Improved method for nondestructive measurement of dynamic pollen source strength from transgenic crops using sonic anemometer

Junming Wang[1], Xiusheng Yang[2]

(1. *Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM* 88003-8003, *USA*; 2. *Department of Natural Resources and the Environment, University of Connecticut, Storrs, CT* 06268-4087, *USA*)

Abstract: Pollen-mediated gene flow from transgenic crops is a concern of the scientific community as well as the general public. Current lack is a nondestructive method to measure dynamic (hourly or shorter time period) source pollen release rate (source strength) from these crops. This paper reports an improved method using Rotorod concentration samplers and sticky slides, together with a fast response sonic anemometer, for measuring source strength from transgenic corn crops. Field measurements were made during the pollination season of 2001 at the Agronomy Research Farm, University of Connecticut, Storrs, Connecticut. Data were analyzed and used to estimate the pollen source strength of the corn crops at hourly time steps by considering both atmospheric transport and gravitational deposition processes. Details of the calculation and assumptions of the method are provided. Results from this study demonstrate that this method is sufficiently robust for use in estimating pollen source strength of transgenic crops with heavy pollen grains in assessing gene flow risks associated with field production of transgenic crops.

Key words: corn, pollen, gene flow, release rate, source strength, transgenic crops

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1 Introduction

Because transgenic crop is economically important and has potential high ecological pollution risks[1,2], numerous gene flow studies have been conducted[3-9]. Many of the experimental studies were done with pollen samplers located within short distances downwind to investigate exclusion distances or within-stand cross-pollination. Comprehensive reviews of the studies were presented by Emberlin et al (1999) [10] and Aylor et al (2003)[11].

Among the current literature, however, there is little work reported in measuring dynamic (e.g., hourly) pollen source release rate (source strength, pollen grains per plant per unit time, or pollen grains per unit ground area per unit time) from transgenic crops. To measure the strength of crop pollen source directly is difficult. Jarosz et al(2003)[9] and Jarosz et al(2005)[12] measured the daily corn pollen source strength by wrapping polythene bags around plant tassels and counting the number of daily pollen grains collected in the bags. The pollen production per plant per day was determined using the same five or six individual plants each day. The hourly pollen release was indirectly estimated from pollen concentration measurement at tassel height. This method has some disadvantages:

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Biographies: Junming Wang, PhD, Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003-8003, USA. Email: jwang@nmsu.edu. **Xiusheng Yang,** PhD, Professor, Department of Natural Resources and the Environment, University of Connecticut, Storrs, CT 06268-4087, USA

Corresponding author: Xiusheng Yang, Professor, Department of Natural Resources and the Environment, University of Connecticut, Storrs, CT 06269-4087, USA. Phone: (860) 486-0135, Fax: (860) 486-5408. Email: xiusheng.yang@uconn.edu

1) Bag wrapping may damage the tassels or affect the natural growth of the tassels;

2) The processes of wrapping and unwrapping may shake the tassels and make tassels release more pollen than from natural release;

3) The bag opening was downward and pollen in the bags can easily leak out during bag unwrapping.

Therefore, there are apparent uncertainties associated with this measurement method. In addition, these directly measured data were at daily steps; and hourly data cannot be obtained using this method because hourly wrapping and unwrapping may cause too much disturbances to the natural processes of the plants.

There have been reported some indirect (modeling) methods to determine pollen source strength. For example, Aylor and Qiu (1996)[13] used a complicated model to measure virus releasing source strength using Similarity Theory. The model sets source strength first and then simulates the concentration in the source center. Then the actual source strength was obtained by scaling the set source strength using the ratio of measured concentration to the simulated concentration. Because crop pollen in general has a much larger size and therefore is quite heavier than a virus (e.g., corn pollen has a diameter of 76−106 um with a settling speed of 0.3 m/s, Aylor et al, 2002[14]) an appropriate model for crop pollen dispersion needs to be parameterized and validated before it can be used to predict corn pollen source strength. However, the parameterization and validation of the dispersion model requires the source strength to be known first. Therefore, using the modeling method alone is very indirect and hard to validate for determining pollen source strength.

The IHF (integrated horizontal flux) method is a widely used experimental way to measure gaseous and light particle source strength[15]. The IHF method measures the vertical particle concentration and wind speed profiles at a strip field edge (downwind direction) or in a circular area where the particles of interest are released. The horizontal flux density can be calculated for each measured concentration location as the product of the concentration and the wind speed (grains/m^2/s). With that, the integral of the flux density over the height from ground to the zero-concentration height is calculated (grains/m/s). Finally, the value of the integral divided by the strip width or the radius of the circular area is used as the source strength (grains/m^2/s). The IHF method was examined by Wilson and Shum (1992)[15] for gaseous source release using model results and was proved acceptably accurate. Because the crop pollen has a larger settling speed than gaseous materials, a big portion of pollen will be deposited. In order to use the IHF method to obtain crop pollen source strength, improvements are needed and the pollen deposition must be considered.

The objective of this study was to develop a nondestructive method to measure the dynamic source strength of heavy pollen from transgenic corn crops. The hypothesis of the study was that the IHF method can be improved to measure the heavy pollen dynamic source strength by considering the downward deposition of pollen grains. In this study, special considerations have been paid to the following four aspects:

1) The method must be nondestructive, i.e., it does not damage the tassels nor affect the tassel normal growth;

2) The method must not touch the flowers or tassels to introduce measurement bias;

3) It must be dynamic, i.e., capable of making short time period (i.e., hourly) observations; and,

4) It has to take pollen deposition into consideration.

Although the method was developed for corn, it has potential applications for other crops with heavy pollen grains.

2 Materials and methods

2.1 Theoretical considerations

Figures 1 and 2 illustrate the overall idea to nondestructively measure the dynamic source strength of a corn crop. The cylindrical coordinating system was used. As shown, the source field of the crop with a circular shape is sized with a radius of R (m). Assume that the plant tassels (or flowers) produce pollen grains with a source strength of Q_0 (grains/m^2/s). A portion of the pollen grains is blown away from the plants by wind in the wind direction from the field edge to the Rotorod concentration samplers (Model 20, Sampling

Technologies, Inc., MN, USA) deployed in the center of the source field. Denote the wind speed at height z (m) as $u(z)$ (m/s). Meanwhile, a portion of the pollen grains is deposited downward due to gravity with a deposition flux density $D(r)$ (grains/m^2/s), where r is the distance from the field center in the wind direction (m).

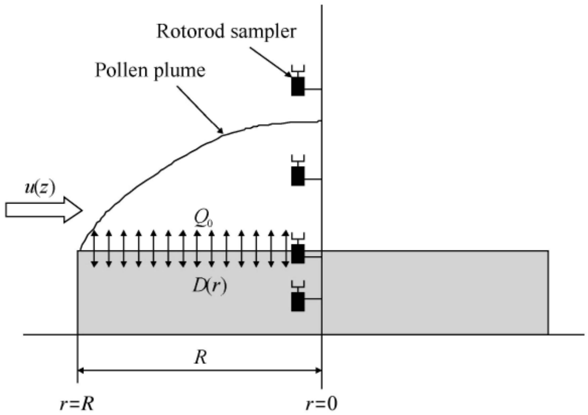

Figure 1 Schematic sketch of source strength measurement in a crop field (following Wilson and Shum, 1992[15]; Aylor and Qiu, 1996[13]), where r is the distance from the center of the plot, R is the radius of the plot, u is the horizontal wind speed above canopy, Q_0 stands for pollen source strength, and D represents the downward deposition of pollen grains.

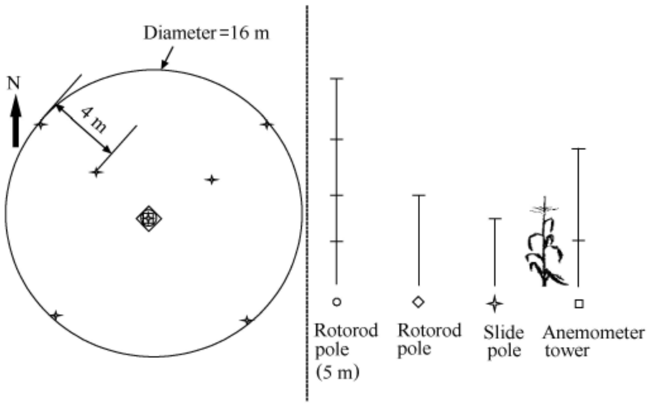

Figure 2 Schematic illustration of the source strength measurement setup, with locations of the sampling poles in the field (left), and vertical positions of the samplers on the poles (right)

The source strength thus can be calculated as:

$$Q_0 = \frac{\int_0^R D(r)dr + \int_0^\infty u(z)C(0,z)dz}{R} \quad (1)$$

where $C(0,z)$ is the concentration of pollen at the center of the source (grains/m^3) at height z. A major improvement of equation (1) over the current IHF method is the inclusion of the deposition term.

The field is set to a circular shape in that even the wind direction is constantly changing over time, the pollen contribution length from the edge to the center of the field, where the Rotorod samplers are deployed, is always a constant (R). This will clearly simplify the definition and calculation of equation (1).

2.2 Field experiments

2.2.1 Site and crop

To evaluate the proposed method, an experiment was conducted in the growing season of 2001. Sweet corn (Jackpot F-1, Hoffman Seeds, Inc.) was used as plant material. Plants were grown in a circular area of 16 m in diameter in the University of Connecticut Agronomy Research Farm (Figure 2) with commercial density of 53376 plants/hm^2. The field was planted in the middle of May. Plants grew to 1.5 m tall and silk height was at 0.7 m.

Two 3-D sonic anemometers (CSAT3, Campbell Scientific, Inc., Logan, UT; V-style, Applied Technologies, Inc., Longmont, CO) were set up during the periods of pollination to measure wind profile in the center of the plot. One V-style anemometer was located at the silk height and the other was at 1 m above the tassel. The three dimensional wind data were measured at 10 Hz, recorded and summarized as 30-min statistics by a CR23X datalogger (Campbell Scientific, Inc., Logan, UT).

2.2.2 Pollen sampling

Pollen concentration was measured by using four Rotorod samplers in a column in the center of the plot with retracting-type sampling heads (Model 20, Sampling Technologies, Inc., MN, USA). One sampler was located inside the crop at the height of the corn silks, one at the height of tassels, one at 3.2 m, and the other one at 5 m above the ground. An additional sampler (the 5th one) was deployed at height of tassels in the center of the source to continuously record concentration when the other samplers were switched off for rods replacement.

Microscope slides (2.5 cm × 7.5 cm) with silicon grease (Sampling Technologies, Inc., MN, USA) were used to measure deposition at a height just below the tassels. Two sampling lines were established in the directions of north-east to south-west and north-west to south-east. Because the prevailing wind was from north to south, the integral calculation of deposition flux density for source strength occurred most in the north half of the area. Correspondingly, two more deposition measurement points were set in the north half of the plot.

Pollen sampling was conducted throughout the pollination season. The sampling period for the collectors was 1.5 h during daytime (7:00−19:30). Timers were used to provide intermittent samplings for the Rotorod samplers based on the capacity of the Rotorod samplers to avoid overloading. Rotorod and slide samplers were then synchronized to correspond to exactly the same time periods. New fresh sampling rods and slides were placed for each new sampling period. No samples were taken during rain periods.

A microscope with power magnification of 3.5X and binocular eyepiece of 15X (B-35-83, American Optical Co., Instrument Division, Bufflo, N.Y.) was used to visually count the pollen grains collected on the Rotorod rods and slides. For each rod, a surface area of 33 mm^2 in the middle was screened for pollen grains. For the deposition slides, random samples (n=8) of circular areas (d=5.3 mm) were drawn from each slide to count the pollen grains to infer the total deposition, following the standard procedures of cluster sampling[16]. The sampling error ranged from 12% to 34%, depending on the deposition density.

2.3 Data processing

2.3.1 Atmospheric parameters

Wind speed (m/s) and direction (degree), atmospheric stability parameter Monin-Obukhov Length L (m), friction velocity u* (m/s) for each sampling period (1.5 h) were calculated from the high frequency measurements of the anemometer located above canopy[17]. The calculated parameters were rotated to the mean wind direction[18]. The wind speed above the canopy was calculated by:

$$\bar{u}(z) = \frac{u^*}{k}\left[\ln\left(\frac{z-d}{z_0}\right) + \varphi\left(\frac{z}{L}\right)\right] \qquad (2)$$

where k is the Karman constant (0.4); d is the displacement distance (m) estimated by Campbell and Norman(1998)[19]:

$$d = 0.65h \qquad (3)$$

where h is the canopy height, m; the parameter z_0 is the roughness length, m, obtained according to Campbell and Norman (1998)[19]:

$$z_0 = 0.1h \qquad (4)$$

The wind speed inside canopy was assumed to take the following profile:

$$u(z) = u(h)\exp[\eta(\frac{z}{h} - 1)] \qquad (5)$$

where η is the attenuation coefficient (η=2) obtained from Campbell and Norman (1998)[19] corresponding to the given canopy structure.

2.3.2 Concentration

The pollen concentration (C, grains/m^3) at each sampling point was determined by dividing the total numbers of pollen grains by the sampled air volume during the corresponding measurement period. The air volume was calculated by:

$$V = \omega ps\Delta t \qquad (6)$$

where V is the total air volume going through each Rotorod sampler during each sampling period, m^3; ω is the rotational speed of the Rotorod sampler, r/min; p is the perimeter of the circular orbit of the rods, m; s stands for the sampling area of each sampling rod, m^2; Δt is the actual sampling time of the system, h. The collection efficiency of the Rotorod samplers was assumed to be 100% for corn pollen, according to Edmonds (1972)[20].

2.3.3 Deposition flux density

The average pollen counts per unit sampling area of the selected clusters on each slide was regarded as the deposition density during each sampling period (grains/m^2). The deposition flux density was defined by the deposition density divided by sampling time (grains/m^2/s). The collection efficiency of the slide traps was assumed to be 100%, according to Raynor et al., (1970a)[5], and Aylor et al., (1989)[21].

2.3.4 Source strength

The source strength Q_0 (grains/m^2/s) for every measurement period was calculated using equation (1). If the mean wind direction was not coincided with any sampling line, then the integral of the deposition term was corrected by:

$$\int_0^R D(r)dr = \frac{\int_0^R D(r_s)dr_s}{\cos(\alpha)} \quad (7)$$

where r_s is the distance from plot center in a sampling line which is the closest sampling line to the wind direction, α is the angle between the sampling line and the mean wind direction (Figure 3).

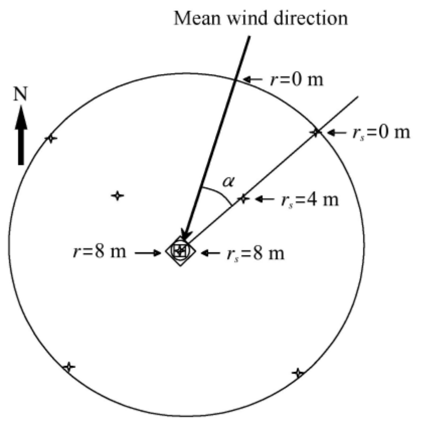

Figure 3 Schematic sketch of the deposition flux density integral calculation when mean wind direction does not coincide with any sampling line. The stars in the circle indicate the sampling points that define the sampling lines

The source strength during each measurement period was normalized by the daily maximum. Daily (grians/m^2/day) and seasonal (grians/m^2/season) source strength was estimated by integrating the source strength over each corresponding day or the whole season, respectively. Daily source strength was normalized by its maximum value during the pollination season. Scatter plots were used to detect the patterns of pollen release.

3 Results and discussion

3.1 Method capability

Figure 4 shows a typical daily pollen release pattern. Pollen release normally began in a couple of hours after sunrise, and then increased quickly with time and reached the maximum at about 10:00 am. After that, the source strength decreased. Little pollen was produced after sunset. The maximum rate of pollen release on the day of the highest pollen production was 391 grains/m^2/s or 73 grains/plant/s (calculated based on plant density). The maximum daily release was about 3.98 million grains/m^2/day, or 0.75 million grains/plant/day.

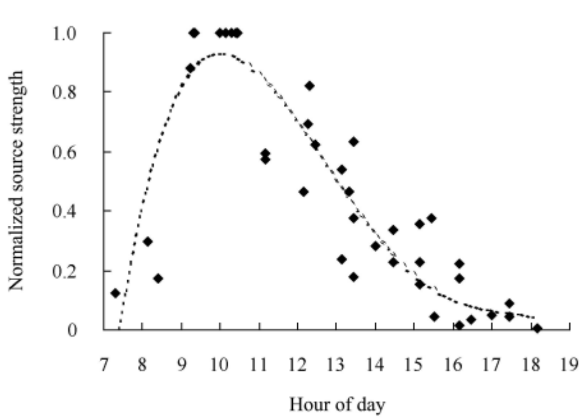

Figure 4 Pollen release pattern during day time

Shown in Figure 5 are the seasonal patterns of the source strength normalized by the corresponding maximum daily value. The daily pollen release increased almost linearly at the beginning of the pollination season. After reaching the peak of pollen shedding when most silks were emerged, the pollen production decreased almost linearly. The pollen shedding season was about two weeks. The seasonal release of pollen grains was 28.72 million grains/m^2/season or 5.41 million grains/plant/season. (Data for days 14 to 16 were not available and daily source strength for these days was extrapolated linearly based on the data from days 8 to 13).

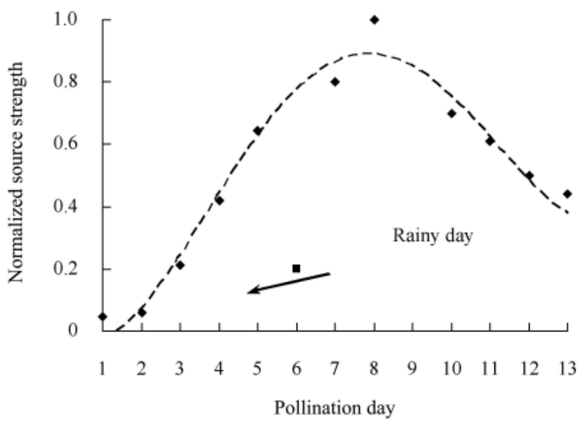

Figure 5 Daily pollen releases during the pollination season

The pollen production was shown to be significantly affected by weather. It rained for a period of time on day 6. The pollen production was much less on that day.

3.2 Comparisons with literature

The source strength data was consistent with the experimental results of Jarosz et al. (2003)[9]. In the study of Jarosz [9] the peak occurred at around 10:30 am (GMT time, close to local solar time) which is similar to the measured peak time around 10:00 am (close to local solar time). The pollen shedding lasted for about two weeks (similar to this experiment) and the peak occurred in about one week after first day of shedding (also similar to this experiment)[9].

The daily release rate (0.75 million grains/plant/day) is reasonable compared with Jarosz et al. (2003)[9] who measured a maximum daily release rate 1.6 million grains/plant/day. The difference between the measurements and that of Jarosz et al. (2003)[9] may have been caused mainly by variety difference. Jarosz et al. (2003)[9] used a France variety, cultivar Adonis (blue grains Pau Semences, France), while a sweet corn variety was used in this research. The seasonal release rate (5.41 million grains/plant/season) is consistent with literature: one corn plant each season can produce 2 million to 5 million grains of pollen[22].

3.3 Advantages and shortcomings of the method

The major advantages of the method in measuring pollen source strength described in this paper over the traditional methods include:

1) The method is nondestructive. It does not damage tassels (flowers), and therefore does not affect the normal growth of the plant elements;

2) It does not introduce experimental bias by using collecting bags;

3) It is dynamic, capable of measuring pollen release over short time periods;

4) It is applicable to plants with heavy pollen grains.

The major shortcoming of the method is that it needs a special shape of planting field. Only with this circular layout the wind direction will not change the effective length between the edge and the center of the plot in the wind direction, i.e. the denominator and the integral upper limit for deposition in equation (1) will not change. More complex mathematical expressions are needed for non-circular source fields.

4 Conclusions

This paper reports an improved method to measure crop pollen source strength. The experimental results were reasonably comparable with literature data. Compared with traditional methods, this improved design offered the following advantages: nondestructive (does not disturb the normal growth and pollen release of the plants), capable of measuring pollen release over short periods of times, and suitable for plants with heavy pollen grains.

For using this method in field studies, the following procedures to be followed are recommended:

1) Plant source plants in a circular area;

2) Measure the vertical concentration profile of pollen concentration in the center of the plot using Rotorod samplers or other instruments;

3) Measure deposition flux density within the plant canopy;

4) Employ a high-frequency anemometer to measure wind speed and direction at the site.

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