

## **CROSS POLLINATION FROM GENETICALLY ENGINEERED CORN: WIND TRANSPORT AND SEED SOURCE**

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Pollen transport from genetically engineered corn was evaluated by testing corn offspring from nearby cornfields. Harvested corn, sown in greenhouse flats, was sprayed with glyphosate at second leaf stage. Corn resistant to glyphosate likely cross-pollinated with GE corn or came from contaminated seed. Overall cross-pollination was less than two percent.

*Key Words:* glyphosate-resistant corn; genetically engineered corn; corn pollen; pollen transport; corn seed purity, transgene.

Organic producers, particularly dairy and sweet corn growers, fear losing their organic certification if genetically engineered (GE) proteins are found in their corn (*Zea mays L.*). Although corn pollen grains are among the largest and heaviest of wind-pollinated plants (Smith, 1990), transport is possible. Corn is a monoecious plant with male and female flowers borne on separate parts of the plant. Pollen shed generally occurs over the course of a week, but may last from 2 to 14 days; a majority of the pollen will be shed around the third day (Purseglove, 1972). Corn produces abundant pollen (2 – 5 million grains plant<sup>-1</sup> (Goss, 1968; Kiesselbach, 1948)), and environmental conditions (temperature, wind speed, and wind direction) affect the likelihood of cross-pollination. Reports of pollen viability vary. Pollen may remain viable for 24 hours (Purseglove, 1972), but could be much shorter in hot weather. Cool temperatures and relatively high relative humidity tend to increase pollen viability.

There has been a great deal of research on corn pollen dispersal conducted during the past 50 years. Critics of GE frequently refer to “gene clouds” of mutant corn pollen traveling distances of many miles to cross-pollinate and contaminate non-GE corn. While physical transport of pollen grains over this distance is theoretically possible, the likelihood of significant contamination is fairly low due to the short viability of the male gamete once shed. Literature reports of measured corn pollen transport distances are highly variable. Many researchers report half distances, or the distance required for pollen to drop by half. Bateman (1942) reported a distance of 3.77 meters (m), and that cross-pollination dropped by 99 percent over a distance of 12 to 15 m. Hodgson (1949) reported a half distance of 8.25 m, while Jones and Newell (1948) reported half distance of 47 m. Given recent legislative attempts to require seed companies to provide growers with set back distances to prevent cross-pollination, more research is needed to understand GE corn pollen transport and potential cross-pollination.

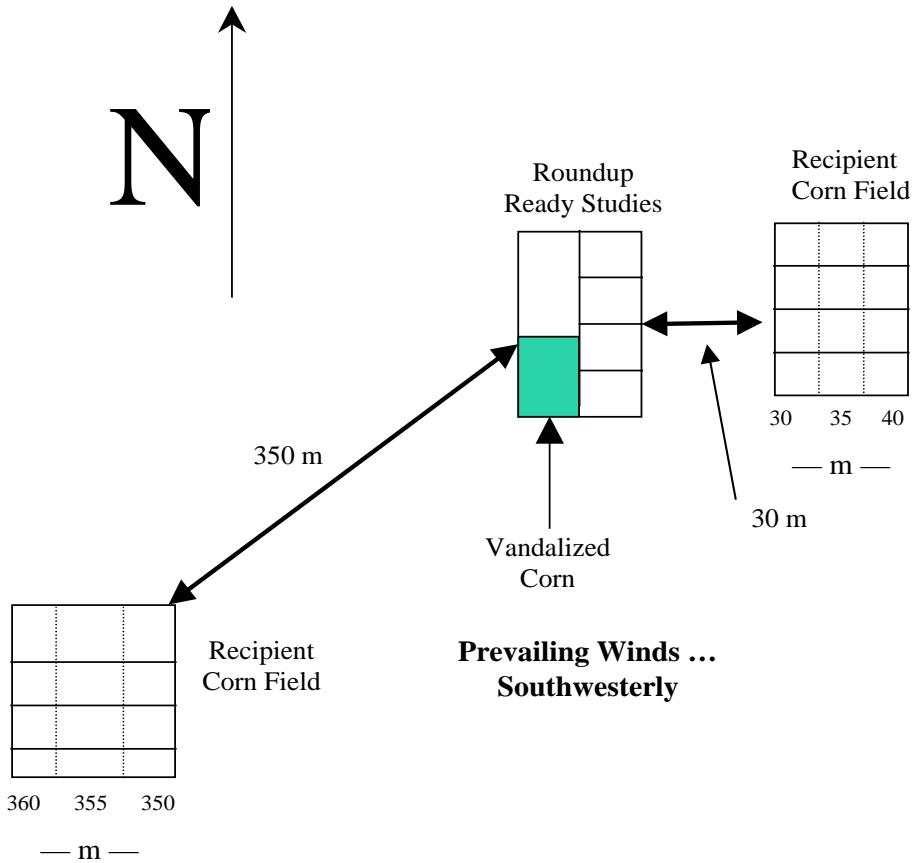
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During the summer of 1999, a Roundup-Ready (RR) corn study was initiated to evaluate application rates, timing, and tank mix combinations for weed control. This cornfield was vandalized in late August after corn pollination. As a result of the vandalism, questions about possible transport distances were raised. So we initiated a study to evaluate the transport of GE pollen. In 1999, we monitored cross contamination to recipient plots, and in 2000 we monitored synchronicity of pollen shed and transport to recipient field plots.

### Field Methods

Roundup-Ready and conventional corn hybrids were planted at the Rogers Farm in Stillwater, Maine between 15 and 17 May 1999 and on 20 May 2000. Each hybrid planted was an 83-85 day maturity hybrid. The RR corn (DeKalb DK335 RR) and the conventional hybrid were planted at 78,496 seeds  $\text{ha}^{-1}$ . Plot locations for 1999 are presented in figure 1. The RR study area was 3454  $\text{m}^2$  located between the two other studies. One recipient field was located 30 m east of the RR study. The other recipient field was located 350 m SW of the GE corn source. Since summer season prevailing winds are predominantly southwesterly, the field 30 m E represented a scenario more favorable for cross-pollination than the weed control study. We subdivided each field potentially receiving GM corn pollen into 12 subplots (each subplot was 23.5  $\text{m}^2$ ) to measure cross-pollination. Corn was harvested 19 September 1999 and 22 September 2000. Similar field methods were followed in 2000. The only change was that the corn planted 350 m SW of the GE source was moved to 100 m due S of the GE source.

**Figure 1: Plot Map of the Field Study Evaluating Cross-pollination.**



## Greenhouse Methods

Corn (50 ears) was harvested from each 23.5 m<sup>2</sup> subplot of each study for a total of 28 plots. Corn was dried to < 12% moisture and shelled. Approximately 200 seeds from each subplot were sown per 0.14 m<sup>2</sup> flats. The greenhouse screening study was replicated four times each year. The conventional hybrid and DK355 were included with the corn offspring to test purity of initial seed source. Plants were watered daily and fertilized twice with NPK fertilizer. At V2, glyphosate was applied at 1.12 kg active ingredient ha<sup>-1</sup>. After 10 days, plants survival was assessed. Selected glyphosate resistant plants were submitted to the Monsanto Corporation for double blind polymerase chain reaction (PCR) tests to confirm the presence of the transgene.

## Results

In 1999, all original seed from the conventional corn seed check died when sprayed with glyphosate. However, some genetic impurity was noted in the original DK 355 corn, as between 2 and 5% in 1999 and 2000, respectively, of that seed source did not survive glyphosate application. Although limited in extent, we did find cross-pollination between the RR corn and the conventional hybrid in the 30, 35, and 40 m E plots in 1999 (table 1 and figure 1). Plants with inherited resistance continued to grow after being sprayed with glyphosate. The highest number of resistant plants came from the RR offspring (70 – 72%), consistent with selfing of a heterozygous dominant trait. The amount of cross-pollination dropped off with distance from the source. This also supports the research by Raynor *et al.* (1972) that indicated that total pollen deposition would be much greater within the source than outside. The highest survival rate found outside of the RR offspring was 1% at 30 m E downwind of the RR pollen source. Cross-pollination dropped off significantly with distance from the source.

**Table 1: Cross-pollination Results from 1999.**

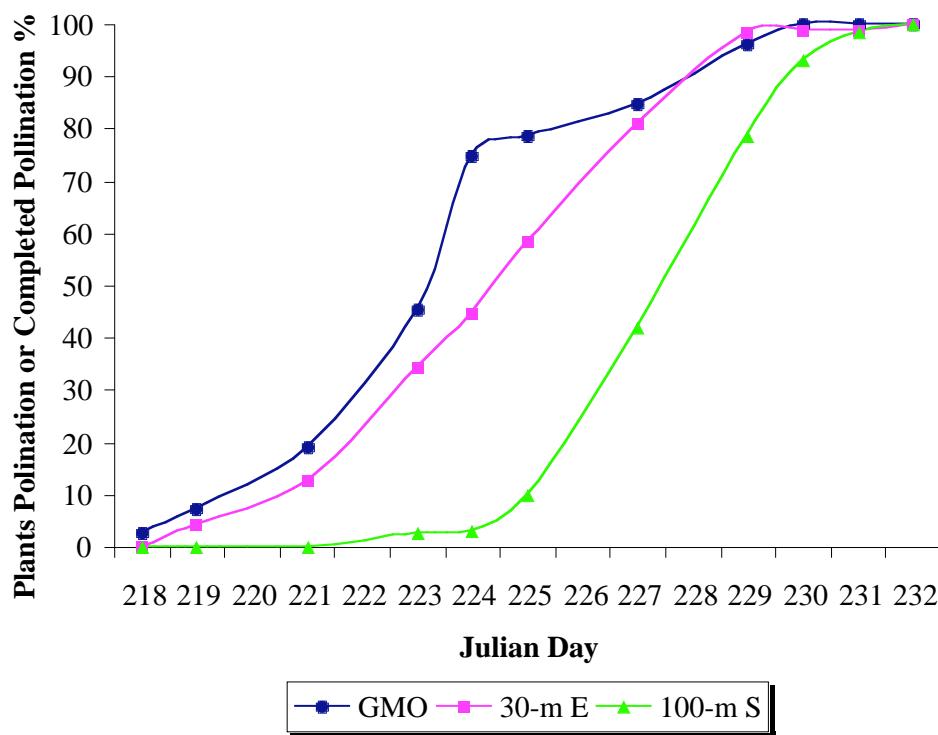
Corn Seed Tested	Mean Germination	Mean Survival	Sum Survival	Percent Survival
<b>DK-355 (GE)</b>	511	502	2008/2044	98.2
<b>Conventional Hybrid</b>	475	0	0/1880	0
<b>DK-355 Offspring</b>	689	503	2016/2756	72.5
<b>30-m E</b>	691	7	29/2764	1.04
<b>35-m E</b>	701	0.75	3/2804	0.11
<b>40-m E</b>	716	0.25	1/2864	0.03
<b>350-m SW</b>	555	0	0/2092	0

Half distances for pollen transport have been reported in the literature from as low as 3.8 to 47.5 m (Bateman, 1947; Jones & Newell, 1948). A number of factors including size of the initial pollen source, wind speed and direction, and frequency of high wind speeds very likely influence this half distance. Bateman (1947) found that cross-pollination dropped 99% over a distance of 12 – 15 m. In our 1999 data, we found similar declines in cross-pollination over distances less than 10 m. Our results agree closer to the 3.77 m half distance reported by Bateman (1947) likely because of our relatively small acreage of initial pollen source. None of the offspring from the 350 m SW plots

survived glyphosate application. Results observed are consistent with predominant wind direction. The distance that corn and the RR pollen source were close to is within the distance generally recommended as safe by corn breeders (220 – 350 m).

In 2000, we monitored environmental conditions and pollination synchronicity among the three study locations. While all corn was sown on the same day, and despite the similarity in maturity, there was some asynchronicity in plant maturity due to one field being wetter and colder longer (figure 2). If most pollen dehiscence occurs in the first three days of the pollination process (Purseglove, 1972), cross-pollination between the GE source and the field 100 m S should have been very low. But cross-pollination was higher than expected (table 2).

**Figure 2: Synchronicity of Pollen Shed.**



Instead of seeing a decrease in cross-pollination with distance from the GE source as we did in 1999, we found low generalized cross-pollination across all sampling sites. In 2000, the conventional hybrid check plot was found to have a low level (0.16%) of glyphosate resistant seed (likely genetic contamination in the breeding or handling of the seed). This observation likely explains the low generalized resistance observed in the 100 m S plots; however, it is difficult to determine whether the glyphosate resistance in the offspring is due to wind-carried pollen from the GE source or due to the contaminated seed. Wind speed and direction were favorable for cross-pollination as northerly winds were the predominant wind direction for four of the days when the GE corn was pollinating. But, given the asynchronicity of pollen timing, it is more likely that the resistance found within the distant site was due from contaminated seed and not wind-blown pollen. We are currently testing several other conventional corn hybrids used commonly in Maine for the presence of low levels of glyphosate resistant seed.

**Table 2: Cross-pollination Results from 2000.**

Corn Seed Tested	Mean Germination	Mean Survival	Sum Survival	Percent Survival
<b>DK-355 (GE)</b>	762	724	2883/3049	95
<b>Conventional Hybrid</b>	576	1.5	8/4941 <sup>1</sup>	0.16
<b>DK-355 Offspring</b>	726	509	2036/2904	70.5
<b>30-m East</b>	682	11.25	45/2729	1.65
<b>35-m East</b>	693	6	24/2770	0.86
<b>40-m East</b>	680	7.75	31/2718	1.14
<b>100-m S</b>	676	4.5	18/2766	0.65
<b>105-m S</b>	722	7.25	30/2887	1.04
<b>110-m S</b>	667	9.25	37/2668	1.38

<sup>1</sup> Upon finding resistance in this seed we tested an additional 2,000 seeds to confirm resistance. This resistance was confirmed through PCR testing.

## Summary And Conclusions

Cross-pollination of GE corn with traditional corn hybrids was demonstrated in this study, but only with a low rate of occurrence. Even with the RR pollen source as close as 30 m away and a wind direction generally favorable for cross-pollination, the amount of cross-pollination was low. However, given the current organic certification regulations, any cross-pollination could threaten grower certification. While in 1999 the distant site (>300 m SW) had no evidence of cross-pollination, in 2000 the distant site (>100 m S) had the same level of resistance as close recipient plots. While distance and tall border rows could mitigate transfer of the transgene to non-GE fields, the observation that the GE trait can be present in conventional seed (as found in 2000) complicates organic certification efforts.

Given the history of ground and surface water contamination with standard pre-emergence herbicides, RR technology may offer a more environmentally friendly alternative. From the 1999 data, we concluded that organic producers concerned about cross-pollination could possibly reduce this threat by not harvesting the outer 3 to 4 m of the cornfield. Beyond this point, the amount of cross-pollination was minimal. However, our 2000 data did not support this. Further, it appears to be increasingly important for organic producers to consider what seed they purchase to plant for corn and possibly test purchased seed stocks prior to planting. They may want to consider growing open pollinated corn varieties or trying to purchase certified seed to prevent losing their certification.

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