# SEED-TO-SEED AND HAY-TO-SEED POLLEN MEDIATED GENE FLOW IN ALFALFA.

Larry R. Teuber*, Shannon Mueller, Allen Van Deynze, Sharie Fitzpatrick, James R. Hagler, and Jose Arias, University of California, Davis 95616, Forage Genetics, Inc, West Salem, WI 54669, and ARS-USDA, Arid-Land Agricultural Research Center, Maricopa, AZ 85239.

Honey bees (*Apis mellifera* L.) are predominantly used in California as pollinators for alfalfa (*Medicago sativa* L.) seed production. In some areas there is an increasing use of leafcutter bees (*Megachile rotundata* Fabricius.) in combination with honey bees. It is well known that honey bees will forage up to several miles from their hive. A study conducted in 2003 by our group conducted a gene flow study with a 6 acre Roundup Ready® source plot and eleven 0.54A trap plots at regular intervals extending East and West of the source. That study demonstrated adventitious presence (AP) in excess of 1.5% 900 ft from the marker gene source plot. Furthermore, the marker gene was detectable at very low frequency out to 2.5 miles – the outer limit of the study. The objectives of the current studies were to 1) evaluate the effectiveness of commercially available test kits in detecting the presence of the CP4 EPSPS (Roundup Ready) protein in seed samples from the 2003 study and known to have low levels of the trait based on extensive seedling growouts, 2) to determine the degree to which genes present in alfalfa fields being produced for hay are transferred to adjacent seed fields located the minimal legal distance of 165 ft from the hay field, and 3) to study gene flow between commercial scale production fields to further determine the extent of potential gene flow between alfalfa cultivars within the foraging range of honey bees.

To assess the effectiveness of the Roundup Ready test strips for seed we used seed produced on each of the 0.54 A trap plots during the 2003 study. All evaluations we conduced in accordance with the manufacturer's instructions. A total of 125 test strips were used to determine if the CP4 EPSPS protein was present in each of the traps. Strip test results from traps with a percentage AP less than 1% as determined by seedling growouts of seventy- to ninety-thousand seedlings provided virtually identical AP percentages based on determinations using "Seed Calc". AP percentages approaching and in excess of 1% could not be quantified because the frequency of AP seeds caused all the test strips to give positive results. For research purposes, we reduced the number of seeds tested when we started getting all positive strips, but kept all the other procedures the same. Results with this modification have also been in agreement with AP percentages found in large scale seedling growouts.

Conventional seed production fields were planted radiating out from a Roundup Ready hay production field. On all sides of the hay field the seed field was planted to within 165 ft and pollinated using honey bees. During the pollination period of approximately 8 weeks, the hay field was allowed to develop approximately 20% bloom (at least one open flower on 20% of the stems in the field) prior to being cut for hay. This is an amount of bloom the will occur with some commercial hay production and results in an opportunity for bees to visit the flowers and tripping does occur. Under this protocol, however, no seed is produced in the hay field. This degree of bloom was allowed to occurred in two consecutive cutting cycles during pollination. Seed was harvested at maturity from the seed fields at 50 foot intervals between 165 ft from the hay field (0 to 3 ft into the seed field) out to 615 ft from the hay field. Based only on test strip assessment, AP percentage was 0.29 % at 165 ft and dropped to less than 0.1% within 200 feet (365 ft

2007 North Central Weed Science Society Proceedings 62:203.

of the hay field). This percentage of AP is well within current standards for varietal purity in the Federal Seed Law.

Seed to seed gene flow was studied in commercial seed production fields in the San Joaquin Valley of California. The source field was a 240A planted to cultivar bred to express the CP4 EPSPS protein. This field was isolated from all other seed production, except fields within the study area, by three miles in all directions. Within in the study area, a conventional cultivars was being produced for seed at 1 mile (240A), 3 miles (40A), and 5 miles (100A). All commercial seed production was pollinated by a combination of honey bees and leafcutter bees. 1.8A bridged trap plots were located on one edge of the study at 900 ft intervals between the source field and the conventional cultivar located 1 mile away. The first of these traps was located 165 ft from the source field. Current results are preliminary and are based on test strips. Equal size (1.8A) study areas were intensely sampled within each of the commercial. Among the small bridged traps, AP averaged 2.3% at 165 ft and rapidly decreased to 0.9% at 900 feet and 0.6% at approximately 4000 ft. At one mile AP percentage was less than 0.2%. At three miles the AP percentage was less than 0.03%. AP was not detected 5 miles from the source plot. Growouts of seedlings from form these test areas are still in progress. However, current data from this study using strip tests is in very close agreement with seedling growout data from our previous study.