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## Indications of Bee Pollination in Sorghum and its Implications in Transgenic Biosafety

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### Introduction

The family Graminae is a large, diverse group that includes some of the world's most important crops such as maize [*Zea mays*], rice [*Oryza sativa* L.], wheat [*Triticum aestivum* L.] and sorghum [*Sorghum bicolor* (L.) Moench]. These crops are generally considered to be wind pollinated, and transgenic risk assessment studies on gene flow studies have focused on wind-mediated pollen only (Arriola 1995; Arriola and Ellstrand 1996; Song et al. 2003; Song et al. 2004). On some occasions, however, bees have been reported to visit Graminae crops and their wild relatives. On some indigenous grasses in South Africa, honey bees were recorded collecting pollen (Anderson et al. 1983). Solitary bees from the genus *Lipotriches* (family Halictidae) are known to collect pollen from 21 different grass species including *Sorghum bicolor* ssp. *arundinaceum*, the wild progenitor of cultivated sorghum (Immelman and Eardley 2000). Honey bees and bees of the genus *Nomia* (family

Halictidae) (The genus *Nomia* was later divided into several genera, one of which is *Lipotriches*) were observed on indigenous grasses in Kenya (Bogdan 1962).

During a field trial (See “Crop-to-crop gene flow in sorghum and its implications for transgenic biosafety” by the same authors in this volume) at the research farm at Roodeplaat near Pretoria, South Africa, investigating crop to crop pollen flow in sorghum, a number of honey bees, wild bees or solitary bees and one beetle species were observed on sorghum flowers. An additional investigation on the flower-visiting insects was therefore conducted. The field trial was not specifically designed to investigate insect pollination, so that the insect role cannot be conclusively demonstrated. Nonetheless, the observations made and samples collected provide strong evidence for bee pollination in sorghum.

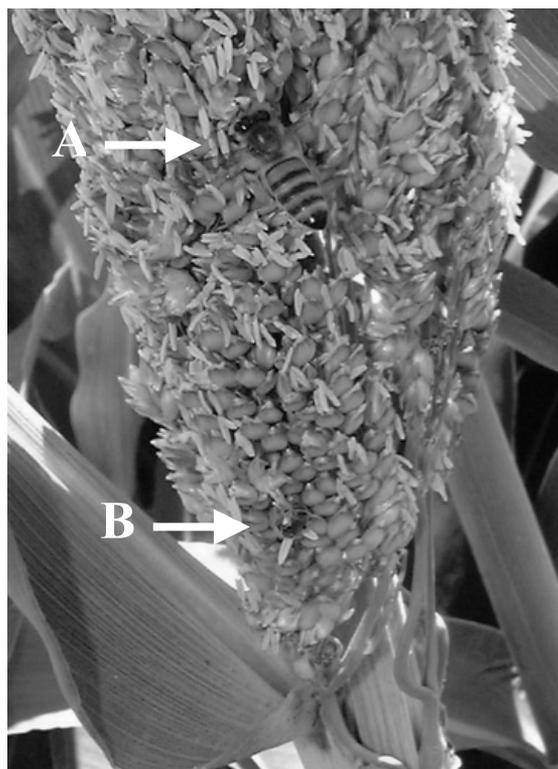
### Materials and Methods

The sorghum field trial was conducted on the 4000 ha Agricultural Research Council (ARC) research farm at 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 non-sorghum growing area, at least 5 km from any other sorghum fields and at least 2 km from wild or weedy sorghum plants. As the pollen source, a central block, measuring roughly 30 X 30 m, was planted with the B-line Redlan Pannar Ps 1051 B / 168(015), containing 35 rows approximately 90 cm apart with 30 cm within-row plant spacing. The surrounding vegetation was dominated by the local veld type (grassland) and some male sterile plants for the original field trial. The sorghum was grown using standard agronomic practices. Trial plants were planted on 28 December 2002. The plants in the central field started to flower in early March, approximately 70 days after planting, and maximum flowering was reached about 75 days after planting. Bees and beetles were observed and photographed on 12, 15 and 17 March and collected on 15 and 17 March 2003 during morning hours (9:00 – 11:15). Insects visiting sorghum flowers were collected with simple plastic boxes and then stored in the refrigerator before preparation for electron microscopy. In addition, fresh sorghum anthers from the central field were collected on 15 March and also prepared for electron microscopy, using standard preparation techniques. Bees and beetle specimens were identified in May 2003 by the Biosystematics Division of the Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa. Halictidae bees were identified by C. Eardley, the Apidae variety by A. Lubbe and the beetle (family Melyridae) by E. Grobbelaar.

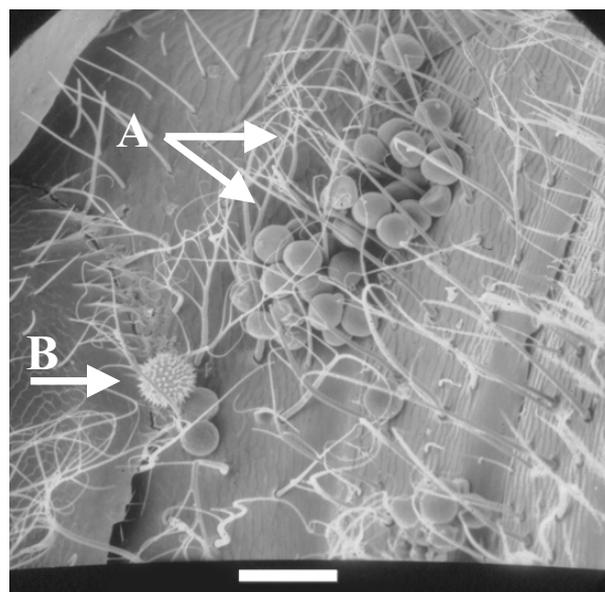
## Results and Discussion

Specimens from 7 genera were recorded visiting sorghum flowers: 6 bee genera and 1 beetle species. The bees were observed to collect pollen from sorghum. They not only visited one flower, but several flowers consecutively. From the 6 bee genera, 5 were from the family Halictidae and one was a local honey bee variety (Table 1). Figure 1 shows *Apis mellifera* and a solitary bee collecting pollen on sorghum flowers. *Apis mellifera*, *Astylus atromaculatus* and two *Lipotriches* species were scanned for pollen

grains under the electron microscope. Pollen grains were found on all the investigated insects; the grains were identical to pollen obtained directly from sorghum anthers. Pollen morphology was additionally compared with reports in the literature, where a good characterisation of sorghum pollen was available. Pollen characteristics matched in terms of pollen size, pore diameter, annulus diameter and exine ornamentation (Chaturvedi et al. 1994). The pollen size of sorghum was 40  $\mu\text{m}$  (37–45  $\mu\text{m}$ ), the pore diameter was approximately 3  $\mu\text{m}$ , the annulus diameter 9  $\mu\text{m}$  and the exine ornamentation can



**Figure 1.** *Apis mellifera* (A) and a solitary bee (B) collecting pollen side by side. Note the size difference.



**Figure 2.** Pollen grains found on the body surface of *Lipotriches* sp.1. (A) Pollen grains from *Sorghum bicolor*. (B) Pollen grain from a different species. Scale bar = 100  $\mu\text{m}$ .

**Table 1: Taxonomy of insects collected from sorghum flowers.**

Pollinator	Family	Species (Genus)
Wild bee	Halictidae	<i>Spatunomia</i> sp.
	Halictidae	<i>Patellapis</i> ( <i>Zonalictus</i> ) sp.
	Halictidae	<i>Lipotriches</i> sp.
	Halictidae	<i>Nomia</i> ( <i>Acunomia</i> ) sp.
	Halictidae	<i>Lasioglossum</i> ( <i>Evylaeus</i> ) sp.
Honey bee	Apidae	<i>Apis mellifera</i> prob. <i>scutellata</i> Lepeletier
Beetle	Melyridae (Melarinae)	<i>Astylus atromaculatus</i> Blanchard

be described as “insular type” (resembling a number of small pieces fitted together). Figure 2 depicts pollen found on *Lipotriches* sp. 1.

The pollen found on the investigated species originated without a doubt from the sorghum in the central field of the field trial. In terms of quantity, *A. mellifera* specimens carried the greatest amount of pollen, followed by the medium-sized Halictidae and the small-sized Halictidae. The beetle *A. atromaculatus* carried the least amount of pollen. (In the honey bees, the pollen was glued to the hind leg but also loosely attached to body hairs, as in Halictidae species.)

Previous studies on the risk of gene flow of transgenic sorghum and outcrossing have focused on wind pollination only (Arriola 1995 and Arriola and Ellstrand 1996). The findings presented in this study are a strong indication for bee pollination in *S. bicolor*. The results cannot be considered conclusive because the field trial was not specifically designed to distinguish between wind and insect pollination. However, the fact that several bees visited several sorghum flowers and collected pollen is strong evidence for this pollination mechanism. The contribution of bee pollination to total pollination in sorghum is difficult to estimate, as wind pollination is still believed to be mainly responsible for sorghum outcrossing. Bee pollination, however, could have effects beyond distances where wind pollination normally plays a major role (up to several hundred meters). Even though sorghum pollen morphology is clearly classified as anemophily (single pollen, no sticky exine, smooth surface), it may be foraged and used as a food source for bees, especially when other nearby pollen sources are unavailable during the sorghum flowering period. Another factor favoring bee visits in crop sorghum could have been the design of the field trial, namely the close vicinity of crop plants to local undisturbed grassland.

The sorghum gene flow field trial was not designed to confirm the occurrence of, nor quantify the extent of, bee pollination in sorghum. No differences between wind and bee pollination could be detected, and, since the receptor plants in the surrounding areas were male sterile, the bees were not rewarded for visiting these flowers. For future studies investigating the role of bee in sorghum outcrossing, wind pollination must be excluded (e.g. by carrying out the study in a glasshouse and providing beehives for pollen active time period) and the receptor plants should be male fertile. The relative frequencies of self pollination and outcrossing will have to be investigated using either molecular or unambiguous dominant phenotypic markers. The observations presented here not only show the flexibility of ecological interactions and the complexity of biological systems but also demonstrate the challenges of comprehensive risk assessment in transgenic sorghum

(as well as in other Graminae crops). The inclusion of bee pollination in the risk assessment of transgenic sorghum adds more uncertainty to the prediction of gene flow, which was previously thought to be driven by wind alone. These considerations should be kept in mind when assessing the risk of gene flow, as they affect three different biosafety aspects:

1. When determining adequate buffering distances between transgenic sorghum fields and other sorghum fields (or wild relatives), the role of bee-transmitted pollen has to be considered. In contrast to wind pollination – which is believed to occur mainly within a distance of a few hundred meters – bee pollination and foraging may extend up to 5 km.
2. The impact on beekeepers active in the vicinity of transgenic sorghum fields and their honey production will have to be investigated within this new perspective.
3. The potential effects of transgenic pollen on honey bees and solitary bees will have to be investigated, especially when new transgenic characteristics deal with insect resistance. The effect of “built-in” insecticides on nontarget organisms such as bees will have to be considered in future studies of formerly-considered “wind pollinated” crops.

One possibility to impede gene flow (whether by wind or bees) has been proposed by Pedersen et al. (2003), namely the use of cytoplasmic male sterility in transgenic sorghum together with male fertile conventional lines. This seed production system could prevent gene flow in transgenic sorghum as no pollen is dispersed by wind and no bees are attracted to the sterile lines.

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## Utilization

### Performance of Layers on Sorghum-Based Poultry Feed Rations

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### Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] in India is grown in the rainy as well as post-rainy seasons, generally by resource-poor small farmers in the semi-arid regions. The rainy season sorghum is often vulnerable to grain deterioration due to grain mold attack, making it unfit for food use. However, normal and molded grain has enormous demand for industrial uses such as preparation of animal/poultry feed and alcoholic beverages. A lack of assured supply of the sorghum grain produced in rainy season limits its use to only about 10% of the potential industrial demand. By 2010, the demand for rainy season sorghum for industrial use is estimated to increase by 10–30%; with the major demand expected to be from the poultry industry, which is growing at a rate of 15–20% per annum (Kleih et al. 2000). But, apprehension about energy levels of sorghum-based feed rations among feed manufacturers and poultry producers is one of the major limiting factors for its use in the poultry industry.

Considering the expected increase in demand and to assess the feasibility of the use of sorghum grain based rations in poultry industry, ICRISAT along with Acharya NG Ranga Agricultural University (ANGRAU) conceptualized and implemented a project (funded by Department for International Development, UK) in collaboration with the non-governmental organizations, Federation of Farmers Associations (FFA), Andhra Pradesh Poultry Federation (APPF) and Janaki Feeds (a private sector partner). One of the project's goals is aimed at enhancing the use of rainy season sorghum in poultry feed rations in layer production as a potential alternative to maize and to create sustainable marketing linkages between sorghum growers and the poultry industry through innovative institutional systems. Performance of layers on rainy season sorghum grain-based