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Pollen-mediated gene flow in Chinese commercial fields of glufosinate-resistant canola (*Brassica napus*)

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One of the most pressing issues surrounding transgenic oilseed rape cultivation is the potential impact of GM genes entering neighboring non-GM oilseed rape plants, related weeds or closely related wild relatives. Following the principle of a case-by-case for transgenic crop safety assessment, we designed experiments to study pollen-mediated gene flow from herbicide-resistant transgenic oilseed rape to Chinese commercial varieties. The pollen dispersal was detected as the progeny of recipient plants that were identified as glufosinate resistant. The results indicated that pollen dispersal occurred up to 2000 m and its rate sharply decreased as the distance from the pollen source increased. However, the rate of long-distance pollen dispersal from 33 to 2000 m was less than 0.015% and did not present a gradual decrease, indicating the randomization of dispersal and pollination. Most pollen dispersed within a 4.5 m area around the transgenic pollen source plot, with a maximum of 1.19% at the sampling site of 1.4 m. Wind direction significantly affected the direction and the distance of pollen dispersal, resulting in the more and farther dispersal in southwest direction. However, the number of potentially pollinating bees was not correlated with direction and distance from the pollen source plot, suggesting that bee density and distribution would not contribute to the differences in pollen dispersal among different directions. This paper is the first report on research in a large-scale experiment of pollen-mediated gene flow under the China's environmental conditions and provided scientific evidence for the effective commercialization of transgenic oilseed rape and its safe management. Our results also provided important data on pollen spatial distribution pattern.

oilseed rape, herbicide resistance, pollen dispersal, transgenic biosafety

Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops in the world. The genetically modified (GM) oilseed rape has demonstrated great advantages in rapeseed production as well as environmental benefits in North America[1]. However, it is still necessary to assess the risks to environments and human health in the context of the Chinese environment before GM crops are extensively adopted. Therefore the safety assessment is indispensable and at present a strict safety assessment is adhered to be conducted for a transgenic cultivar and followed by a strict governmental approval procedure before a transgenic cultivar is released. Prior to the launch of any novel technology it is not uncommon for general concerns of risk to be expressed by stakeholders. In this situation confidence in the safety of a product prior to launch can be increased by targeted safety research. One specific area of safety research is to examine transgene flow. In the case of oilseed rape which has a considerable potential for outcross and can pollinate some relatives in the genus Brassica[2−4], transgenes could flow to neighboring traditional cultivars or related wild populations or weeds. Concerns have been ex-

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pressed that transgene flow could affect farming practices, contaminate non-GM crops or irreversibly alter the ecosystems[5]. Therefore, the likelihood of pollen-mediated gene flow has been investigated in several studies[6]. These studies will help establish sustainable land-management practices. The principle of the safety assessment for GM crops is the case-by-case. However, under the China environmental conditions, there is no report to date to the public on study of the farm-scale gene flow of oilseed rape.

In the fields of oilseed rape, cross-fertilization is mediated by physical contact between neighboring plants as well as by insects and wind. Bees have been identified as one of the most prolific pollinators of oilseed rape[7] and both short- and long-distance pollen dispersal by bees was reported, but not necessarily in equal proportions[8]. Some studies demonstrated that insects may play the key role in long distance pollination events[9,10]. Other studies have documented wind dispersal of oilseed rape pollen[11−14]. The dispersal range of pollen varies from a few meters to several kilometers, and the dispersal distances vary with the environmental and topographic conditions[15,16]. Timmons et al.[11] provided evidence of viable pollen wind dispersal up to a distance of 1.5 km. Longer distance dispersal occurs when pollen grains are caught by upward air movements, resulting in transport above the height of the local vegetation and air currents created by surface features[6]. The relative importance of wind and insects as pollen vectors has not been fully elucidated and varies regionally and seasonally[17]. There is no report for such studies in China.

The characteristics of pollen-mediated gene flow support a critical need to assess the risks associated with gene flow from transgenic crops to conventional crops, and such risk studies have been conducted worldwide[6]. Several models have been proposed to predict the dynamics of intra- and inter-specific gene flow and the subsequent potential risks for circumstances[8,18−21]. Despite the attention to transgenic pollen transfer in other parts of the world, the lack of this line of research is clearly evident in China. Although transgenic oilseed rape is not commercialized in China, many organizations are developing transgenic oilseed rape for commercialization. Therefore there is an urgent need to conduct research and to accumulate data on transgenic environmental risk assessments under the Chinese special natural circumstances. In light of the lack of data assessing the environmental risks of transgenic plants in China which is necessary information for transgenic crop management practice, this avenue of research should have a high priority[22].

To date, herbicide resistance has been favored and is the most common transgenic trait for oilseed rape. In addition, it serves as an excellent marker to assess gene flow. In this study, we investigated the pollen-mediated gene flow from herbicide-resistant transgenic oilseed rape variety to the non-transgenic ones in China. Factors known to influence pollen dispersal, including rapeseed varieties, wind direction and pollinating insects were monitored during the flowering period.

1 Materials and methods

1.1 Plant materials

Transgenic oilseed rape Ms8Rf3 harboring the gene *bar* which confers resistance to the herbicide glufosinate, provided by Bayer (China) Co. Ltd, was used as the pollen donor. Ms8Rf3 had not been planted in China before and its flowering time was unknown. So in order to meet the flowering period of Ms8Rf3, two varieties of non-transgenic oilseed rape Qingyou 14 (spring oilseed rape, released by Qinghai Academy of Agricultural Sciences) and Zhongyou 821 (winter oilseed rape, released by Oil Crops Research Institute, OCRI) were used as the pollen recipients.

1.2 Pollen dispersal experiment

The principle of the experiment methods is detection of non-transgenic oilseed rape plants for the transgene *bar* conferring resistance to glufosinate due to pollen-mediated gene flow from transgenic donor oilseed rape plants to non-transgenic plants. Thus, the experiments comprised two parts: pollen dispersal and progeny detection for herbicide resistance in non-transgenic oilseed rape.

Transgenic oilseed rape pollen dispersal experiment was performed from September, 2002 to May, 2003 in farm fields at Hannan, Wuhan, China. An isolation distance of 2000 m was established around the experimental fields. In this area, growth of oilseed rape and cruciferous vegetables was prevented, and cruciferous weeds and oilseed rape volunteers were cleared.

Transgenic oilseed rape Ms8Rf3 was sown in a 15 m × 15 m square plot in the middle of a 120 m × 120 m field (Figure 1). The area surrounding the central plot (donor plot) was sown with two pollen recipient varieties (Qingyou 14 and Zhongyou 821) arranged alternately. The central plot was divided into 5 strips, each 2.5 m × 15 m with 0.5 m strip spacing. Ms8Rf3 was drilled in rows arranged from north to south in each strip of the central plot alternately on September 30 and October 15, 2002. Seeds were sown at a rate of 1 g per 2.5 m row in length (in total 225 rows with 33 cm row spacing). Every early sowing row (112 rows) was neighbored by a late sowing row (113 rows) to ensure an overlap between flowering time of pollen donor and recipient varieties. Seeds of the recipient varieties was broadcasted in the area outside the central plot on 30 September 2003 at a rate of 6 kg/ha.

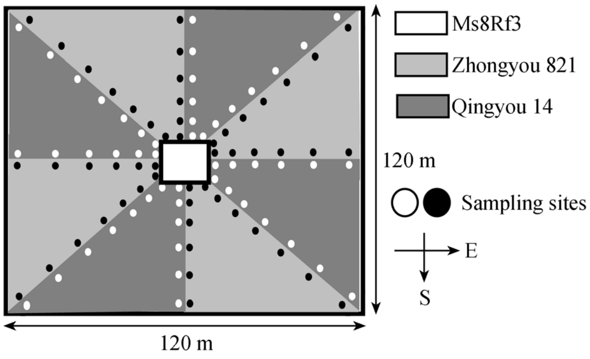

Figure 1 Schematic representation of the experimental design. Transgenic herbicide-resistant plants (Ms8Rf3) were grown in the 15 m × 15 m central plot and recipient plants (Zhongyou 821 and Qingyou 14) in the remainder of the field. E indicated east and S indicated south.

To see if there was predominant influence of wind direction and insect behavior on pollen dispersal during the experiment, we sampled using the following two approaches: (1) along diagonals, resulting in seven sampling distances (1.4, 4.5, 11.5, 20.5, 33.5, 49.5 and 65 m from the donor plot) in the direction of northwest (NW), northeast (NE), southeast (SE) and southwest (SW); and (2) along the horizontal and vertical lines, resulting in six sampling distances (1.4, 4.5, 11.5, 20.5, 33.5 and 49.5 m from the donor plot) in the direction of north (N), east (E), south (S) and west (W) (Figure 1).

In order to detect pollen dispersal distance longer than 120 m, we sowed a traditional cultivar Zhongshuang 4 (released by OCRI, maturity is similar to that of Qinyou 14) in 1 m × 2.5 m plots at the distances of 1000—2000 m away from the south borderline of the above experiment field. At crop maturity, oilseed rape seeds were harvested from these plots.

1.3 Observation of plant growth stages, weather conditions and insect pollinators

The observations were recorded from seed germination to plant maturity for the donor and the recipient oilseed rape, which included the following variables: germination percentages, the dates of each growth stage, weather conditions and the number of presumably pollinating bees.

Various growth stages of oilseed rape were differentiated according to the criteria described by Liu[23]. During the oilseed rape flowering period, anemoscope/dogvane and a remote rainfall recorder were equipped to record wind velocity and direction, rainfall and temperature in the experimental field. Frequency of wind direction and mean velocity were recorded every hour from April 1 to 20, 2003.

To determine if insect pollinating behavior was influenced by direction and the distance from the donor plot, we surveyed the population of insects at three sampling sites (1—5, 30—35 and 60—65 m away from the donor plot) in recipient field at four directions (N, E, S, W). Insects were hand caught (using nets) at 9:00—11:00 am every day from March 19 to April 16, 2003. At each sampling site, five net sweeps were conducted above the plants with a sweeping diameter of 4—5 m. All insects were identified to the bee and other groups described by Wu[24].

1.4 Detection for herbicide resistance in progeny of non-transgenic oilseed rape

At crop maturity, seeds were harvested separately on each sampling site. A total of 108 sets of seeds (104 from the adjacent recipient plants and 4 from the long distance recipient plants) were collected (Figure 1). Resistant individuals were identified in the experimental fields of OCRI farm, Chinese Academy of Agricultural Sciences (CAAS). A split-split plot design was adopted in which two recipient varieties were arranged as the main plot, sampling distances as the subplot and directions as the sub-subplot. Three replicates were conducted, each with a plot size of 2 m × 2 m. Distance between plots was 0.5 m. On May 21 and 22, 2003, the above seeds were sown with a maximum of 20 g seeds per plot. The number of seedlings was spot-checked by using an iron rectangular frame with the dimension of 40 cm × 20 cm. Two frames of each plot were spot-checked at the cotyledon stage or one-true-leaf stage in order to estimate the number of seedlings. All plots were afterwards treated with the herbicide glufosinate (750 g/ha) at the two-true-leaf stage and again 14 d later. Survivors were confirmed resistant by a third application 10 d after the second application. A seedling was considered resistant when it exhibited no signs of chemical burn. The number of resistant seedlings per plot was counted.

These resistant plants were checked by PCR to exclude false-positive plants (i.e. non-transgenic survivors). Genomic DNA from leaves was extracted using a sodium dodecyl sulfate (SDS) extraction method described by Jiang and Chen[25]. Primers were specific for the gene *bar* (forward, 5′-TTTCGGTGACGGGCAGGAC-3′, reverse, 5′-CTGCACCATCGTCAACCAC-3′). Amplifications were started with 5 min at 95℃, followed by 30 cycles of 94℃ for 45 s, 58℃ for 45 s and 72℃ for 1 min, and a final extension step at 72℃for 7 min. PCR products were examined by electrophoresis on a 1.2% agarose gel.

1.5 Statistic analysis

The proportion of herbicide-resistant vs. total progeny was calculated. Variance analysis for the split-split plot experiment was done using JMP 4.04 (SAS Institute Inc.). χ^2-tests were conducted to determine if the number of bees differed significantly between distances and directions. Pollen spatial distribution shapes were constructed using Microsoft Excel 2003.

2 Results

2.1 PCR detection for the gene *bar* in the progeny of non-transgenic plants

The number of seedlings estimated by spot-check in each plot of the progeny detection experiment ranged from 6734 to 6800. In total, about 1134126 and 1137589 seedlings from non-transgenic recipient progeny of Qingyou 14 and Zhongyou 821, respectively, were tested for glufosinate resistance, 1594 and 171 individuals survived after three herbicide applications. In order to exclude seedlings which had survived due to not enough absorption of herbicide, a subsequent PCR analysis was performed with *bar*-specific primers in randomly selected survival individuals (30% for Qingyou 14 and 40% for Zhongyou 821). PCR results showed the amplification of a *bar*-specific DNA fragment with the expected size of 485 bp (Figure 2) and 2.1% of individuals in Qingyou 14 and 1.8% in Zhongyou 821 had no visible amplified products. The final number of the resistant individuals per plot was corrected using the PCR-positive rate for the next analysis.

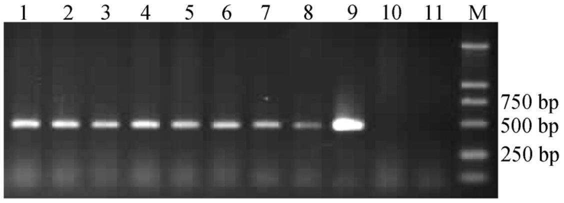

Figure 2 PCR detection from genomic DNA of the survivors after three herbicide applications. 1－8, survivors; 9, the glufosinate-resistant plant of Ms8Rf3; 10, sensitive plant of Qingyou 14; 11, sensitive plant of Zhongyou 821; M, DNA marker DL2000.

2.2 Variance analysis for variety, distance and direction

In the split-split plot experiment, the corrected number of the resistant seedlings in each plot was transformed

Table 1 Variance analysis of the proportion of resistant progeny within variety (Qingyou 14 and Zhongyou 821), and pollen dispersal distance and direction from transgenic oilseed rape Ms8Rf3 to recipients

Source of variation	DF	SS	MS	F	P
Block	2	44.44	22.22	0.38	
Main plot					
Variety	1	14749.03	14749.03	251.58	0.004
Residue	2	117.25	58.62	0.70	
Subplot					
Distance	5	69584.85	13916.97	165.58	<0.001
Variety×distance	5	45522.57	9104.51	108.32	<0.001
Residue	20	1680.97	84.05	1.35	
Sub-subplot					
Direction	7	1761.27	251.61	4.03	<0.001
Distance×direction	35	5938.12	169.66	2.72	<0.001
Variety×direction	7	1106.83	158.12	2.53	0.017
Variety×distance×direction	35	3814.40	108.98	1.75	0.011
Residue	168	10480.00	62.38		
Total	287	154799.75			

into proportion to total seedlings for variance analysis. The results indicated that effect of distance and direction and recipient variety on pollen dispersal was significant ($P<0.01$) (Table 1). Interactions were also significant ($P<0.01$ or 0.05) in any two factors: variety, distance and direction. However, variances of the block and the residue were not significant, indicating that the proportion of resistant progeny from recipient plants was not significantly affected by the soil and the environmental factors of the experiment field.

2.3 Pollen dispersal distance

The proportions of resistant progeny at different distances away from the central donor plot were significantly different ($P < 0.001$, Table 1). The highest proportion was 1.19% from the sampling site 1.4 m away from the donor plot at the southern direction. We averaged the proportion at each direction to generate a subjective overview of the decrease in resistant progeny over distance. In terms of the proportions, pollen dispersal decreased significantly with distance increasing (Figure 3). There was a sharp decrease in the proportion with distance from 1.4 to 4.5 m in Qingyou 14. Up to 20.5 m, just about one in ten thousand seeds were set as consequence of pollination by transgenic pollen. The mean proportions of resistant progeny in Qingyou 14 and Zhongyou 821, at 1.4 m from the donor plot in all directions were 0.7837% and 0.0838%, respectively. The proportions decreased to 0.1171% and 0.0121% at 4.5 m, and to below 0.0400% at 11.5 m. A significant difference ($P<0.001$, Table 1) in the proportion of resistant progeny was observed between Qingyou 14 and Zhongyou 821. However, correlation of the proportion was significant ($r = 0.9998$, $P<0.01$) between the two varieties. Thus significant interaction in variety × distance would originate from a big difference in the proportions at 1.4 m between the two varieties.

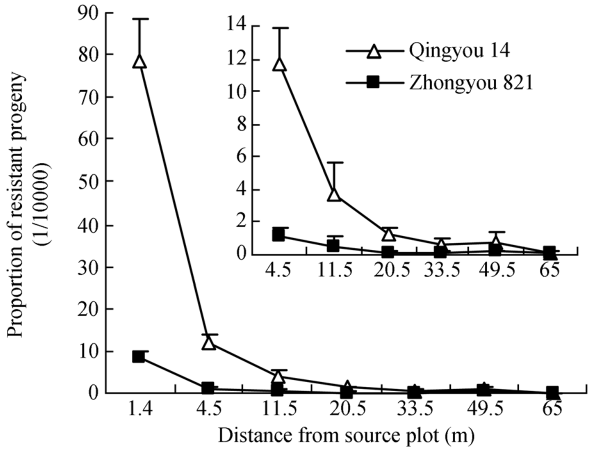

Figure 3 Mean proportions of resistant progeny in Qingyou 14 and Zhongyou 821 at different distances from the pollen source plot. Data for each point was mean from 24 plots (8 directions sampled × 3 replicates for herbicide application). The vertical bars stand for standard error.

For detection of long-distance pollen dispersal, a total of 134945 seeds were harvested from non-transgenic Zhongshuang 4 grown in the fields at the distances of 1000 to 2000 m away from the south borderline of the above 120 m × 120 m experiment field. Among the progeny, nine herbicide-resistant and PCR-positive individuals were detected and survival rates ranged from 0.0030% to 0.0132% (Table 2). The results indicated that long-distance pollen dispersal was at least 2000 m, but the dispersal rates were very low. The rate for pollen dispersal and pollination was less than 0.015% and did not present a gradual decrease at the distances of 33.5—2000 m from the central donor plot, indicating the randomization of dispersal and pollination.

Table 2 Survival rates of herbicide-resistant individuals in long-distance pollen dispersal of Ms8Rf3

Distance and direction	Seedling number	Survival seedlings	Survival rate (%)
1000 m, south	31894	1	0.0031
2000 m, south	32625	2	0.0061
1100 m, south by west 22°	32513	1	0.0030
1200 m, south by west 31°	37913	5	0.0132
Total	134945	9	0.0254

2.4 Pollen dispersal direction

The distributions of pollen dispersal were not isotropic. In terms of proportion of resistant progeny, the variance for the direction component was significant ($P < 0.001$, Table 1). A higher proportion of resistant progeny was observed in the sampling sites situated in southwest of the donor plot than in any other direction (Figure 4(a)). The south was the second highest pollen dispersal direction. These also resulted in a significant interaction in distance × direction. A significant difference in the two recipient varieties was observed ($P<0.001$, Table 1 and Figure 4(a)). However, correlation of the proportions was not significant between the two varieties. Significant interaction in variety × direction would originate from big changes in the proportions at different directions in Qingyou 14.

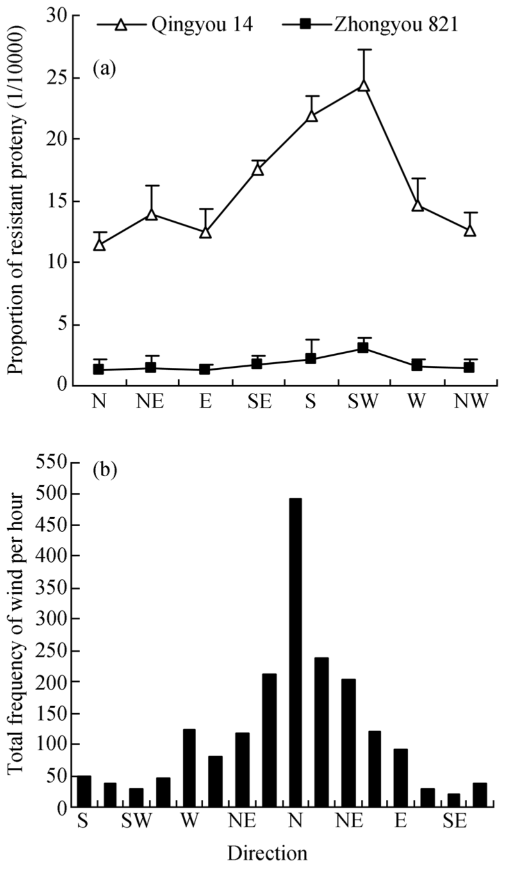

Figure 4 The relationship between mean proportions of resistant progeny in Qingyou 14 and Zhongyou 821 at different directions and wind direction. S, south; SW, southwest; W, west; NW, northwest; N, north; NE, northeast; E, east; SE, southeast. The vertical bars in (a) stand for standard error.

2.5 Pollen dispersal difference within recipient varieties

In this study, significant differences ($P<0.001$, Table 1) of pollen dispersal within recipient variety were observed. The proportions of resistant progeny from Zhongyou 821 were less than that of Qingyou 14 (Figures 3 and 4(a)). Overlapping of flowering time between the donor and recipient varieties is one of the key factors affecting cross pollination. So we observed differences in the phenology of these varieties. The onset of flowering and peak flowering stages in Zhongyou 821 were 12 d earlier than those of transgenic oilseed rape Ms8Rf3 (Table 3). In contrast, Qingyou 14 flowered 1－2 d later than Ms8Rf3. Therefore, Qingyou 14 and Ms8Rf3 experienced a longer overlapping flowering period, resulting in increased opportunities for pollen transfer from Ms8Rf3 to Qingyou 14.

Table 3 Growth stage timing for different oilseed rape varieties

Variety	Emergence date	Start flowering date	Peak flowering date	Date of end of flowering
Zhongyou 821	Oct. 12, 2002	Mar. 12, 2003	Mar. 19, 2003	Apr. 8, 2003
Qingyou 14	Oct. 12, 2002	Mar. 26, 2003	Apr. 1, 2003	Apr. 20, 2003
Ms8Rf3	Oct. 12, 2002	Mar. 24, 2003	Mar. 31, 2003	Apr. 20, 2003

2.6 Factors affecting pollen dispersal distance and direction

Our results indicated that oilseed rape pollen dispersal was affected by certain environmental factors, such as wind and insects. We chose a period of 13 d spanning peak flowering time (mid-flowering stage) of Ms8Rf3 to record the wind direction and velocity. Data showed that northerly wind was most frequent or dominant during the 13-day period, and winds from other directions were rare (Figure 4(b)). These results were consistent with the distribution of resistant progeny as detected in the recipients (Figure 4(a)) and higher proportions of resistant progeny occurred at far distances in the two directions than that in the other ones.

Bees also play a role in pollen dispersal of oilseed rape[7]. During the flowering period, insects at different sites of the recipient field were surveyed. Most insects were identified as bees and the rest were mainly syrphid flies and mosquitoes. There were no significant differences in the number of bees between different directions and distances, indicating that bee distribution was not affected by direction and distance from the donor plants (Figure 5). Therefore, bee density and distribution would not contribute to the differences in pollen dispersal

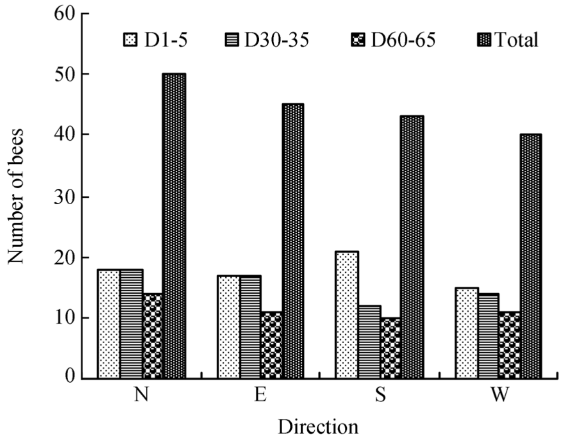

Figure 5 Bee distribution in the experimental field during the flowering period of oilseed rape. Data were taken from March 19 to April 16, 2003. The number of bees was cumulated from 21 samples in 4 directions. D1-5, D30-35 and D60-65 were distances (m) from the source plot. N, north; E, east; S, south; W, west.

among different directions. Exactly differential contribution of wind and bees to short-distance pollen dispersal was not able to be identified in this study.

2.7 Pollen spatial distribution

By using transgene *bar* as a marker, we obtained a set of data reflecting pollen spatial distribution at distance and direction. A quantitative examination of the frequency data suggested the relationship between pollen dispersal distance and direction in the shape of an inverse petunia flower (Figure 6). The higher proportions of resistant progeny were in the vicinity of the source field, and decreased sharply as the distance from the pollen source increased. Pollen dispersal was predominant in a south and southwest direction. This pollen spatial distribution suggested that an increased level of gene flow occurred mainly in a small area around the source plot.

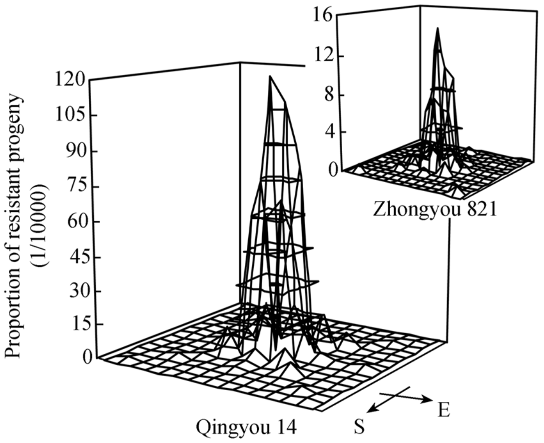

Figure 6 Spatial distribution of herbicide-resistant oilseed rape Ms8Rf3 pollen detected in recipient *cv.* Qingyou 14 and Zhongyou 821. S, south; E, east.

3 Discussion

In outcrossing plants a transgenic plant may hybridize with wild relatives, including both native species and non-native species, or GM crops may cross with non-GM crops of the same species. Therefore, a number of current risk assessment practices for oilseed rape focus on pollen-mediated gene flow. In general, two types of experimental designs are used in pollen dispersal experiments: a continuous design, as described here, where the recipient plants are spaced around the source plot, and a discontinuous design, where recipient plants form patches are situated adjacent or at various distances from the source. The latter is not natural, as in farm fields oilseed rape is usually planted continuously on a large scale and pollen fertilization is competitive or opportunistic between different plants at different sites. Thus some studies suggest that the second experimental design favors long-distance dispersal[26,27]. Irrespective of the design, a number of experiments used small-scale field trials[12,28], which are considered inadequate for assessing gene flow from GM crops grown on a large agricultural scale[29]. To date, only a few studies have carried out large-scale experiments[5,16,29,30]. Rieger et al.[16] performed one of the most realistic studies in pollen-mediated gene flow, who were the only ones investigating cross-fertilization over fragmented landscapes, almost in parallel with this study. This study provides a set of data from a large scale experiment using a continuous design.

To our knowledge, it is the first report on research in a large-scale experiment of pollen-mediated gene flow under the China's environmental conditions. The frequency of detected gene flow up to 2000 m and decreased sharply with distance from the pollen source increased. The proportion of resistant progeny varied, with a maximum of 1.19% at the sampling site of 1.4 m southwest from the source plot, and occurred up to 2000 m southwest. The results were primarily congruent with those from the previous studies[7,16,18]. The results of Scheffler et al.[7] came from the designed experiments conducted in a 1.1-ha field with a pollen source plot of 9 m in diameter. Lavigne et al.[18] conducted their experiments by sowing the source plants in a 10 m × 10 m in the middle of a 90 m × 90 m field. The results of Rieger et al.[16] came from a strategy of designed sampling in farm fields over a wide scale. They reported that resistance was detected in only 30% samples, and the resistance frequencies varied up to a maximum of 0.197%. Their results showed that gene flow via pollen movement occurred between oilseed rape fields, consistent with our study. However, even between fields in Australia gene flow was low and resistance was not detected in rapeseed fields more than 3 km from the source plants. Furthermore, pollen spatial distribution in this study showed that the rate for pollen dispersal and pollination, at the distances of 33.5 to 2000 m from the central donor plot, was less than 0.015% and did not present a gradual decrease, indicating the randomization of dispersal and pollination at distances farther than 33.5 m.

Among factors affecting pollen-mediated gene flow such as environmental conditions, plant species or variety and density, and insect behavior[16], wind in this study was a key factor for the flow between oilseed rape varieties in both short and long distance, which resulted in an asymmetric pollen distribution[29]. A number of other studies have documented pollen wind dispersal in oilseed rape[11 – 13]. Most wind-dispersed pollen travel less than 10 m and the amount of pollen decreases as the distance from the pollen source increases[11,31]. In our study most pollen dispersal was within a 4.5 m area around the transgenic pollen source plot. Furthermore, the direction of predominant pollen dispersal was coincident with wind direction. Lavigne et al.[18] observed similar results. They obtained the results that in the pollen distribution 80% of the pollens fell within the source plot and 12% within 3 m. Moreover more pollen drift was observed on the dominant wind directions.

Data from our experiments indicated no significant differences in bee numbers at different directions and distance from the source plot, suggesting that bee pollinators had no effect on pollen dispersal in any direction. There might be a fact being buried that high proportion of pollen dispersal or cross-fertilization at the sampling sites adjacent to the donor plot would include contribution from bee pollination, i.e. bees might mainly play a role in short distance dispersal. We noted slightly higher bee density near to the central plot than at far distance (Figure 5), which might be explained by the fact that there was no oilseed rape surrounding the experiment fields in the range of 2000 m and bees tend to fly to the field center with more flowers. Other studies indicated that pollen is predominantly deposited by bees on plants close to the pollen source[32,33]. Bees are often observed spending time on one plant, visiting several flowers before moving to an adjacent plant. They are more likely to remain in a single field if sufficient resources (e.g., flowers) are readily available, resulting in localized pollen dispersal[16]. Many studies have shown that up to 80% of bee flights are less than 1 m in distance, with most pollen transported no father than 5 m[9,31]. However, some studies demonstrated that bees transported pollen long-distance, occasionally reaching a maximum of 4 km[6,34]. Our data in the present study did not support the long-distance transfer.

The present research together with previous studies[6,8,14,35] provides scientific data for decision-making of management strategies to minimize gene flow from GM oilseed rape. According to our data, a 5 m discarded buffer zone can achieve less than 0.5%, a maximum value of the benchmark for crop-to-crop cross-fertilization and much less than 0.9%, a threshold set by the EU labeling legislation[36]. Outside the 50 m buffer zone, we did not detect any sampling site with more than 0.01% resistant progeny. Previous studies showed that isolation distance and buffer zone are the most effective physical strategies to regulate gene flow[8]. Damgaard and Kjellsson[35] demonstrated that in small fields, an isolation distance of 50 m reduced the GM-pollination percentage to 0.3% and a 5 m discarded buffer zone surrounding a non-GM-field is expected to reduce GM-pollination by approximately one third. According to our results, a 4.5 m discarded buffer zone could reduce the GM-pollination to below 0.15%. It is worth pointing out that pollen dispersal distance should vary in different regions as topography and wind may different[31,37]. Thus strictly speaking our results are not applicable to a region where topography and wind are different. Moreover, gene flow is only one potential source for the unintended or adventitious presence of GM alleles. Seed admixture, spillage and volunteers as examples can also contribute to the detection of GM alleles outside of areas that have been sown to GM crops. However, from our points of view, a potential environmental risk of transgenic cultivars which have gone through strict safety assessment and approved for release by the governments is controllable because such potential risk in future has been predicted prior to release of the cultivars and a counterplan for the risk prevention has been prepared. Particularly, transgenic cultivars in which “non-target” transgenes are deleted by molecular biology approaches and only a target gene cloned from the same crop or other crops are retained in the targeted genome does not comprise any risk to the environments.

The pollen spatial distribution in this study presented a pattern of inverse petunia flower. The pattern provides not only overall view on pollen-mediated gene flow from the source plot to the surrounding area, but also overall view to guide other production practice, for example, it can be applied to decide isolation distance for production of commercial hybrid seeds and its parent seeds. The pattern also provides estimation of seed purity if there are different varieties growing around the seed production fields. It is especially useful in China where each farmer has small land.

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