



## Controlling the release of pollen from genetically modified maize and increasing its grain yield by growing mixtures of male-sterile and male-fertile plants

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Received 27 December 2001; accepted 30 July 2002

**Key words:** cytoplasmic male sterility, genetically modified crops, non-isogenic pollination, maize, pollen dispersal, xenia, *Zea mays* L.

### Summary

There is public concern about the consequences of pollen dispersal from genetically modified (GM) crops. The release of viable pollen from GM maize can be controlled by growing mixtures of cytoplasmic male-sterile plants and male-fertile non-transformed pollinator plants. Our experiments indicate that such associations can bring about grain yields as high or even higher than those produced by pure male-fertile maize crops, especially when the male-sterile component is pollinated non-isogenically. The grain yield benefits from cytoplasmic male sterility and xenia as well as the fact that seed of male-sterile varieties can be produced cheaply and reliably in large quantities would facilitate the implementation of the proposed system in agricultural practice.

### Introduction

The advent of genetically modified (GM) crops has raised great public concern and political debate in many countries. One major criticism of gene technology is that the dispersal of GM pollen may cause several ecological and economical problems: (i) GMO crops may pollinate non-transformed crops on adjacent fields with grave consequences for product quality and seed purity, (ii) transgenes may be transferred by means of pollen dispersal to landraces (Quist & Chapela, 2001) and related weed species (Mikkelsen et al., 1996), (iii) GM pollen may harm non-target organisms (Losey et al., 1999), and (iv) honey may be contaminated by GM pollen. Genetically engineered maize (*Zea mays* L.) usually sheds large amounts of pollen, which can be transported over long distances in a viable state (reviewed by Feil & Schmid, 2002).

Cytoplasmic male sterility (cms) occurs in 140 plant species (Levings, 1990), many of which are of great agricultural interest. In the USA, large areas are planted with maize crops that contain male-sterile

(cms was not restored) and male-fertile components (Strachan & Kaplan, 2001). Cytoplasmic male sterility can raise grain yield (Stamp et al., 2000). Male-sterile plants do not release viable pollen; consequently, growing cms versions of GM maize varieties can prevent the spread of transgenes into the environment. We report on a maize cropping method that can control the dispersal of GM pollen and increase grain yield at the same time.

### Materials and methods

The problems linked to the release of pollen from GM maize can be reduced or even prevented by growing cytoplasmic male-sterile GM maize plants in a mixture with male-fertile non-transformed maize plants, whereby the latter function as pollen donors for the GM plants (Stamp & Feil, 2001; Feil & Schmid, 2002).

To determine how cms and pollinator variety affect grain yield, the male-sterile dent × flint single-cross hybrid Silpro was grown together with six European

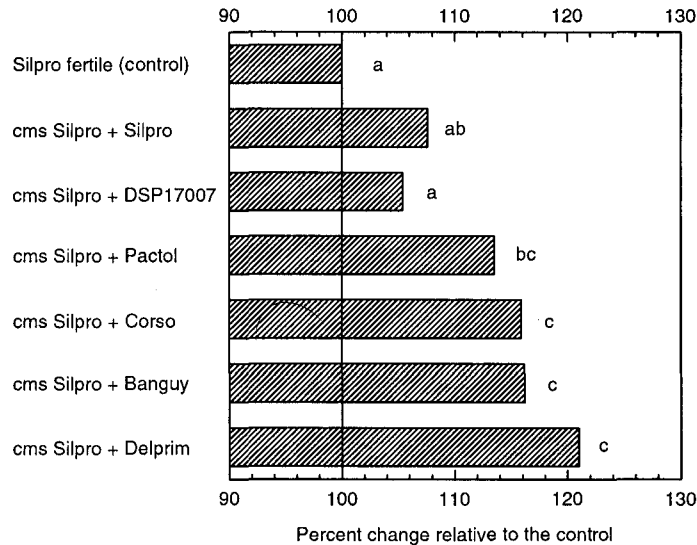


Figure 1. Combined effects of cytoplasmic male sterility and different pollinators (cms Silpro + pollinator hybrid) on grain yield. Data are means across six environments. Isogenically pollinated male-fertile Silpro was the control ( $100 = 9.4 \text{ t ha}^{-1}$ ). Bars with the same letter are not significantly different at the  $p = 0.05$  probability level.

dent  $\times$  flint male-fertile single-cross pollinator hybrids (Silpro, DSP17007, Banguy, Corso, Delprim, and Pactol). The male-sterile version of Silpro carried the T-cytoplasm (Liu et al., 2002). The male-fertile, isogenically pollinated version of Silpro was the control. Pollinator hybrids and cms hybrids were planted in a randomized complete block design with five replicates in six environments (two years, three locations per year) in Switzerland. The pollinator blocks consisted of 18 rows, 16 m long and 75 cm apart. The population density was  $11.5 \text{ plants m}^{-2}$ . The control plot and the cms Silpro plots were in the pollinator blocks; the plots consisted of two rows, each 4 m long. Row spacing and population density were the same as on the pollinator area. At the black layer stage of maturity, about 35 ears were harvested from the central  $3 \text{ m}^2$  of each plot. A subsample of grains (500 g) was dried at  $65 \text{ }^\circ\text{C}$  to constant weight to determine the grain yield on a dry weight basis. Plant density was used as covariance factor in the analysis of variance. Environment was treated as a random effect. The comparison of means was performed with the Student-Newman-Keuls test.

## Results and discussion

The cms version of Silpro, pollinated by male-fertile Silpro (= isogenic pollination), yielded 7.6% more than the male-fertile, isogenically pollinated version of Silpro (Figure 1). Several researchers reported similar effects of cms (e.g. Rogers & Edwardson, 1952; Stamp et al., 2000; Kaeser et al., 2003). Other investigators found no, inconsistent, or negative effects of cms on grain yield. Studies on the effect of non-isogenic pollination (= xenia) on grain yield produced inconsistent results (Tsai & Tsai, 1990; Weiland, 1992). According to Bulant & Gallais (1998), the general advantage of xenia is related to the genetic distance. In this study, statistically significant increases in grain yield occurred when the male-sterile Silpro was pollinated by Banguy, Corso, Delprim, and Pactol. Compared to isogenically pollinated cms Silpro and the male-fertile, isogenically pollinated control, male-sterile Silpro, pollinated by Delprim, showed grain yield advantages of 12 and 21%, respectively (Figure 1).

These yield data refer to pure male-sterile crops; the gain in yield would be about one-fifth smaller on farmers' fields if mixtures of 20% male-fertile and 80% male-sterile plants were used. The strong positive grain yield responses of male-sterile Silpro to xenia demonstrate that the grain yield of this hybrid was not limited by the plants' capacity to deliver assimilates to the growing grains ('source capacity').

In the USA, a large hectareage is planted with blends of male-sterile and male-fertile varieties for the production of high-oil maize (Thomison & Geyer, 1999; Strachan & Kaplan, 2001; Thomison et al., 2001). This indicates that growing mixtures of male-sterile and male-fertile plants is principally feasible. Needless to say, the pollinator variety must release sufficient pollen at silking to ensure complete pollination. In high-oil maize, about 8 to 10% of pollinator plants is considered to be sufficient (Thomison et al., 2001).

Thus far, little attention has been paid to blends of male-sterile and male-fertile varieties for the purpose of controlling the dispersal of GM pollen. In the case of transgenic herbicide-resistance, mixtures of GM varieties and non-transformed pollinator varieties cannot be used, because the herbicide would kill the latter component. Furthermore, it must be taken into account that transgenic quality traits are diluted by large portions of non-transformed plants in the mixture. In such cases, male-fertile GM plants could replace the non-transformed pollen donors in the mixture. This approach cannot completely prevent the dispersal of GM pollen from GM crop stands, but it would be reduced by about 80%. Bt maize was primarily developed to control the European corn borer (*Ostrinia nubilalis* Hübner). Widespread use of Bt maize could lead to Bt toxin resistance in the European corn borer. Therefore, farmers in the USA must plant a portion of their maize hectareage with non-Bt maize. Blends of 80% male-sterile Bt-maize and 20% male-fertile non-transformed maize may help prevent the development of Bt toxin-resistant insect populations.

## Conclusions

Our results demonstrate that the combination of male sterility and xenia can bring about grain yields as high or even significantly higher than those produced by pure male-fertile maize crops. Growing mixtures of male-sterile and male-fertile maize varieties could be put into practice in the near future, because inexpensive seed of cms varieties can be produced in

large quantities using standard methods. We, therefore, recommend that male-sterile versions of GM maize varieties be grown together with male-fertile non-transformed pollinator varieties to control the release of viable GM pollen. The proposed method for controlling the dispersal of GM pollen can be applied to crops that produce a sufficient surplus of pollen.

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