



Pollen flow of cultivated rice measured under experimental conditions

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Abstract. The pollen flow pattern of a cultivated rice variety, Minghui-63, was studied at horizontal and vertical levels under experimental conditions. Data obtained from pollen traps for six designed populations (as pollen sources) at different intervals showed that the dispersal of rice pollen decreased with the increase of distance from pollen sources and that the rice pollen flow was significantly influenced by weather conditions, particularly by wind direction and speed. For a mean wind speed of 2.52 m/s in a downwind direction, the observed distance of rice pollen dispersal was 38.4 m, indicating that rice pollen grains normally disperse at a relatively small range. However, the maximum distance of rice pollen flow could be up to 110 m, using regression analysis of pollen flow and wind speed, when the wind speed reached 10 m/s in this study. The frequency of pollen flow was positively correlated with pollen source size within a given range, suggesting that pollen flow will occur effectively at a considerable rate in rice fields with sufficiently large pollen sources. In addition, many more pollen grains were detected at the height of 1.0–1.5 m than at 2.0 m, indicating that rice pollen mainly disperses at relatively low heights. Results from this study are useful both for minimizing transgene escape from transgenic rice and *in situ* conservation of wild relatives of rice, as well as for hybrid seed production, where an effective isolation buffer zone needs to be established.

Introduction

With the rapid development of transgenic technology, an increasing number of transgenic crop varieties have been released into the environment, since the first transgenic tomato modified for delayed ripening was officially approved for commercial production. Alongside this, there have been growing debates about the biosafety of transgenic crops, e.g. concerning the degree to which release into the environment might bring ecological risks, including transgene escape to non-transgenic crop populations or to close wild relative species through cross-pollination. Transgene escape could lead to contamination of non-transgenic varieties or cause serious weed problems (Arriola 1997; Raybould 1999; Dale et al. 2002). Therefore, effective measures should be taken to minimize transgene escape, in addition to cautiously assessing environmental impacts and monitoring ecological consequences of transgenic crop release.

Pollen flow is one of the essential components in the ecological risk assessment of transgenic crops, because pollen can act as a vehicle to disseminate transferred alien

genes. Such an assessment usually includes pollen spreading rate, the maximum distance of pollen dispersal (horizontal), spatial dynamics of pollen movement (vertical), and the patterns of pollen dispersal of a crop (Amand et al. 2000). On the other hand, the understanding of pollen flow is also significant for the *in situ* conservation of wild relatives of crop species, particularly for those that are important genetic resources for crop improvement but are endangered or under great threat either by direct human activities or other factors. Efficient *in situ* conservation of wild relative species should consider the strategic selection of conservation sites where an effective isolation distance between a crop (particularly the transgenic crop) and its wild relative species needs to be determined to avoid contamination of the target species by pollen flow. Considerable pollen flow between crops and wild relatives will result in outcrossing and, consequently, alter the gene pool of wild and cultivated species (Ellstrand et al. 1999). Moreover, pollen pollution must be avoided in conventional crop breeding, especially for hybrid rice. Therefore, solid studies of crop pollen flow can provide valuable information for determining the minimum isolation distance for the buffering area between crops and their wild relatives or between breeding fields, for both prevention of transgene escape and successful *in situ* conservation guidance, or for harvesting high quality commercial seeds.

It is shown in the literature that airborne pollen flow can follow an exponential leptokurtic pattern and can be greatly influenced by meteorological factors, especially microclimatic conditions, such as wind speed and direction, ambient temperature, and relative air humidity (Tonsor 1985; Jackson and Lyford 1999; Galan et al. 2000). It is generally understood that pollen dispersal is linked to height, that herbaceous species have more intensive pollen content at lower heights (Alcazar and Comtois 2000), and that airborne pollen flow and source size are more significant than density, as is the case in anthophilous pollen flow (Rognli et al. 2000). However, the pollen flows of different plants mediated by various vectors differ significantly in their dispersal patterns (Alcazar et al. 1998). It is therefore necessary to carry out a detailed investigation of pollen flow of a particular species, where ecological risk assessment requires an accurate estimation of pollen flow including both distance and intensity of pollen dispersal (Amand et al. 2000).

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops, providing staple food for over 50% of the global population. To enhance rice productivity for food security of the world's growing population, a number of new rice varieties with agronomically beneficial genes have been developed by transgenic methods. Some of these transgenic rice varieties have been released into the environment for field testing in China (Huang et al. 2002). Detailed studies on the potential ecological risks associated with these transgenic rice varieties should be carried out before they are extensively released into the environment. Rice is an anemophilous plant and has airborne pollen flow, but little is known about the pattern of its pollen flow, although there is some literature recording pollen flow of Poaceae plants, which may be useful as a predictive reference for rice pollen flow (McDonald 1980; Norris-Hill 1997; Fernandez et al. 1998).

Common wild rice (*O. rufipogon* Griff.) is the ancestral species of cultivated rice

and has served as an important genetic resource for rice improvement (Oka 1988). In some Asian countries, such as China, Vietnam, Thailand, and Bangladesh, the survival of *O. rufipogon* is under threat. Therefore, effective conservation of this species has been prioritized in genetic resource programs in these countries. It has been shown that spontaneous hybridization and gene flow between *O. rufipogon* and *O. sativa* occur frequently in nature due to their limited reproductive isolation (Lu 1999; Song et al. 2002). This poses a serious risk of transgene escape to wild relative species when transgenic rice varieties are released into their vicinity, through contamination of some original and rare *O. rufipogon* populations. Accurate data on rice pollen flow are therefore important for determining effective isolation or buffer zones between the cultivated and wild rice species, which can be imperative both for the safe release of transgenic rice and *in situ* conservation of wild rice genetic resources. Therefore, we designed this experiment to study rice pollen flow, particularly the effects of pollen source size and the height of pollen recipients on pollen dispersal under the influence of various weather conditions.

Materials and methods

Plant material

The cultivated rice variety, Minghui-63, donated by Prof S.M. Mu from Hubei Academy of Agricultural Science of China, was used as the pollen source. Minghui-63 is a universal paternal line used for hybrid rice breeding with a 90–95 day maturation period, and it usually produces a relatively large number of pollen grains.

Experimental design

A total of six Minghui-63 populations, MP-1–MP-6, were constructed at a selected site in Chaling, Hunan Province, China. MP-1–MP-5 were designed as round populations with radii of 0.3, 0.6, 1.2, 2.4, and 4.8 m, respectively, and MP-6 was planted as a rectangular population sized $10 \times 6 \text{ m}^2$ (Table 1). The study site was selected in a rice field isolated by south-to-north hills, where no pollen grains from other rice fields could reach the experimental populations. Distances between experimental populations were more than 50 m to avoid interference with each other. The MP-1–MP-5 populations were constructed in 1999 and 2000, whereas

Table 1. The cultivated rice Minghui-63 populations designed for the pollen flow experiment.

Population code	Size of population	Shape of population
MP-1	Radius = 0.3 m	Circle
MP-2	Radius = 0.6 m	Circle
MP-3	Radius = 1.2 m	Circle
MP-4	Radius = 2.4 m	Circle
MP-5	Radius = 4.8 m	Circle
MP-6	$6 \times 10 \text{ m}^2$	Rectangle

the MP-6 population was constructed in 2000 at the same site. Construction of the additional population MP-6 is based on the findings in the 1999 experiment that a relatively prevailing wind blew from north to south during the Minghui-63 flowering period, and that the wind speed had a detectable effect on pollen flow. Seeds of Minghui-63 germinated in the middle of June, and seedlings were transplanted with 10×18 cm intervals in the middle of July. Soil preparation and irrigation management were typical for the rice agricultural systems of Hunan Province.

Airborne pollen measurement

Pollen flow was recorded during the flowering period of Minghui-63 between mid-September and mid-October. The measurement of pollen flow included two parts: pollen flow distance from each source, and pollen density at particular intervals. The pollen trap method described by Kearns and Inouye (1993) was used to detect the presence of pollen and to measure its density in all experimental plots. A microscope glass slide (7.62×2.54 cm²) was coated with vaseline and attached to a wooden stake; in each plot three stakes were placed at particular intervals. The glass slides were arranged at flower height level for all populations, except for the MP-5 population where the glass slides were arranged at different heights of 0, 0.5, 1.0, 1.5, and 2.0 m, respectively. All the plots were arranged downwind with 1.2 m intervals. Plots for the MP-1–MP-5 populations were arranged from 1.2 to 24 m, and those for the MP-6 population were arranged between 1.2 and 48 m from pollen sources. In addition, for the MP-5 population, plots with the same intervals as above were arranged around the pollen source in eight geographical orientations in order to detect the relationship between pollen flow and wind direction (Figure 1). These were east, southeast, south, southwest, west, northwest, north, and northeast,

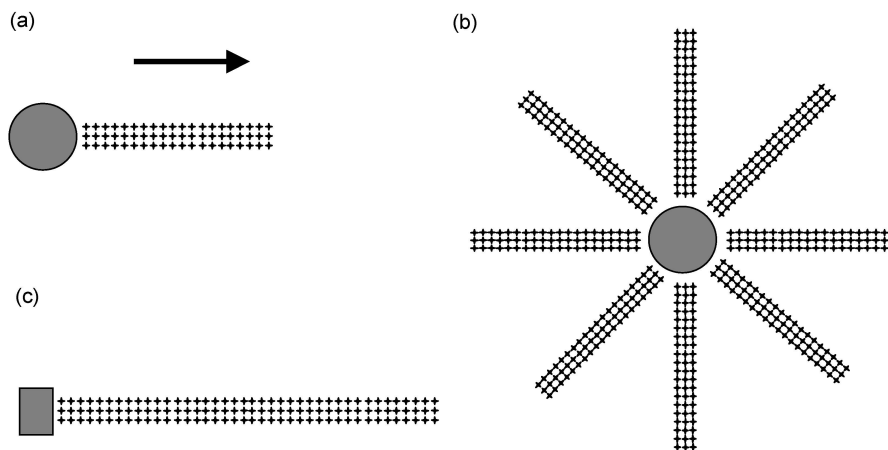


Figure 1. Illustration of pollen flow measurement. (a) MP-1–4 populations; (b) MP-5 population; (c) MP-6 population. The symbol '+' indicates the positions of pollen traps; the shaded circles or rectangles represent Minghui-63; the arrow shows the prevailing wind.

respectively. On sunny days, pollen traps were set up around 8:30 A.M. before flowering and collected around 4:30 P.M. after most flowers had discharged. The atmospheric parameters, including temperature, air humidity, atmospheric pressure, wind speed, and wind direction, were recorded on the same day as pollen flow was measured, using a portable microclimate meter at 09:30 and 11:00 A.M., and 1:30, 2:00, and 3:30 P.M., respectively. The collected smears of glass slides were observed under a microscope after staining with aniline blue in lactophenol (Kearns and Inouye 1993). The number of pollen grains was counted and pollen density was calculated as number of grains per cm^2 .

Data analysis

The ANOVA procedure was used to analyze the effectiveness of pollen flow, including pollen dispersal distances and pollen density at certain intervals, against the size of pollen source, wind speed and direction, and the height of receptors. Regression analysis was conducted for variants with significant correlation between pollen flow at a certain interval and the size of pollen source, in the MP-1–MP-5 populations. The regression module was also used to analyze relationships between pollen flow and wind direction (MP-5) or wind speed (MP-6). All statistics analyses were performed using the STATISTICA for Windows software package (single user version (5.0), StatSoft Inc. 1995).

Results

The size of pollen source and pollen flow

The data of downwind pollen flow from different populations (MP-1–MP-5) on the same day were compared with each other. The results showed that the intensity of pollen flow (density) was significantly correlated with the size of the pollen source within the range of our experiment (ANOVA, $F = 63.472$, $P = 0.001$). The greater the size of the Minghui-63 population used as a pollen source, the higher the pollen density detected at the same distance. It was evident that pollen density radically increased in the MP-5 population with the largest size (72 m^2) (Figure 2). The mean pollen density detected at the 4.8 m interval in the MP-1–MP-5 populations was selected to demonstrate relationships between pollen flow and the size of pollen sources. The result clearly indicates that pollen density at the range of pollen flow increased with the increase of size in pollen sources $\{y = 2.995 \exp(0.704x), r = 0.805, P < 0.001\}$. The same trend of size effect was observed at the different intervals in our experiment (Figure 2). All downwind pollen traps set for the MP-1–MP-5 populations captured rice pollen grains with different densities, clearly indicating that the distance of rice pollen flow should be beyond 24 m. Thus, a relationship between range (maximum distance) of rice pollen flow and pollen source size could not be detected in this study.

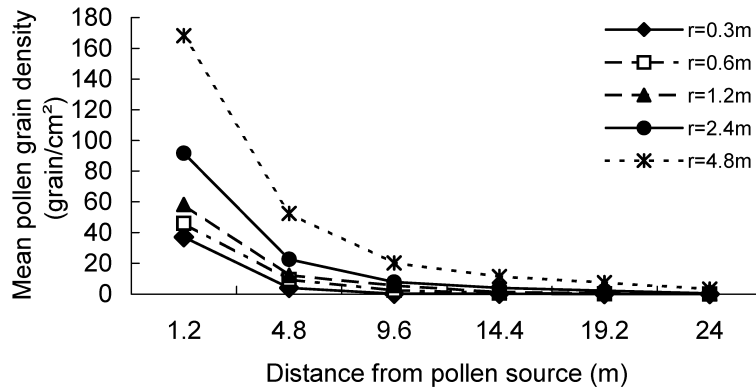


Figure 2. Downwind pollen flow of the MP-1–MP-5 Minghui-63 populations under the following weather conditions: temperature = 33.1 °C, air humidity = 67.4%, and wind speed = 1.72 m/s. r = radius of pollen sources.

Wind direction and pollen flow

The ANOVA analysis with geographical orientation as independent variant and distance as co-variant showed significant differences in pollen dispersal among geographical orientations around the MP-5 population (radius = 4.8 m) ($F = 4.888$, $P = 0.004$). More pollen grains were found to disperse for southern and southeastern orientations (Figure 3). The regression analysis indicated that pollen dispersal generally reduced in all orientations with increasing distance ($r = -0.624$, $P < 0.001$), but the distance of pollen flow in different orientations was significantly different. Figure 3 shows that pollen dispersal occurred within 24 m from the pollen source for the northern, northeastern, western, and southwestern orientations, but over 24 m for the other orientations. The ratio of pollen density between 24 and 1.2

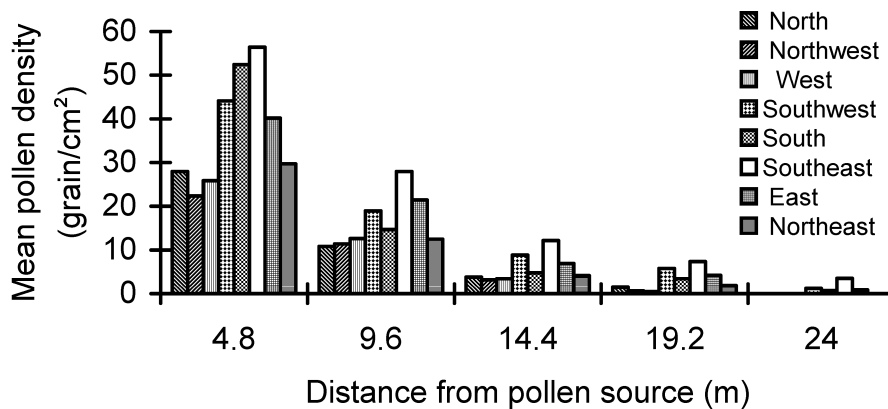


Figure 3. Pollen flow at eight orientations around the MP-5 Minghui-63 population under the same microclimate conditions, temperature = 33.4 °C, air humidity = 65.2%, and wind speed = 1.52 m/s.

Table 2. One-way ANOVA for the microclimate parameters recorded during monitoring of pollen flow in the MP-6 population.

	September 25	September 26	September 27	ANOVA					
				df1	MS	df2	MS	F	P
Temperature (°C)	35.2 ± 2.77	35.9 ± 3.23	34.2 ± 3.16	2	14.47	12	9.38	1.54	0.253
Humidity (%)	60.6 ± 7.98	62.4 ± 7.02	63.2 ± 8.79	2	0.27	12	0.63	0.43	0.660
Atmospheric pressure (mmHg)	99.3 ± 0.16	99.5 ± 0.18	99.6 ± 0.28	2	0.01	12	0.05	3.21	0.076
Wind speed (m/s)	1.84 ± 0.88	1.61 ± 0.67	2.52 ± 1.12	2	10.55	12	1.75	6.04	0.015

m intervals was 0.19% for the southeast orientation, indicating a slow reduction of pollen density with the increase of distance, compared with that for southern, southwestern, and eastern orientations (0.05, 0.08, and 0.07%, respectively). The longest distance of pollen flow could therefore be expected for this orientation, as can be inferred from Figure 3. This pattern of pollen flow correlated with the prevailing wind that was monitored at the study site, where the wind mainly blew from the northwest or north, although it could vary occasionally during the flowering period.

Wind speed and pollen flow

Pollen flow of the MP-6 population was detected for three consecutive days with relatively calm weather conditions. The weather parameters for the three days were not significantly different, except for wind speed (ANOVA test, Table 2). This means that the possible variation in pollen flow was mainly attributed to variation in wind speed on different days. The ratios of pollen density at intervals between 24 and 1.2 m under different wind speeds were 0.3% (vs. 1.61 m/s), 0.5% (vs. 1.84 m/s), and 2.0% (vs. 2.52 m/s), respectively. The longest distances of pollen flow observed under wind speeds of 1.61 and 1.84 m/s were about 28.8 m. The ratio of pollen density between 38.4 and 1.2 m intervals at a wind speed of 2.52 m/s was 0.4%. The distance of 38.4 m was also the maximum distance where rice pollen grains were found in this study. This indicates that pollen dispersal of rice was influenced by wind speed. The ANOVA method was used to analyse the influence of wind speed on pollen movement using wind speed as an independent variant and distance as co-variant. It was further revealed that wind speed had a significant influence on pollen dispersal ($F = 21.043$, $P < 0.001$); both intensity and distance of pollen flow increase with the enhancement of wind speed (Figure 4). The correlation between distance of rice pollen flow and wind speed was analyzed using the present data. It was shown that the distance of pollen flow significantly increased with the enhancement of wind speed ($r = 0.897$, $P = 0.006$). The maximum distance of rice pollen flow could be as far as 110 m at a wind speed of 10 m/s, based on the regression analysis.

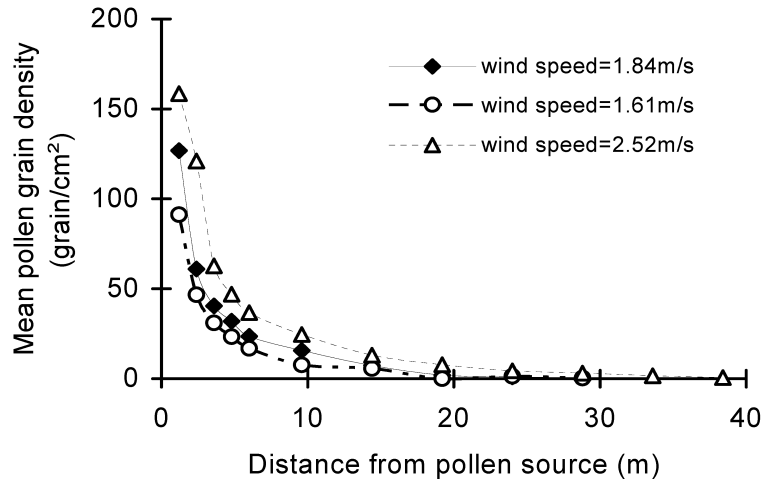


Figure 4. Pollen flow of the MP-6 population detected at wind speeds of 1.61, 1.84, and 2.52 m/s.

Pollen flow at different height

The study designed an experiment to compare the number of pollen grains deposited at five different vertical levels of 0, 0.5, 1.0, 1.5, and 2.0 m, respectively. The results showed that the densities of deposited pollen grains at different height levels were significantly different (ANOVA, $F = 4.888$, $P = 0.004$). The lowest pollen grain density was detected at the 2.0 m height at all intervals. At the 1.2 m interval, moderate pollen density at heights of 0.0 and 0.5 m, and high pollen density at the 1.0 m height were found. Within 6 m, a significantly higher pollen density was found at heights of 1.0 and 1.5 m compared to other heights (Figure 5). However, a relatively high pollen density was found at the 0.0 m level beyond the distance of 6 m, then followed by the 1.5 and 1.0 m levels. The ratios of pollen density between 24 and 1.2 m intervals at different heights (from 0.0 to 2.0 m height) were 8.8, 2.6, 2.5, 2.7, and 5.7%, respectively. This indicates that pollen dispersal at heights of 0.0 and 2.0 m declined relatively slowly compared with that at the other levels.

Discussion

Studies of pollen flow in rice are essential for establishing an effective buffering zone to prevent transgene escape to wild rice relatives or transgene contamination of non-transgenic rice varieties through cross-pollination (Dale et al. 2002). Usually, patterns such as maximum distance of pollen flow, pollen density at a given horizontal and vertical interval, size effect of a pollen source, and factors like wind

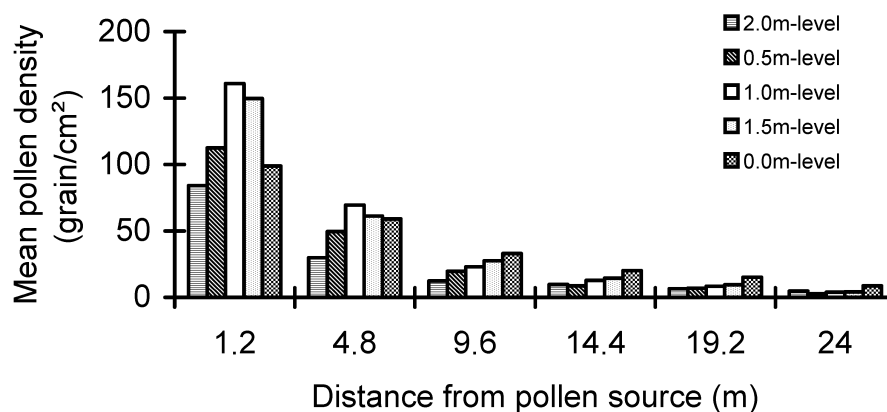


Figure 5. Pollen flow of the MP-5 population detected downwind at different height levels under the following micro-climate conditions: temperature = 34.2 °C, air humidity = 63.2%, and wind speed = 2.87 m/s.

direction and speed, that can significantly affect pollen flow, are important pieces of information for us to gain a better understanding of pollen flow. Our data in this study show that the intensity of rice pollen flow is significantly correlated with the size of a pollen source at the given range. This can be explained as the reason why the larger pollen sources with the same plant density produced more pollen grains that were released from anthers simultaneously, and more pollen grains were certainly disseminated by wind at a given range. Therefore, the intensity of rice pollen flow showed a size effect on pollen source, rather than a density effect as reported in anthophilous cases (Rognli et al. 2000). Theoretically, for a wind pollinating species, the size of pollen sources would not considerably affect the distance of pollen dispersal, because wind-mediated pollen depends mainly on pollen grain architecture and shape when weather parameters were fixed (Jackson and Lyford 1999). However, the present study did not show this relationship due to the limitation of the sampling range (within 24 m). Nevertheless, our present study suggests that pollen flow will occur effectively at a considerable rate in the rice field where sufficient pollen sources are available.

Our results indicated that rice airborne pollen flow was significantly influenced by wind direction and speed, which is in agreement with the viewpoint that meteorological/weather factors have an effect on airborne pollen flow (Jackson and Lyford 1999; Galan et al. 2000), although influences of temperature, humidity, and air pressure were not demonstrated because the measurement of pollen flow in this study was carried out on sunny days with calm atmospheric conditions (Table 2). This result is also in accordance with the data of gene flow from transgenic to non-transgenic rice studied by Messeguer et al. (2001) and is further supported by the study of rice to wild rice gene flow detected with specific SSR markers (Song et al. 2003). The experimental site was surrounded by south-to-north hills that allowed wind to blow mainly from north to south on calm days when pollen flow was tested,

although whirlwinds occurred occasionally. This special landscape also enables us to set pollen traps for downwind orientations, to analyze relationships between pollen flow and wind speed, and to obtain regression for pollen flow with different wind speeds. Apart from the downwind orientations, a small amount of pollen grains at other orientations might be due to whirlwinds that would result in a relatively uniform spatial distribution of pollen flow at all orientations (Jackson and Lyford 1999). Nevertheless, the cardinal wind mainly accounted for the distribution of pollen flow in this study. Undoubtedly, a detailed study is needed to find out what the influences of other weather parameters are on the distribution of rice pollen flow under normal field conditions.

Data from our experiment indicated that the observed distance of pollen dispersal of the cultivated rice Minghui-63 was about 40 m for calm weather with a relatively moderate wind speed. It is slightly shorter than the 43.2 m distance found for gene flow between cultivated rice (with the same pollen donor Minghui-63) and wild rice (Song et al. 2003). These distances are significantly longer than the isolation distance of 10 m that was considered sufficient in hybrid rice seed production to avoid contamination with pollen from adjacent fields (Khush 1993). Apparently, this observed value does not reflect the actual range of rice pollen flow at the horizontal level. Like other wind-pollinated crops (Timmons et al. 1995; Lavigne et al. 1998), pollen flow of cultivated rice observed in this study could be strongly influenced by wind, both in terms of direction and strength. The distance of pollen dispersal increased significantly with the increase of wind speed. Under weather conditions where the wind speed reached 10 m/s, the calculated maximum range of rice pollen flow could reach as far as 110 m. The wind speed of 10 m/s is about grade 5 wind power, which occurs frequently during the rice flowering period in southern China – the region of major Chinese rice production and wild rice *O. rufipogon* distribution. Transgene escape or contamination of wild rice populations and hybrid seed production will occur under these conditions if no effective isolation measures are taken. However, when the wind power gets stronger and exceeds grade 5, the capture of pollen grains by rice stigmas and germination of the captured pollen grains become difficult; moreover, such strong wind will significantly affect fertilization of the rice plants. Although rice pollen longevity is relatively short (Khush 1993; Song et al. 2001), which can influence cross-pollination and may shorten the distance of gene dissemination or decrease pollination efficiency, it is impossible that all distantly dispersed pollen grains are unviable. In addition, airborne pollen or seed dispersal distribution has a thick tail (Bullock and Clarke 2000), which implies that the dispersal distance might be much longer. Based on the above discussion, we suggest in general that an isolation distance up to 110 m should be considered between transgenic rice and its close wild relatives (or non-transgenic rice varieties) and between hybrid seed-production fields under normal weather conditions, both to prevent transgene escape or *in situ* conservation of wild rice species such as *O. rufipogon*, as well as to avoid pollen pollution in rice breeding.

Knowledge about vertical rice pollen flow is very limited. Our present study demonstrated that the movement of pollen grains at different heights was sig-

nificantly different, which supports the conclusion that the height placement of the pollen traps is related to efficiency in pollen grain capture (Alcazar et al. 1998). It was shown that more pollen grains were captured at the 1.0 and 1.5 m heights in this study. This result also supports the conclusion that greater pollen flow is distributed at relatively lower heights for herbaceous species (Alcazar et al. 1998; Alcazar and Comtois 2000), suggesting that an appropriate height of pollen trap placement was crucial for a reliable estimation of airborne pollen flow. Pollen flow (density) detected at 0.5–1.5 m heights was not significantly different, indicating that the placement of pollen traps around the height of Minghui-63's panicles (ca. 0.7 m) in this study was properly designed, and the results of pollen flow in this study were reliable.

In addition, the results of vertical rice pollen movement can serve as a guide for effective isolation setting in the management of transgenic rice. The data, showing relatively few rice pollen grains detected at the 2.0 m level and the majority of pollen grains moving below the height of 1.5 m, suggest that tall crops such as sugarcane can be used as an effective buffer objective to limit the distant dispersal of transgenic pollen, when there is insufficient space between transgenic rice and its wild relative species or non-transgenic counterparts.

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