Frequency of Pollen Drift in Genetically Engineered Corn

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Cross fertilization and concerns over cross contamination

Corn (*Zea mays* L.), also called maize, is a monoecious crop with male (staminate inflorescence) and female (pistillate inflorescence) flowers formed in separate parts of the same plant, leading to a high degree of cross-pollination between plants. It is reported that cultivated corn freely crosses with nearly all members of the genus, including several hundred mutants[1]. The male inflorescence (tassel) of corn can produce considerably more pollen grains than are required for pollination of a single plant. Westgate *et al*.[2] estimated that individual tassels produced 4.5 x 10^6 pollen grains, and pollen shedding often lasts 5 or 6 days.

Interest in pollen movement has increased recently due to possible contamination of conventional crops from pollen of genetically engineered (GE) genotypes. Under natural conditions, pollen can travel from field to field, but the majority of pollen grains are assumed to fall within the row space, as corn pollen is one of the heaviest and largest (about 90-100 µm in diameter) among wind-dispersed pollen grains[1,4]. For corn producers, the major issue is that contamination of conventional hybrids by pollen from neighboring GE hybrids will restrict marketing the grain harvested from the contaminated field. Such grain is declared as essentially transgenic and will only be accepted by specific elevators and processors[3]. Thus, there is an urgent need to understand pollen-mediated gene flow and the minimum distance required to isolate conventional hybrids from neighboring GE cornfields.

The endosperm of corn kernels can be yellow or white, with yellow dominant to white. These colors are easily observable and can be used as markers in studies designed to measure cross-fertilization. It is possible to quantify the extent of out-crossing between genotypes by planting a white-kernel hybrid next to a yellow hybrid and measuring the incidence of yellow kernels on white cobs. Using this production system, we determined: i) the frequency of cross-fertilization of a corn genotype by foreign pollen of neighboring hybrids; and ii) the practical distance from neighboring GE corn fields required to grow non-GE corn.

Site description for field experiment

Field experiments to quantify the level of cross fertilization of white corn by pollen from neighboring Bt yellow corn were planted at three locations in Ottawa, Canada, (45°22'N, 75°43'W) for three growing seasons (2000, 2001, and 2002). All sites were open fields, and in all cases, there were no corn crops, fences, or blocks to stop wind flow within at least 200 m in all directions. At each site, the yellow Bt corn was planted in the center (36 rows of 27 x 27 m) while a white corn hybrid was planted in the surrounding area (**Fig. 1**). In this region, the prevailing wind in July and August is generally from the northwest direction. Therefore, white corn planted east and south of the yellow Bt corn was designated as "downwind," while white corn west and north was "upwind." Hybrids and planting dates for each site-year are listed in **Table 1**.

Assessment of the frequency of cross-fertilization

At maturity, a thorough systematic sampling of white corn was conducted to determine out-crossing of white corn kernels by the yellow Bt pollen (**Fig. 2**). In each site, in both downwind and upwind directions, ears of white corn were sampled from rows No.1 (the first row of white corn adjacent to the yellow Bt hybrids), 7, 13, 19, 25, 31, 37, 43, and 48 (37 m) bordering the yellow Bt corn. In the northern and southern ends of yellow Bt corn rows, white corn ear samples were taken from rows 1, 7, 13, 19, 25, and 33 (row number was arbitrarily defined, but plant number was counted always starting from the white kernel plant bordering the yellow corn). In all designated rows, ears from every 10th plant of white corn (i.e., 1st, 11th, 21st, and so on) were collected, marked, and measured. The relative distance to the yellow corn determined the position of all sampled plants in the field. A plant was considered as 0% cross-fertilized if there were no yellow-colored kernels in the sampled ear as well as in the ears of two adjacent plants (e.g., if the target was an 11th plant, plants 10th, 11th and 12th were also field-checked to insure no yellow kernels were present). Within a single ear, the total number of rows, number of kernels per row, and the total number of yellow kernels were counted. Percent out-cross was calculated as the number of yellow kernels divided by the total number of kernels (white+yellow) per ear.

An exponential decline model was used for fitting the cross-fertilization data as a function of distance to the yellow Bt corn pollen:

$$Y = Y0\ e^{-BX} \quad [1]$$

where Y is cross-fertilization (%), Y0 is cross-fertilization extrapolated to X = 0, B is a shape coefficient, and X is the distance (m) of the sampled ear to the pollen source of the yellow corn.

Results and discussion

Pollen shedding and silking dates, time taken to reach the stages, and CHU accumulated for both yellow and white corn hybrids are presented in **Table 1**. The hybrid 4521Bt (yellow) at Site #2 (year 2000), V414W (white) at Site #3 (year 2001), and both hybrids at Sites #2 and #3 in 2002 had non-uniform plant growth (uneven plant size) and development (phenological stages) requiring longer periods to complete their flowering (**Table 1**), and probably asynchronous pollination within the population.

Seasonal weather conditions affected the level of cross-fertilization. The observed maximum out-crossing was over 82%, indicating that, in corn, cross-fertilization between genotypes is more favored than that within the population (>50% kernels are yellow in the white kernel corn). The average level of out-crossing in the first adjacent row was greater in 2000 (18.2%) than in 2001 (12.3%) or 2002 (13.3%). A consistent pattern was also observed in subsequent rows.

The level of cross-fertilizations across site-years fluctuated greatly because of wind speed and directions, but as a rule, the farther away from the yellow Bt pollen source, the lesser was the percent out-crossing (**Fig. 2**). Overall, maximum cross-fertilization occurred in the first row of the white corn adjacent to the yellow Bt hybrid. The extent of cross-fertilization in the subsequent rows declined exponentially to 0 or near 0% toward the edge of the field. Greater than 1% cross-fertilization was found after the 37th border row (28 m) downwind from the prevailing wind direction or the 13th row (10 m) in the upwind direction in all site-years. In the white corn rows on the straight northern and southern sides of the yellow Bt corn field, a considerable amount of out-crossing (7-15%) was recorded, mainly within 7.4 m (41st plants) from the pollen source, with substantial differences in level of out-crossing among site-years. The rate of cross-fertilization considering distance to the pollen source was well represented by the exponential decline function ($P<0.01$), with R2 = 0.64 for downwind and 0.58 for upwind (**Fig. 3**).

The Eq [1] also fits the data of cross-fertilization in the northern and southern sides of yellow Bt corn. According to Eq [1], the extrapolated zero (or 0.0001%) cross-fertilization would have occurred in the white corn at about 28–30 m downwind or 18–23 m upwind from the pollen source (**Fig. 3**), suggesting that pollen traveled shorter distances, or cross-fertilization declined more quickly, along the same row direction than along cross row directions. In general, although the model fits the data, the R^2 values in all cases were not very large, indicating factors other than distance, e.g., wind speed and directions during the peak pollination period, also played important roles in the extent of cross-fertilization. The risk of cross-fertilization of white corn (or other non-GE corn) by pollen from neighboring yellow Bt corn was very low beyond the 37th row (28 m) from the source.

Summary and Concluding Remarks

Using yellow kernel corn as a marker of cross-fertilization in white corn is a useful tool to estimate pollen dispersal. The experimental results showed that the majority of corn pollen grains settle close to the source. There was an exponential decrease in pollen dispersal as the distance from pollen source increased. From a practical point of view, and considering differences in planting dates from neighboring cornfields and/or different maturity of two hybrids involved, our data suggest it is possible to produce non-GE corn grains by removing the outside rows of corn plants (about 30 m) adjacent to the GE corn field in concern, if the acceptance level is set at <1% cross-fertilization. Although the chances of cross-fertilization of white corn by pollen from neighboring yellow corn at the distance of 28 m was minimal, this study did not examine the situation in which Bt and non-Bt corn were separated by a non-corn space[3].

References

1. Burris JS (2001) Adventitious pollen intrusion into hybrid maize seed production fields. *Proc. 56th Annual Corn and Sorghum*

Research Conference 2001. American Seed Trade Association, Inc. Washington, D.C.

2. Westgate ME, Lizaso J & Batchelor W (2003) Quantitative relationship between pollen-shed density and grain yield in maize. *Crop Sci*. **43**, 934-942

3. Ma BL, Subedi KD & Reid LM (2004) Extent of cross-fertilization in maize by pollens from neighboring transgenic hybrid. *Crop Sci*. **44**, 1273-1282

4. Stewart DW, Ma BL & Dwyer LM (2001) A mathematical model of pollen dispersion in a maize canopy. 2001 Annual Meeting Abstracts, The ASA-CSSA-SSSA Headquarters, Madison, WI.

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