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Risk Assessment for Transgenic Sorghum in Africa: Crop-to-Crop Gene Flow in *Sorghum bicolor* (L.) Moench

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ABSTRACT

A crop-to-crop gene flow risk assessment study was conducted with *Sorghum bicolor* subsp. *bicolor* to estimate the impact of transgenic sorghum in (South) Africa. The trial was conducted with a central sorghum field (30 × 30 m) with male fertile donor plants that was surrounded by eight arms planted with male sterile recipient plants at a distance of 13 to 158 m from the central field. Gene flow was relatively high within the first 40 m and relatively low beyond that distance, but gene flow was detected even at the greatest distance investigated (158 m). The average hybridization or outcrossing rate for male sterile plants was 2.54% at 13 m, below 1% at a distance of 26 m or greater, and eventually dropping to 0.06% at 158 m. Outcrossing rates are expected to be even lower for male fertile plants, which were not investigated in this study. Mathematical models were used to estimate the maximum gene flow distance that is expected to be between approximately 200 and 700 m. These values are in line with observational data from sorghum plant breeders, who use an isolation distance of 100 m to achieve less than 1% genetic pollution. On the basis of the presence of fully fertile crop wild relatives and the weedy relative johnsongrass [*S. halepense* (L.) Pers.], which may form hybrids with crop sorghum, and on the fact that gene flow takes place, there is strong evidence that introgression of genetically modified- (GM)-sorghum into crops and crop wild relatives will take place once GM-sorghum is deployed.

The majority of world crops originated in today's developing countries in so-called Vavilov centers, regions that hold a high diversity of traditional crop varieties and crop wild relatives. These regions hold the main genetic resources for crops and are especially important to secure current and future plant breeding programs. They serve as "genetic insurance" against upcoming pests or changing abiotic conditions (Swanson, 1995; Tanksley and McCouch, 1997). In light of the increasing deployment of GM crops in developing countries (James, 2003), scientific risk assessment of transgenic crops and its impact on conventionally bred crops and crop wild relatives is needed to further establish adequate biosafety regulations. Information on probable introgression of transgenes into wild relatives and its economic and ecological consequences is still rare despite increasing study efforts. Therefore, data are needed on outcrossing rates, outcrossing distance, and the consequences of gene flow. Gene flow has been investigated for some crops, such as rice (*Oryza sativa* L.), rapeseed (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), beet (*Beta vulgaris* L.)and maize (*Zea mays* L.) (Ellstrand et al., 1999; Hall et al., 2000; Lavigne et al., 1998; Reboud, 2003; Snow et al., 2003; Song et al., 2004; St. Amand et al., 2000). Nonetheless, for one important crop, sorghum, little information on gene flow and especially on the outcrossing distance is available, despite the fact that transgenic, herbicide-resistant sorghum could be developed soon (Arriola, 1995; Arriola and Ellstrand, 1996; Arriola and Ellstrand, 1997; Tadesse et al., 2003). If this gene were to "escape" into the environment to a weed such as johnsongrass, it would have a major impact on the control of this weed. To assess the environmental risk of possible future deployment of transgenic sorghum, data on the outward flow of genes through sorghum pollen to nontarget relatives is needed. This study investigates crop-to-crop gene flow in sorghum. In the case of sorghum, a special emphasis must be placed on Africa—its center of origin—where a large number of sexually compatible weeds, wild relatives, strains, and races of cultivated sorghum occur (Harlan, 1976; Doggett, 1988). Also, sorghum is an important staple food crop in Africa, South Asia, and Central America and was ranked the seventh most important crop worldwide in terms of harvested area. It is the dietary staple for more than 500 million people in more than 30 countries and more than 50% of the global harvest takes place in Africa (FAOSTAT, 2002; NRC, 1996). In terms of plant-derived energy uptake, only rice, wheat (*Triticum aestivum* L.), maize, and potatoes (*Solanum tuberosum* L.) surpass sorghum in feeding humans worldwide. Sorghums are remarkably drought-resistant and vitally important where the climate is too dry for maize, i.e., at annual rainfalls ranging from 350 to 750 mm (FAO, 2003; Wenzel, 2003), and it tolerates an astounding array of soils. These characteristics make sorghum an ideal food crop in semiarid areas of Africa (and elsewhere) where other crops, such as maize, would fail.

Sorghum Taxonomy and Outcrossing

Sorghum belongs to the family Poaceae, and the genus is subdivided into five sections. The section sorghum includes three species (de Wet, 1978; Doggett, 1988; Smith and Frederiksen, 2000; Raemakers, 2001; McGuire, 2004): *S. halepense* (weed, johnsongrass), *S. propinquum* (Kunth) Hitchc. (perennial, fully fertile with *S. bicolor*), and *S. bicolor* with three subspecies. The three *S. bicolor* subspecies are *S. bicolor* subsp. *bicolor* (cultivated species, five main races), *S. bicolor* subsp. *arundinaceum* (Desv.) de Wet & J. R. Harlan ex Davidse [synonym to subsp. *verticilliflorum* (Steud.) Stapf] (the wild progenitor of cultivated

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sorghum, four main races), and *S. bicolor* subsp. *drummondii* (Steud.) de Wet ex Davidse (sudangrass, weed).

No reproduction barrier exists between cultivated *S. bicolor* subsp. *bicolor* and its wild progenitor *S. bicolor* subsp. *arundinaceum* (hybrids form shattercane-type weeds) and the weed *S. bicolor* subsp. *drummondii* (de Wet, 1978; Doggett, 1988). *Sorghum bicolor* × *almum* Parodi is a rhizomatous hybrid between *S. bicolor* and *S. halepense* and is occasionally cultivated as a fodder grass. If backcrossed to *S. halepense*, it can give rise to aggressive weeds, including johnsongrass, which was classified as one of the world's most noxious weeds (Pope and Martins, 2002; Holm et al., 1977). Sorghum is largely self pollinated, but wind pollination between plants does occur (McGuire, 2004). Subspecies or varieties of sorghum with open, grass-like panicles, such as sudangrass (*S. bicolor*), have a higher rate of outcrossing than sorghum, with compact heads typical of commercial hybrids. Outcrossing also varies by location on the panicle, with much higher rates at the top of the panicle, where flowering initiates (Maunder and Sharp, 1963).

Gene Flow

Gene flow can be expected to occur in many crop–weed complexes in cases where the crop and the weed have sympatric ranges, are sexually compatible, have overlapping flowering times, and share a common pollination mechanism. Several studies demonstrated that hybridization in the sorghum crop–weed–wild relatives complex takes place (Arriola and Ellstrand, 1997; Baker, 1972; Doggett, 1988; Ellstrand et al., 1999; ICRISAT, 2002). Molecular and genetic analyses revealed crop-specific alleles in sorghum wild relatives when it co-occurs with the crop in Africa, suggesting that intraspecific hybridization and introgression are common (Aldrich and Doebley, 1992; Aldrich et al., 1992; Smith and Frederiksen, 2000). Arriola and Ellstrand (1997) concluded that a transgene that is either neutral or even beneficial to johnsongrass is likely to persist in populations growing under agricultural conditions under continued gene flow from the crop. Although the triploid progeny of johnsongrass × sorghum hybrids are usually sterile, such hybrids are expected to propagate vegetatively through rhizomes and to persist under agricultural conditions. Furthermore, limited viable seed production on johnsongrass × sorghum hybrids has been reported (Hoang-Tang and Liang, 1988). Introgression from crop sorghum has been implicated in the evolution of increased weediness in one of the world's worst weeds, *S. halepense* (Holm et al., 1977). Although not classified as a noxious weed, weed-like *S. bicolor* (e.g., shattercane that is a hybrid between subsp. *bicolor* and subsp. *arundinaceum*) is common and can cause great economic damage to commercial maize, soybean [*Glycine max* (L.) Merr.], and sorghum production (ICRISAT, 2002).

The objectives of this study were to investigate the crop-to-crop gene flow in *Sorghum bicolor* subsp. *bicolor* (race kafir) depending on the distance between pollen source and pollen recipient, and—on the basis of the experimental data—to estimate proper distances, or buffer zones, to avoid gene flow between sorghum crops and/or its wild relatives.

MATERIALS AND METHODS

Sorghum Gene Flow Field Trial

The sorghum field trial was conducted on the 4000-ha Agricultural Research Council (ARC) research farm Roodeplaat, approximately 20 km northeast of Pretoria, South Africa (25° 31′ S and 28°21′ E, altitude approximately 1160 m). The trial took place in a nonsorghum growing area, at least 5 km from any other sorghum field and at least 2 km from wild or weedy sorghum plants.

Trial Design

Serving as the pollen source, a central block, roughly 30 × 30 m, was planted with the B-line of Redlan Pannar Ps 1051 B/168(015). The central block contained 35 rows approximately 90 cm apart; within a row, the individual sorghum plants were about 30 cm apart. Radiating from the central block, small blocks of the male sterile A-line of Redlan Pannar Ps 1051 A/52(015) were planted, serving as pollen receptors. The small blocks consisted of three rows, each 3 m long. The small blocks were 13 m apart for the first 10 blocks, thereafter 28 m apart until about 158 m from the central block. Eight of these radiating arms were planted at an angle of 45° from each other (see Fig. 1). The trial was planted under dry-land conditions and irrigated when necessary. The area where the trial was

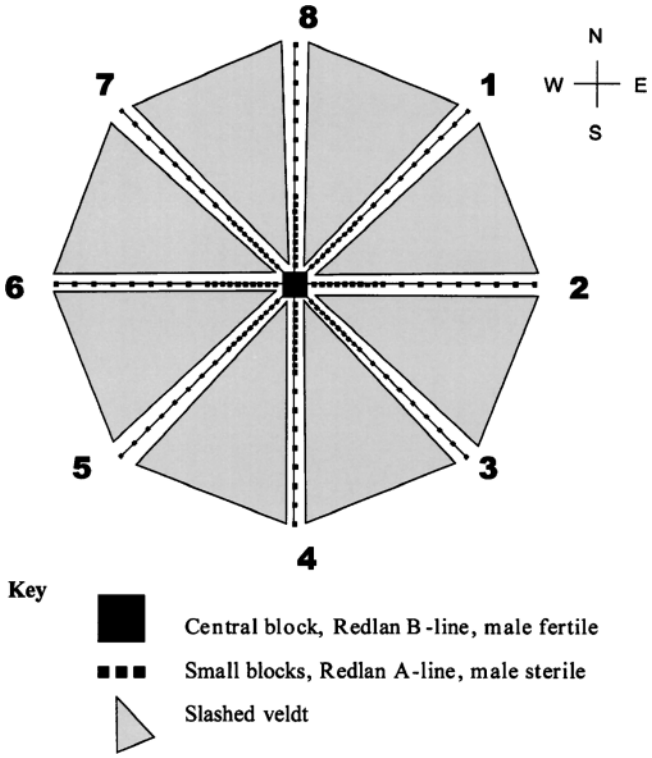

Fig. 1. Plan of the sorghum gene flow trial layout. Numbers were assigned clockwise to radiating arms, starting with the north-eastern arm. The central block with Redlan B-line (male fertile) was the pollen source, the arms with Redlan A-line (male sterile) were the pollen receptors. Each small square represents a block with sorghum plants. The first 10 blocks closest to the central field were located 13 m, the blocks farther away were located 28 m from each other. Veld grass were located between the arms not containing wild sorghum grasses.

planted was slashed to keep the veld grass short. The ground of the central block was tilled before planting. The ground along the eight radians was also plowed and about 4 m wide. The sorghum was grown under standard agronomical practices. Standard pest and disease control measures were applied, including ergot (*Claviceps africana* Frederickson, Mantle & De Milliano) spraying at the end of the flowering period (male sterile or nonfertilized flowers are particularly susceptible to this disease). After pollination, all panicles were covered with paper bags to protect immature seeds from being damaged by birds.

Flowering Period and Harvesting

Trial plants were planted on 28 Dec. 2002. The flowering period of every single plant in each small block in the radiating arms and of plants from a sample of four of 35 rows in the central field was monitored. Sorghum panicles from the radiating arms were covered with paper bags on Day 95 and 96 to avoid bird damage. Plants were harvested on 1 and 2 May (Day 125 and 126), approximately 55 d after the plants' main flowering period. The exact location of each harvested panicle was recorded (arm and distance) and the orientation toward the central field also was marked directly on the harvested plant. Seeds were counted on the hemisphere that faced the central field as well as on the hemisphere showing away from the central field. The sum of both hemispheres—the total amount of seeds per panicle—was also recorded. The extension of the sorghum gene flow trial layout was somewhat restricted by surrounding fences and a gravel road (1–3 cars passing per day). Therefore not all of the theoretically possible blocks could be planted. Still, 63 blocks were planted and used in the study.

Weather Data

A mobile weather station was installed in the middle of the central field to record temperature, relative humidity, rainfall (additional irrigation took place during March and April.), wind speed, and wind direction. Weather data was sent in 15-min intervals to the main station and was then electronically recorded in the database. Weather data were recorded after 11 February. The average daily temperature (T_{aver}) was 21.3°C (SD: 2.1), ranging from T_{min} = 13.5°C (SD: 3.1) to T_{max} = 30.4°C (SD: 2.6). The average daily relative humidity (RH_{aver}) was 61.8% (SD: 11.3), ranging from RH_{min} = 30.8% (SD: 13.1) to RH_{max} = 91.6% (SD: 7.5). Data on wind speed and wind direction were used only for the "pollen active" time of the day, i.e., when pollen was shed. Pollen release takes place in the morning, approximately from 0600 to 1130 h and sometimes also briefly in the afternoon, from approximately 1600 to 1800 h. Afternoon pollen shed is optional, depending even more on weather conditions than in the morning hours. Wind data are therefore presented for both cases, first for morning hours only and second for morning and afternoon hours together. During the flowering period (data were used from 1–31 March), wind direction was recorded more frequently blowing to the south and to the west (see Fig. 2). Wind speed records showed a distribution with frequent slow winds (average wind speed during morning hours: 4.49 km/h, SD = 2.93; during morning and afternoon hours: 4.25 km/h, SD = 2.79) and some peak wind speed of up to 16 km/h. Wind speeds exceeding 10 km/h were mainly recorded in the morning hours. No storms, hurricanes, or other extreme events were recorded during the flowering period (see Fig. 3).

Dispersion Model and Maximum Distance Gene Flow

Regression lines were calculated with the software Curve Expert 1.3 (http://www.ebicom.net/~dhyams/cmain.htm; verified 21 November 2005), a comprehensive curve fitting system for MS Windows. It employs a large number of regression models (both linear and nonlinear) as well as various interpolation schemes. In addition, the user may define any customized model desired for use in a regression analysis.

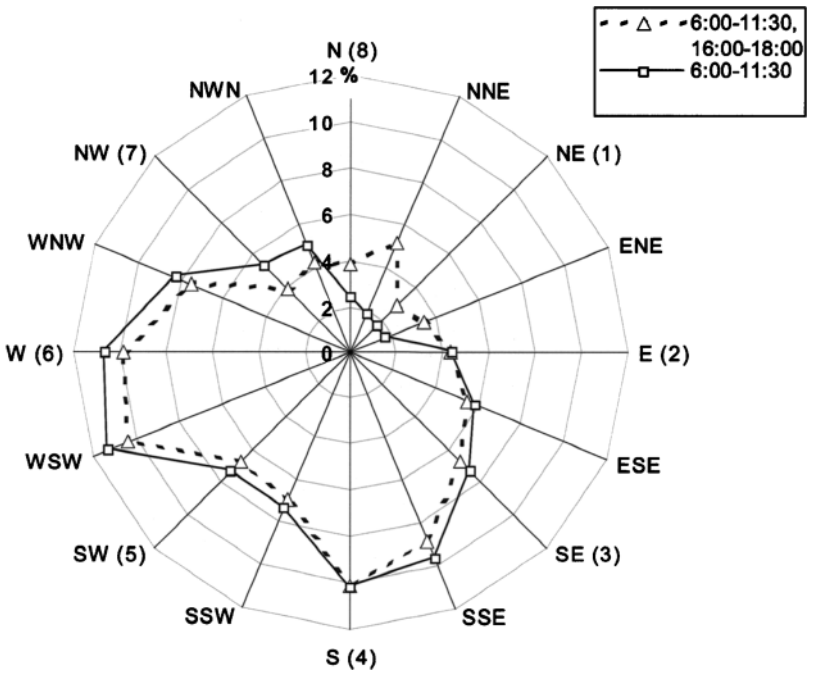

Fig. 2. Relative wind direction recorded at the trial at 15-min intervals (values in %). The solid line represents morning wind direction (0600–1130 h) and the dashed line represents morning and afternoon wind direction 0600–1130 h and 1600–1800 h). Note that the wind direction is presented as blowing to a direction, not from a direction. Arm numbers are shown in brackets.

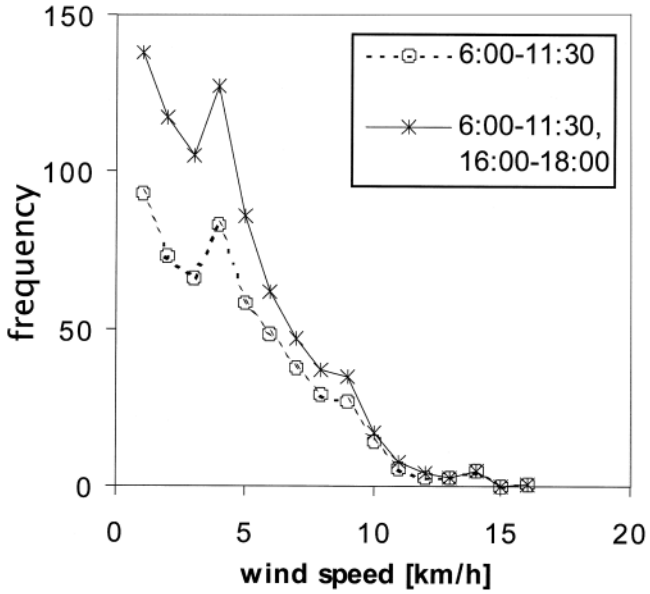

Fig. 3. Frequency of wind speed recorded at the trial in 15-min intervals during the flowering period. The light line represents morning wind speed (0600–1130 h) and the black line represent morning and afternoon wind speed together (0600–1130 h and 1600–1800 h).

Relative Pollen Flow

Relative pollen dispersal was estimated from the average amount of seeds produced per plant, multiplied by $2\pi r$, where r is the distance to the central, pollen-donating field. Calculated values are therefore estimates of relative pollen flow based on observed seed production rates (see e.g., Arriola, 1995).

Distance-Dependent Hybridization Rate and Error Intervals

The total amount of fertile female flowers per plant was counted for the experimental test plants (*S. bicolor*). Based on the counted total amount of female flowers per plant, including the standard error (SE), and based on the average amount of produced seeds per distance class, including the standard error (SE), the hybridization rates and the error intervals were calculated using the following formulas:

$$\text{average hybridisation rate } (\%) = 100 * \frac{\text{average amount of produced seeds}}{\text{amount of female flowers per plant}}$$

$$\text{minimum hybridisation rate } (\%) = 100 * \frac{\text{average amount of produced seeds} - \text{SE}}{\text{amount of female flowers per plant} + \text{SE}}$$

$$\text{maximum hybridisation rate } (\%) = 100 * \frac{\text{average amount of produced seeds} + \text{SE}}{\text{amount of female flowers per plant} - \text{SE}}$$

Data analysis was performed by MS Excel, SPSS, and Curve Finder 1.3 software.

RESULTS

Overlap in the Flowering Times

The plants started to flower in early March, about 65 to 70 d after planting, and maximum flowering was reached approximately 75 d after planting. Plants in the surrounding arms started to flower approximately 3 d after those in the central field. The total flowering period continued for about 20 d, so overlapping flowering time was ensured.

Bees

Honey bees (*Apis mellifera*) and wild bees (*Apis* ssp.) were observed in the central field; however, no bees were observed at male sterile plants in the surrounding arms. Only fertile male plants generate pollen; the sterile ones did not produce a bee reward, and thus bee visits to the central field had no notable impact on pollination of sterile recipient plants.

Pollination Pattern

Seed production and therefore crop-to-crop gene flow was detected on receptor plants at all investigated distances from the pollen source. Of the 656 plants used in the study, 428 produced at least one seed. A trend toward decreased pollen fertilization probability and seed production at farther distances from the pollen source was observed. Combining the plants from the same distance category, fertilization probability was highest at 13 m, the closest distance investigated, with 82.9%, and lowest at 130 m, with 48.9%.

Seed Production in Blocks and Arms

The amount of seeds produced per panicle tended to increase at closer distances (considering only those panicles that produced at least one seed). Combining all arms and only considering the distance to the central field, a sharp decrease within the closest three distance classes was apparent. At the closest distance, 13 m, an average of 125.1 seeds per panicle (SE = 22.4) was recorded, compared with 39.8 seeds (SE = 7.0) at 26 m and 19.0 seeds (SE = 2.9) at 39 m. This decrease was statistically significant. The continuous decrease in seed production with increasing distance from the pollen donor field was interrupted by one plant in Arm 4 (distance: 104 m). This plant had an unusually high number of seeds (379) probably because of partial cytoplasmic male sterility (CMS) instability. Therefore, the average number of seeds per panicle was higher in this distance category than would be expected.

Wind Direction and Seed Production

Seed production differed between different arms at the same distance. Variation in seed production was compared with wind direction patterns, and correlation coefficients (r^2) were calculated. Correlation coefficients for two different wind data sets (morning hours only and morning and afternoon hours together) for each distance class showed a reasonable correlation for distances between 13 and 117m, with r^2 values ranging between 0.54 to 0.89. On the basis of wind data from morning and afternoon hours, the first three distances classes show a statistically significant correlation with seed production. On the other hand, using wind data from the morning

hours only, the correlation is statistically significant for plants at 26, 39, and 64 m. A negative correlation resulted for the most distant blocks at 130 m (see Table 1).

Orientation of Seeds

The panicle hemispheres facing the pollen donor field yielded significantly more seeds than the hemispheres facing the opposite direction. From a total of 11 932 seeds counted from all panicles in all blocks and arms, 8951 seeds (75.02%) were produced on the hemispheres facing the central pollen donating field, and 2877 seeds (24.11%) were collected from hemispheres showing away from the central field. (Another 104 seeds (0.87%) were found unattached inside the paper bag that surrounded the panicles at the time of harvest and could not be attributed to a hemisphere). On the level of single plants, only 6.1% had more seeds at the backside than on the front; another 4.9% produced the same number on both sides. Most plants (89.0%), however, had more seeds on the front hemisphere than on the backward-oriented hemisphere.

Modeling the Gene Flow Gradient

Gene flow gradients for both the percentage of panicles that produced at least one seed and the amount of seeds produced per panicle were calculated. Regression analyses for the percentage of panicles with seeds were done by two dispersion models, a linear (A1) and an exponential (A2) model. The linear dispersion model was as follows: A1, $y = a_1 + b_1 \times x$. Here y is the percentage of plants with seeds, x is the distance from the pollen-donating field, and a_1 and b_1 are model parameters that were estimated by ordinary least square regression: $a_1 = 73.53$; $b_1 = -0.01124$; (SE = 7.72). The resulting regression line had a correlation coefficient of $r^2 = 0.57$ with a significant level of $p = 0.033$ (one-sided) and $p = 0.065$ (two-sided), with $n = 11$. The exponential dispersion model was as follows: A2, $y = a_2 \times e^{-b_2 \times x}$, where $a_2 = 74.64$, $b_2 = -0.00018631$ (SE = 7.60), $r^2 = 0.59$, $p = 0.028$ (one-sided) and $p = 0.055$ (two-sided), and $n = 11$. Another regression analysis was calculated for the amount of seeds produced per panicle with data from all 428 seed-bearing plants. Again, two dispersion models were used to fit to the gradient data: a power fit (B1) and an exponential (B2) dispersion model. The power fit dispersion model was as follows: B1, $y = a_3 \times x^{-b_3}$. Model parameters were estimated by ordinary least square regression after double log linearizing transformation, resulting in the coefficient data $a_3 = 912.93$ and $b_3 = -1.258$. The resulting regression line had a correlation coefficient of $r^2 = 0.506$ at a highly significant level of $p < 0.001$ (two-sided) ($n = 428$). The exponential dispersion model was as follows: B2, $y = a_4 \times e^{-b_4 \times x}$. Model parameters were $a_4 = 25.74$, $b_4 = -0.0217$, $r^2 = 0.448$, $p < 0.001$ (two-sided), and $n = 428$ (see Table 2).

Prediction of Maximum Distance Gene Flow

The dispersion models (A1, A2, B1, B2) were used to estimate the maximum gene flow distance. The models A1 and A2 were calculated with data from all plants investigated. For models B1 and B2, calculations were repeated with three data sets: (i) data from all 428 plants with seeds were used; the other two data sets were used for worst-case scenario with (ii) the upper 5% (95-percentile) and (iii) the upper 1% (99-percentile; thus, only plants were considered that produced more seeds than the lower 95% or lower 99% of all other plants). The maximum distance gene flow was estimated by specific criteria for each dispersion model. For the linear dispersion model A1, the criterion for maximum gene flow was assumed at a distance where the probability for a plant to produce at least a single seed was 0% (criterion: 0% of plants with seeds). For the exponential dispersion model A2, a different criterion was assumed because the exponential gradient never approaches 0%; here, the limit of 1% was chosen. In that case, the probability that a plant would produce seeds was 1% (criterion: 1% of plants with seeds). For the dispersion models B1 and B2 (regarding the amount of seeds produced), one criterion that could be applied to both models was chosen. The criterion was the distance where only one seed per plant would be produced (criterion: 1 seed per plant). (Note that the production of one seed would represent a hybridization rate of only 0.02%.) According to the four dispersion models, the maximum gene flow distance for the trial would be between 150 and 2315 m (see Table 2). As an example, the regression line calculated with dispersion model B1 is shown in Fig. 4.

Relative Pollen Flow

The amount of pollen needed to produce the observed hybridization rates at each distance—the relative pollen flow—was highest at 13 m and lowest at 78 and 91 m. In general, the relative pollen flow decreased with

Table 1. Correlation coefficients (r^2, Pearson) between wind direction and amount of seeds produced per plant for each distance category. *n* is the number of blocks used per distance category.

		Distance from central field (m)†									
Time period (h)		**13**	**26**	**39**	**52**	**65**	**78**	**91**	**104**	**117**	**130**
0600–1130 and 1600–1800	**r^2 (Pearson)**	**0.76***	**0.89****	**0.82***	**0.57**	**0.6**	**0.6**	**0.66**	**0.63**	**0.73**	**−0.62**
	n	**7**	**7**	**7**	**6**	**8**	**8**	**6**	**6**	**3**	**4**
0600–1130	**r² (Pearson)**	**0.67**	**0.87***	**0.81***	**0.63**	**0.72***	**0.53**	**0.81**	**0.54**	**0.59**	**−0.55**
	n	**7**	**7**	**7**	**6**	**8**	**8**	**6**	**6**	**3**	**4**

*** $p < 0.05$.**
**** $p < 0.01$.**
† Because there was only one block available at 158 m, no correlations could be calculated for this distance.

Table 2. Estimation of maximum distance of sorghum gene flow. For the percentage of plants with seeds, see dispersion models: (A1) the linear regression fit and (A2) the exponential fit. For the amount of seeds per (pollinated) plant, see dispersion models: (B1) the power fit and (B2) the exponential fit. Regression coefficient (r^2) and its statistical significance (p) for each fit are shown. Specific criteria were used to estimate the maximum distance gene flow. For models A1 and A2, all data was used, for B1 and B2, additional calculations were made also at the 95 and 99 percentile to simulate "extreme events".

Dispersion model		r^2	p (Two-sided) of r^2	Criteria	Distance (m)		
					All data	95 percentile	99 percentile
A1:	$y = a_1 + b_1x$	0.57	0.065	0% of plants with seeds	654	–	–
A2:	$y = a_2 \times e^{b_2 \times x}$	0.59	0.055	1% of plants with seeds	2315	–	–
B1:	$y = a_3 \times x^{-b_3}$	0.51	<0.001	1 seed per plant	225	708	773
B2:	$y = a_4 \times e^{-b_4 \times x}$	0.45	<0.001	1 seed per plant	150	234	238

increasing distance to the central field, with the exception of the distance category at 104 m, where the unusually high number of seeds in one plant needed the highest relative pollen flow (see Fig. 5).

Estimated Hybridization Rates

Crop sorghum had an average of 4916.8 female flowers per panicle (SE = 642.9; n = 12). On the basis of the amount of female flowers per plant (and its standard error) and on the average amount of produced seeds per plant and distance class, the hybridization rate of pollinated flowers per plant was calculated. The hybridization rate was relatively low, not surpassing 3.45%, even at the closest distance to the central field. The rate was even below 1% for plants at a distance of 39 m or greater and below 0.1% at the two farthest distances at 130 and 158 m (see Fig. 6).

DISCUSSION

This study shows an observable, sharp decrease of pollination within the first 40 m of the pollen-donating field. Although only a very small percentage of plants became pollinated beyond that distance, pollination continued to be observable at a very low level up to the farthest distance investigated (158 m). In brief, sorghum gene flow was notable within approximately the first 40 m and very low beyond 40 m. In a similar study on the gene flow of rice, Song et al. (2003, 2004) also observed outcrossing distances of up to 40 m under "normal" weather conditions. A serious discussion of the results obtained here and the estimations of the maximum pollination distance, however, require considering the two following points.

1. The results are only valid for the observed weather and especially wind conditions. The results indicate

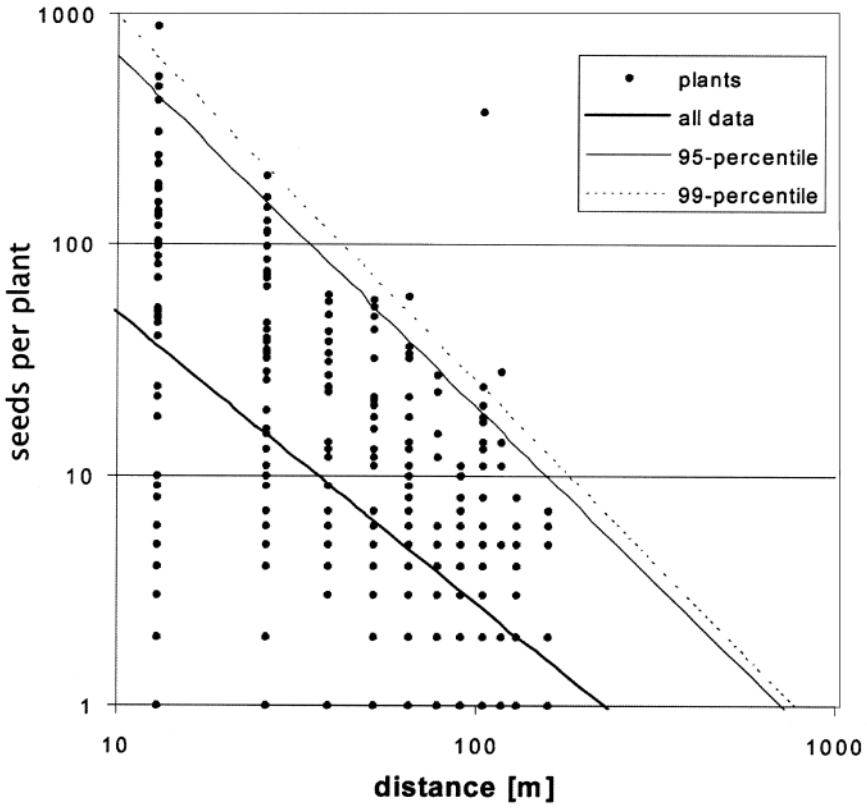

Fig. 4. Amount of seeds produced per plant and model of seed production gradient, calculated with a power fit model from three data sets: (i) all data, (ii) 95 percentile, and (iii) 99 percentile. Note the double logarithmic scale.

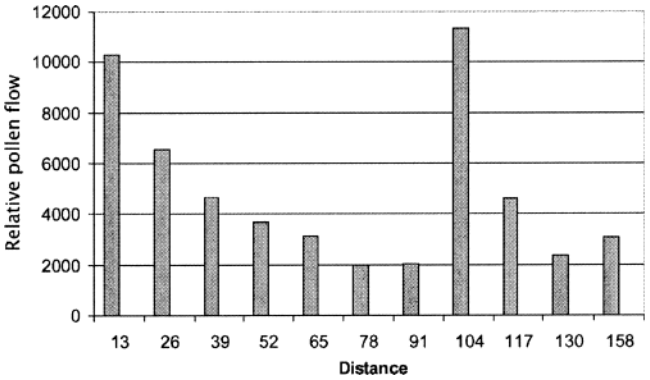

Fig. 5. Relative rate of pollen flow at male sterile plants. Values represent the amount of seeds produced $2\pi r$, where r is equal to the distance from the pollen-donating field.

that sorghum airborne pollen flow was significantly influenced by wind direction, which agrees with the viewpoint that weather factors have a strong effect on airborne pollen flow (Song et al., 2004). Changes in the pollination gradient can be expected when extreme wind conditions occur. Long-distance transport of pollen (roughly 3000 km) has been reported in the case of pine (*Pinus bankasia* Lambert) and spruce [*Picea glauca* (Moench) Voss)] pollen (Campbell et al., 1999). Under normal temperature and humidity conditions, however, pollen has an "inbuilt obsolescence" because longevity is limited. In sorghum, pollen longevity is approximately 30 min to 2 h after release (Lansac et al., 1994; Personal communication with sorghum plant breeder W. Wenzel, Agricultural Research Council, South Africa, 2003). Desiccation and reduced viability determine pollen longevity and restrict fertilization of female flowers after this time period, even though the pollen itself may be transported several hours or days by the wind. Nonetheless, 2 h in an unusually strong wind could transport the pollen several kilometers and it would still be able to pollinate a female flower. Assuming a uniform distribution of the pollen from the pollen source, the pollen rain would be highly diluted with distance because pollen density is approximately related to dispersion distance ($1/r^2$). In the case of unusually strong winds (singular events), we can expect inhomogeneous or even chaotic dispersion rather than a uniform pattern (see e.g., Di-Giovanni and Kevan, 1991).

2. The field trial was conducted with male sterile pollen receptor plants. Thus, the pollen from the central donor field did not have to compete with pollen from the receptor plant, which is normally responsible for approximately 70 to 95% of its pollinated female flowers (Ellstrand and Foster, 1983; Pederson et al., 1998; Djé et al., 1999; Smith and Frederiksen, 2000). Moreover, the flowering period of a single plant is completed in 4 to 7 d, but the female flowers can remain receptive up to 16 d in the absence of pollination (Shertz and Dalton, 1980). Under the conditions of our field trial, the absence of self-pollination and the increased receptive time period of female flowers should lead to a higher outcrossing and gene flow rate than under natural conditions. Still, the percentage of pollinated female flowers per plant only ranged from 0.04 to 3.45%, values similar to the results from a gene flow study conducted with maize (Biosicherheit, 2004). Therefore, under natural conditions, the outcrossing rates can be expected to be even lower than the values obtained here.

The gene flow observed in this study fit well into the experiences of sorghum plant breeders, who—for conventionally bred varieties or hybrids in southern Africa—use an isolation distance of 100 m to achieve less than 1% genetic pollution (Personal communication with ARC-Grain Research Institute sorghum plant breeder Dr. W. Wenzel and ICRISAT sorghum plant breeder Dr. E.S. Monyo, 2003). Other sources give an isolation distance of 400 to 800 m for hybrid seed

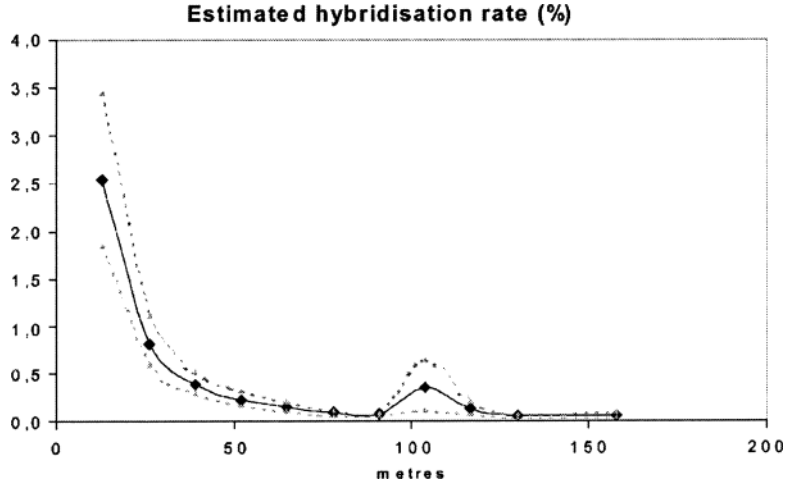

Fig. 6. Distance-dependent hybridization rate of pollinated sorghum plants. The dark solid line represents the average rate and the grey lines indicate the error interval.

production but without additional information on seed purity (Chopra, 1987). Thus, the estimations obtained here agree with the experiences from sorghum hybrid plant breeding. Apart from buffering zones, there is also another way to restrict gene flow, i.e., by means of cytoplasmic male sterility (McVetty, 1997; Smith and Frederiksen, 2000; Pedersen et al., 2003). The use of cytoplasmic male sterility to prevent the release of viable pollen, e.g., from transgenic maize, has been recently proposed (Feil and Stamp, 2001). In this system, transgenic plants are male sterile and are grown in a mixture with fertile nontransgenic pollen donors. Exploiting this technique has proven successful for the production of high oil maize (Bergquist et al., 1998a) and high grain quality maize (Bergquist et al., 1998b) and may be used to restrict gene flow in genetically modified sorghum as well. The robustness of cytoplasmic male sterility, however, is an important issue. Kidd (1961) noted that higher temperatures tend to increase male fertility of the A-lines (male sterile line). In a study by Pedersen et al. (2003), the probability of a cytoplasmic male sterility plant becoming fertile was estimated to be about 0.39%, or approximately 1 out of 252 plants. In our field trial, there was an unusually high number of seeds in a plant 104 m from the central field. Considering their findings, it is possible that this plant was fertile (or partly fertile) because of cytoplasmic male sterility instability. The number of plants used in our study (656) also supports this hypothesis. If cytoplasmic male sterility is to be used as a biosafety measure to avoid transgene flow, the involved cytoplasmic male sterility instabilities must be taken into account.

Limitations of the Study

Under field conditions, many factors will affect gene flow, i.e., the particular cultivar, field size (especially of the pollen donor), or planting density. The weather conditions (temperature, humidity and strength and direction of the wind and rainfall) during the growing period and especially during pollination will also affect the quantity and quality of the pollen available to the receptor (Arriola, 1995; Wenzel, 2003; Song et al., 2004). This is obviously the case for all field studies. One limitation, however, must be mentioned in particular, namely, the size of the pollen donating field. Compared with the field size of large-scale industrial sorghum production, the pollen source used in this study was rather small (30×30 m). As shown in our study, however, most of the gene flow occurs within the first 40 m, and the contribution to gene flow of plants growing inside a large-scale field—more than 40 m away from the field's edge—will be rather small. Still a larger pollen donor field would better resemble industrial sorghum production in terms of gene flow.

CONCLUSIONS

The outcome of this study shows that gene flow in sorghum will take place, and introgression of transgenic characteristics into crops and crop wild relatives is likely. Some of the novel characteristics (e.g., pest, disease, and drought resistance) envisioned for future transgenic development could favor the survival of hybrids with crop wild relatives outside the agroecosystem (Smith and Frederiksen, 2000; deVries and Toenniessen, 2001). Further research should therefore also focus on the prevention of gene flow from transgenic sorghum to other sorghum crops, landraces, and wild relatives before hand, e.g., by using adequate buffer zones and the possibilities (and limitations) of cytoplasmic male sterility.

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