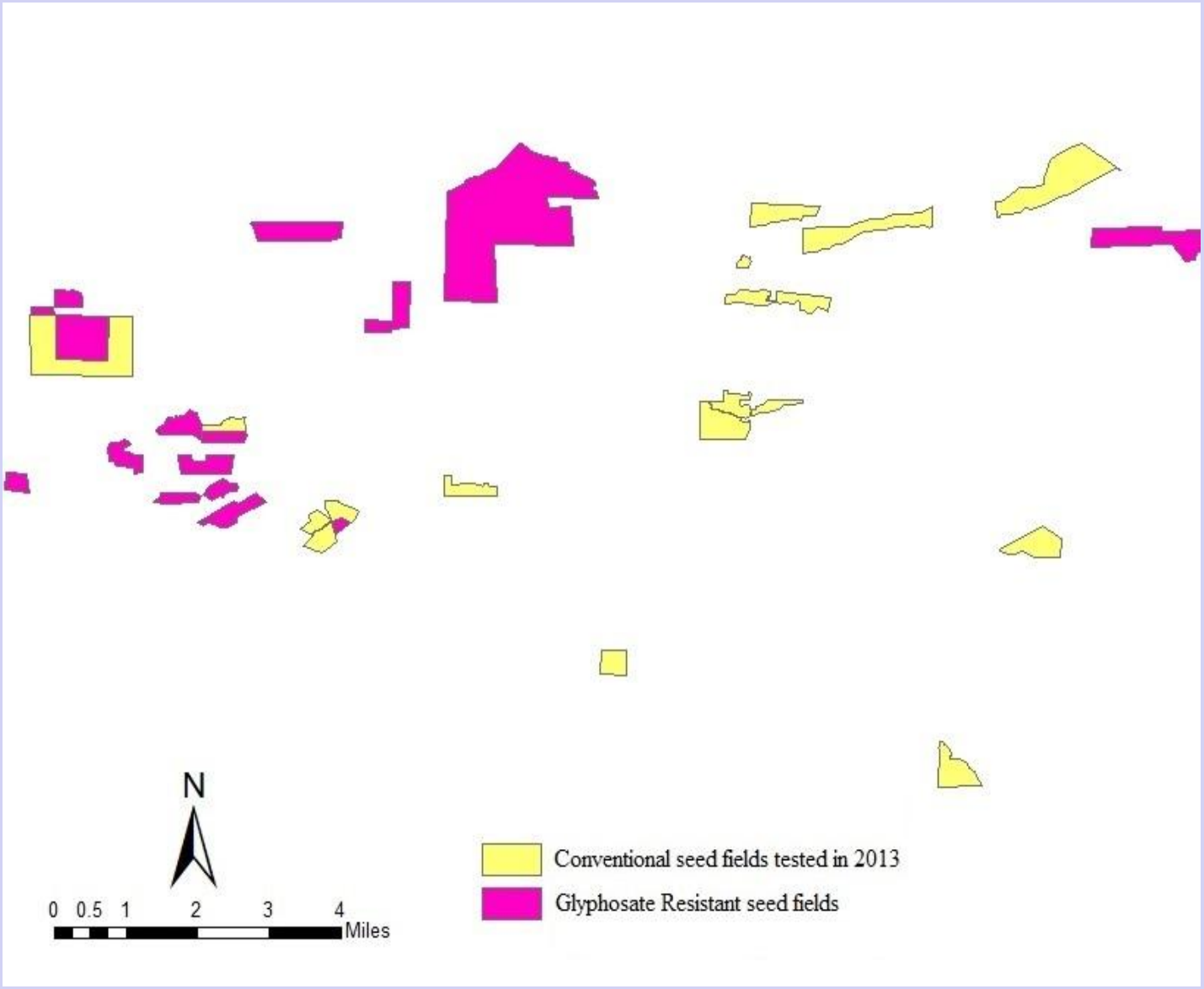
*Determine extent of GR transgene movement **from** GR seed fields, GR feral plants and GR hay fields **to** conventional seed fields to better understand how transgene flow impacts the occurrence of adventitious presence of the transgene in conventional seed lots*

Location	# of conventional commercial seed fields sampled	# of seed samples
Fresno Co., CA	16	249
Canyon Co., ID	10	157
Walla Walla Co., WA†	17	968

† Key fields were resampled in 2014

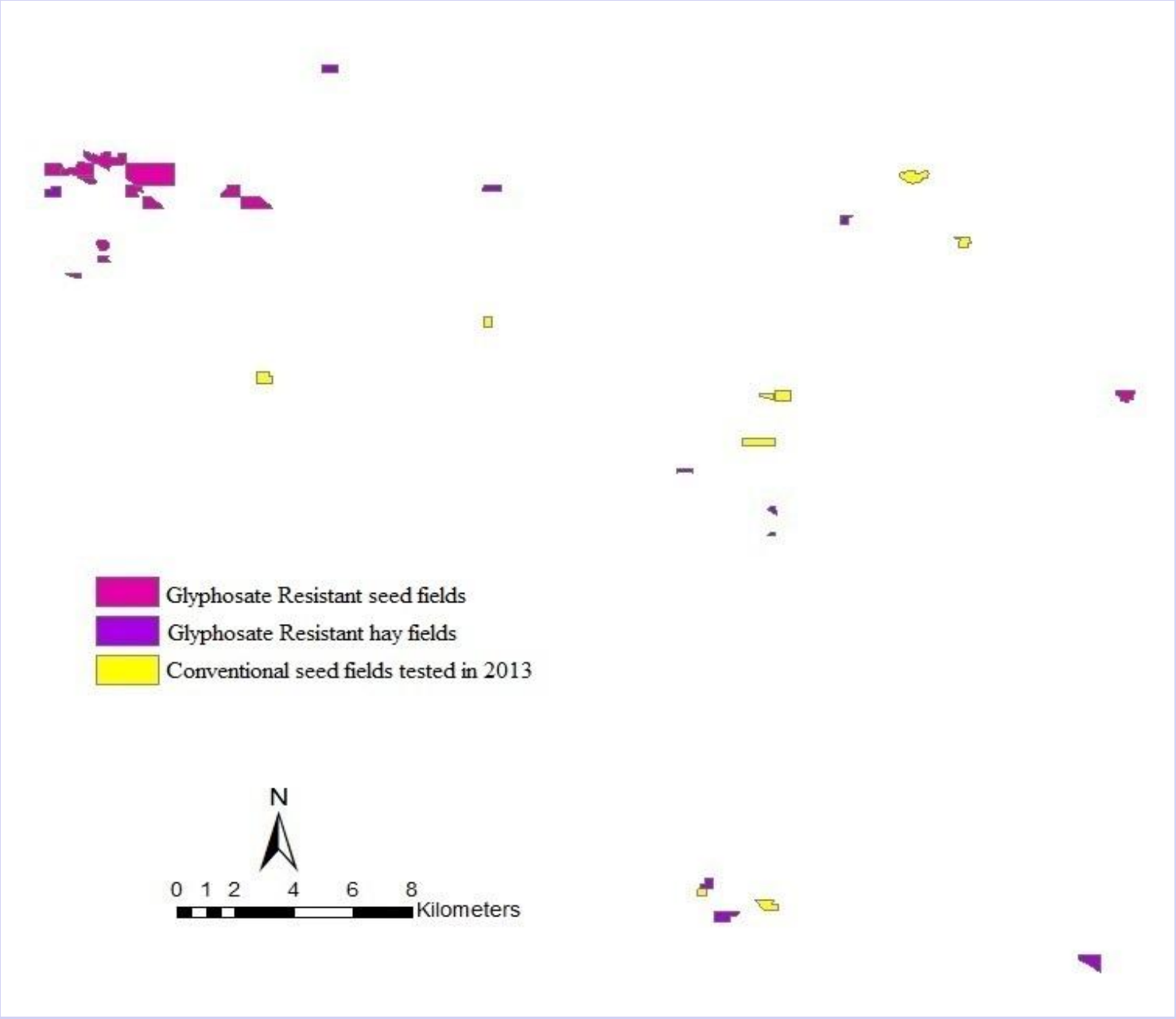
Walla Walla

Map shows the location of all commercial GR seed fields, but only shows conventional seed fields that we sampled for transgene presence.



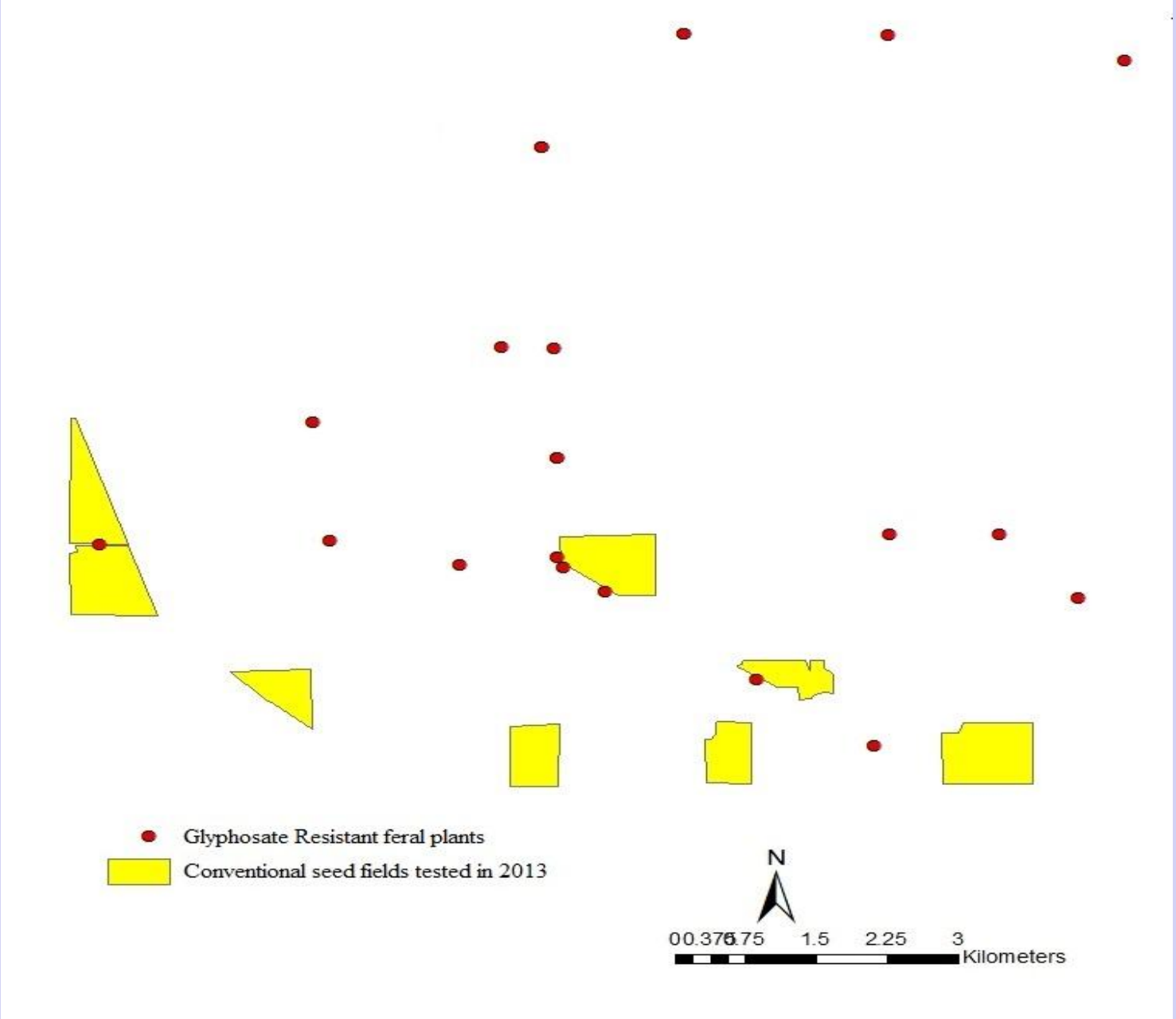
Canyon Co., ID

Map shows the location of all commercial GR and hay seed fields, but only shows conventional seed fields that we sampled for transgene presence.



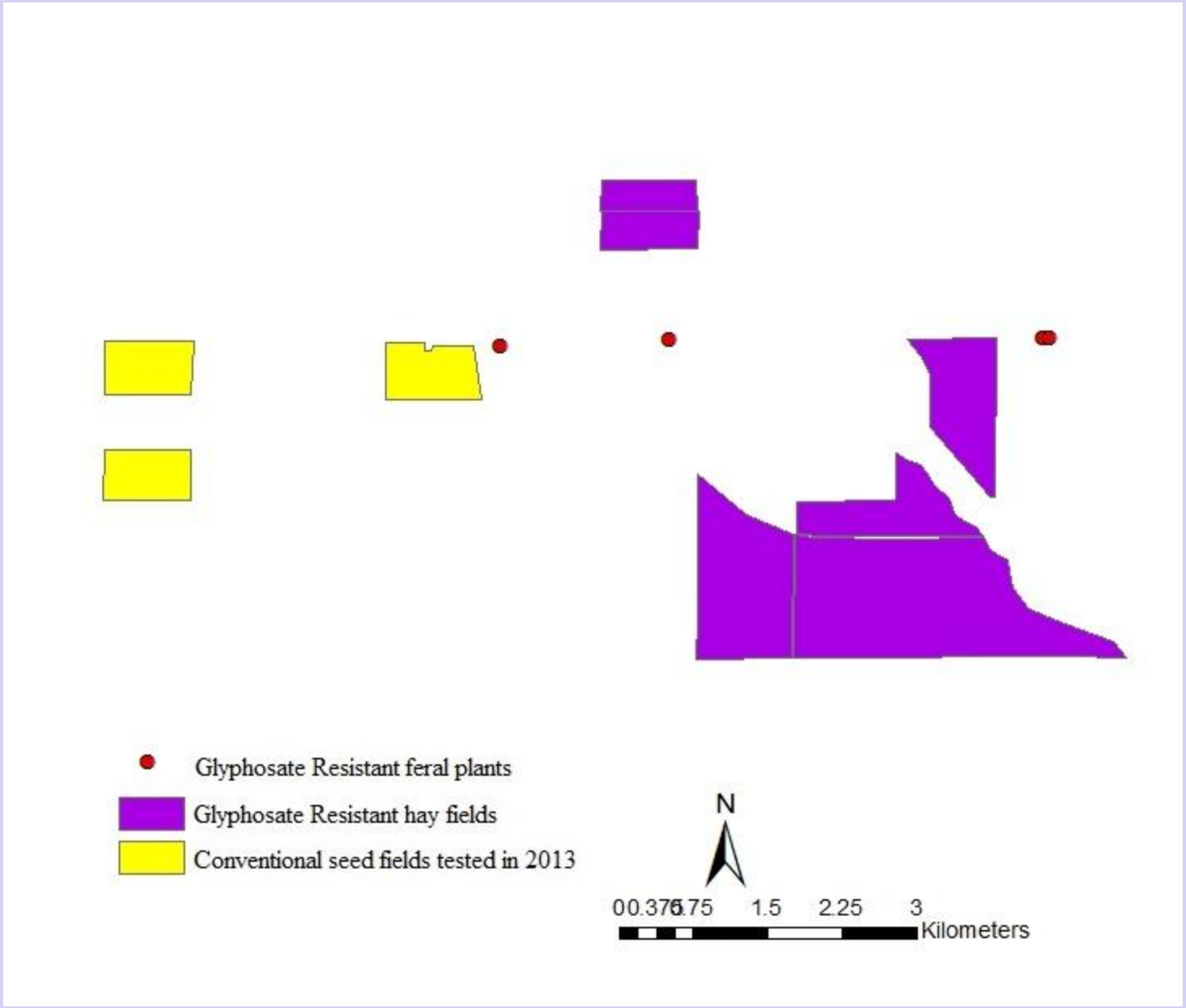
Fresno Co., CA

Map shows the locations of GR feral plants, but only shows conventional seed fields that we sampled for transgene presence.



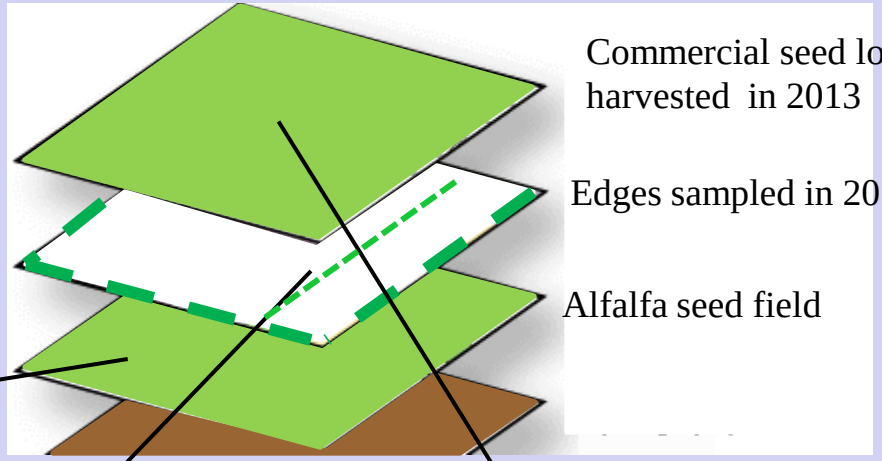
Fresno Co., CA

Map shows the location of all commercial GR hay fields and GR feral plants, but only shows conventional seed fields that we sampled for transgene presence.



Method

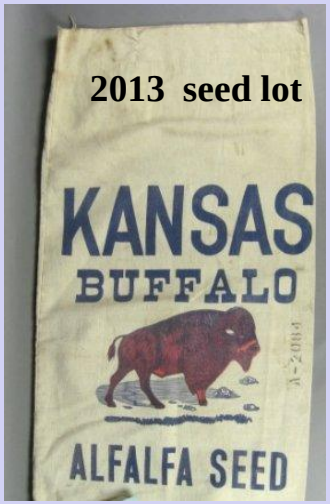
Sampling conventional fields



Overall sample from original seed lot used to plant field (baseline AP assessment)

Combine samples every 100’ along field edge and every 50’ on transects within fields; Fresno, Canyon- some hand harvest

Overall sample of commercial seed lot produced from 2013 harvest (overall AP assessment)



Method

Assessment of % adventitious presence (AP)

- Similar method used by industry, but use 3X the number of seedlings

- Eight samples of 300 seeds were vacuumed planted onto germination towels, and replicated three times, for a total of 7200 seeds tested. Positive and negative controls were also included. Seeds were germinated in an 80 ppm glyphosate solution for10 days then scored. GR seedlings were confirmed using AgraStrip test strips or PCR. % germination in water was also determined.

Method

- Seed also assayed by crushing 600 seeds and using AgraStrip test strips to qualify presence/absence of transgene (detect level is 1 in 600 seed). Number of tests depends on distance from transgene source

Method

Data Analysis

Our focus is to assess adventitious presence, that is quantify the occurrence of individuals pollinated by GR pollen having one or more dominant GR alleles.

$$\%AP = 100\left(\frac{+GR}{Total\ \#\ of\ seeds * Proportion\ of\ seeds\ germinated}\right)$$

The relationship between % AP along field edges and within field transects and distance (m) to GR source fields are being spatially modeled using a model similar to an exponential decay model, but that asymptotes to zero. Adjustments to account for baseline AP in original seed lots are made.

Work to date- Objective 2

- We are currently completing our assessment of fields in Walla Walla. Our aim is to define the landscape-level GR pollen dispersion curve. Once we have identified fields where we no longer observe AP, modeling can help us determine if current isolation distances are sufficient. We can also estimate specific distances needed to ensure conventional seed lots remain below various market thresholds.

- Once we have completed our study examining GR seed field dispersal to conventional fields, we will examine GR feral plant and GR hay field to conventional seed field dispersal

Acknowledgements

Research was supported by Biotechnology Risk Assessment Grant Program competitive grant no. 2011-33522-30733 from the USDA NIFA, USDA OSEC grant and the USDA, ARS

Field and Lab Crew :

Estela Cervantes

Jesse Prieto

Gwen Pentecost

Manuel Acala

Ines Ferjado

Miguel Cervantes

Samantha Goodman

Jose Acala

Enrique Acala

Rafael Atilano

Luz Barrantes

Special thanks goes to all the alfalfa seed growers and seed companies who have been such great cooperators!


Thank you
Questions?