P. 1

Parecer sobre a avaliação de biossegurança do milho geneticamente modificado MIR 162

Por Nagib M. A. Nassar

I – Avaliação de efeito sobre frangos de corte

A avaliação apresentada pela empresa produtora restringiu-se ao exame de peso após 44 dias de dieta padrão. Não há dados sobre o efeito dessa variedade de milho sobre os órgãos internos dos frangos adultos ou em crescimento, como rins e componentes do sangue. A literatura se refere à mudança anatômica e química (referência 1).

Chowdhury *et al.* (2003) detectaram alterações em gastrointestinos de animais alimentados por milho BT. Journal of Animal Science 81: 2546-2551.

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: 23.06.08

MCT / CTNBio
20 / 06 / 2008
Número de Controle:
21897 / 08

Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11 [1]

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ABSTRACT: Genetically modified corn has been approved as an animal feed in several countries, but information about the fate of genetically modified DNA and protein in vivo is insufficient. Genetically modified corn Bt11 is developed by inserting a recombinant DNA sequence encoding insecticidal Cry1Ab protein from *Bacillus thuringiensis* subsp. *kurstaki*. We examined the presence of corn intrinsic and recombinant *cry1Ab* gene by PCR, and the Cry1Ab protein by immunological tests in the gastrointestinal contents of five genetically modified corn Bt11-fed and five nongenetically modified corn-fed pigs. Fragments of corn zein (242 bp), invertase (226 bp) and of ribulose-1,5-bisphosphate carboxylase/oxygenase genes (1,028 bp) were detected in the gastrointestinal contents of both Bt11 and nongenetically modified corn-fed pigs. Fragments of recombinant *cry1Ab* gene (110 bp and 437 bp) were detected in the gastrointestinal contents of the Bt11-fed pigs but not in the control pigs. Neither corn intrinsic nor *cry1Ab* gene fragments were detected in the peripheral blood by PCR. The gastrointestinal contents were positive for Cry1Ab protein by ELISA, immunochromatography, and immunoblot; however, these methods did not work for blood and precluded conclusions about any potential absorption of the protein. These results suggest that ingested corn DNA and Cry1Ab protein were not totally degraded in the gastrointestinal tract, as shown by their presence in a form detectable by PCR or immunological tests.

Key Words: Feed, Maize, Recombinant DNA, Pigs

 J. Anim. Sci. 2003. 81:2546–2551

Introduction

Genetically modified (**GM**) corn has been approved as an animal feed in several countries; however, there is growing anxiety in European countries and in Japan about the safety assessment of foreign genes and proteins expressed in plants by recombinant DNA (**rDNA**) technology (Hino, 2002). For the evaluation of GM feed safety, compositional and nutritional properties and animal performance of GM crops as feed have been examined (FAO/WHO report, 2000). Further information on the fate of rDNA after ingestion is additional concern. Genetically modified Bt11 corn (**Bt11**) is developed by inserting rDNA sequence encoding insecticidal Cry1Ab protein from *Bacillus thuringiensis* subsp. *kurstaki*. Polymerase chain reaction for rDNA has been used to differentiate GM and non-GM corn (Matsuoka et al., 2000), but information on the fate of the rDNA and its products (Cry1Ab protein) in the gastrointestinal (**GI**) tract after ingestion of GM feed is limited. The objective of the present study was to determine if the *cry1Ab* gene and Cry1Ab protein, as well as corn-intrinsic genes, could be detected in GI tract contents and peripheral blood of pigs fed Bt11 and non-Bt isoline corn.

Materials and Methods

Diet and Animals

Diet. Genetically modified Bt11 (N58-D1 Lot No. 2608611, Novartis Seed, Inc., Greensboro, NC) and non-

[1]This study was partly supported by the Research project system for urgent administrative requests by the Ministry of Agriculture, Forestry and Fisheries and a STA fellowship by Japan International Science and Technology Corporation. The authors wish to thank H. Murata, Safety Evaluation Laboratory, NIAH, Tsukuba, Japan for his valuable suggestions and to K. Miyamoto, National Institute of Agrobiological Science, Tsukuba, Japan, for his providing anti Cry1Ab rabbit serum.

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Received October 16, 2002.
Accepted June 12, 2003.

II - Avaliação da referida variedade de milho sobre organismos do solo

A avaliação feita pela empresa em solo do estado americano de Illinois, usando proteinaVip3Ha19. Essa avaliação falha em seu mérito científico para considerar os aspectos de biosegurança desse milho pelas seguintes razões:

a) a avaliação não foi realizada em solo e em condições brasileiros, conforme manda a constituição brasileira.
b) foi usada uma proteína pronta na evolução e não tratou com solo após o plantio das próprias plantas transgênicas, por um período completo de estação de crescimento. Isto faz uma grande diferença em produtos de raízes e seus efeitos no solo.
c) A duração do experimento realizado pela empresa produtora foi de 29 dias, que não são suficientes para ter condições realísticas do solo após uma estação de crescimento, que dura no milho até 5 meses de crescimento e amadurecimento.

Recomendo para ter avaliação do mérito científico o seguinte:

a) que se faça a avaliação de biosegurança da referida variedade em solo e sob condições ambientais brasileiros
b) que se faça a avaliação uma estação completa de crescimento de plantas que dure pelo menos 5 meses
c) a avaliação tem que tratar com produto das plantas no solo e não com proteína externa

(veja documento anexado) : Saxena, Flores, Stotzky, Nature 1999

pag. 3

Transgenic plants

Insecticidal toxin in root exudates from *Bt* corn

Bt corn is corn (*Zea mays*) that has been genetically modified to express insecticidal toxins derived from the bacterium *Bacillus thuringiensis* to kill lepidopteran pests feeding on these plants. Here we show that *Bt* toxin is released into the rhizosphere soil in root exudates from *Bt* corn.

The insecticidal toxin produced by *B. thuringiensis* subsp. *kurstaki* remains active in the soil, where it binds rapidly and tightly to clays[1] and humic acids[2]. The bound toxin retains its insecticidal properties[3] and is protected against microbial degradation by being bound to soil particles[4], persisting in various soils for at least 234 days (the longest time studied), as determined by larvicidal bioassay[5]. Unlike the bacterium, which produces the toxin in a precursor form, *Bt* corn contains an inserted truncated *cry1Ab* gene that encodes the active toxin.

In laboratory studies, caterpillars of the monarch butterfly (*Danaus plexippus*) were killed as a result of feeding on milkweed (*Asclepias curassavica*) that had been artificially contaminated with pollen from transgenic corn that expressed the *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki*[6], and green lacewings (*Chrysoperla carnea*), which are insect predators of insect pests, were killed by ingesting European corn borers (*Ostrinia nubilalis*) reared on *Bt* corn[7].

We germinated surface-sterilized seeds[8] of *Bt* corn (NK4640Bt) and of the isogenic strain without the *cry1Ab* gene on agar. The seedlings were aseptically placed on plastic screening (6-mm mesh), which, to minimize contamination by products of endosperm hydrolysis, was suspended over 200 ml of Hoagland's solution in 4-litre beakers covered with aluminium foil, which was removed after 18 to 20 days when the tops of the plants had reached it. After 7, 15 and 25 days of growth in a plant-growth room (26 ± 2 °C, 12 h light–dark cycle), the soil-free medium was replaced with fresh solution and analysed.

Total protein in the medium (average of 105 μg protein per plant) was determined by the Lowry procedure[9]. A major band migrating on SDS–PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) to a position corresponding to a relative molecular mass of 66,000 (66K), the same as that of the Cry1Ab protein, was evident after 7 and 15 days only in the [illegible] tein bands of smaller relative molecular mass were seen in exudates from both *Bt* and non-*Bt* corn. We confirmed the presence of the toxin in the exudates from *Bt* [illegible] Flow Quickstix (from EnviroLogix, Maine; detection limit < 10 parts per billion) and verified that it was active in an insecticidal bioassay using larvae of the tobacco hornworm (*Manduca sexta*), a model for testing antilepidopteran activity[3,5].

Larvae placed on medium containing exudates from *Bt* corn stopped feeding and began to die after 2 to 3 days and had a mortality of 90 to 95% after 5 days (dose lethal to 50% of larvae, LC_{50}, was 5.2 μg protein). There was no immunological reaction or larval mortality obtained with the exudates from non-*Bt* corn. After 25 days of growth, when the medium was no longer sterile (as demonstrated by streaking it on various microbiological media), the 66K band had disappeared, although there were several new protein bands of smaller relative molecular mass, and the immunological and larvicidal assays were negative, indicating that microbial, and probably also corn, proteases had hydro-lysed the toxin.

Samples of soil from the rhizosphere of seedlings that had been transplanted into either sterile or non-sterile soil in test-tubes were taken from randomly selected tubes, vortexed with extraction buffer (EnviroLogix) and centrifuged. We analysed the supernatants using the immunological and larvicidal assays and found that these were positive, even after 25 days of growth, for samples from *Bt* corn (100% mortality; $LC_{50} = 1.6$ μg protein per soil tube) but were negative for non-*Bt* corn. Moreover, particles of rhizosphere soil in suspension placed directly on the bioassay medium caused mortality comparable to the supernatants.

Although the concentration of protein in the rhizosphere soil was approximately 95 μg per g soil, the concentration of the actual toxin in the extraction buffer was apparently too low to be detected by SDS–PAGE. These results agree with earlier findings showing that the toxin binds rapidly to surface-active soil particles and that the bound toxin retains its larvicidal activity and is protected by this binding against biodegradation[1–5].

About 15 million acres of *Bt* corn were planted in the United States in 1998, which was just under 20% of the total acreage of corn[10]. The *Bt* toxin that is released into soil from roots during the growth of a *Bt* corn crop would add to the amount of toxin introduced into soil from pollen during tasselling and as a result of the incorporation of plant residues after harvesting the crop.

We have no indication of how soil com- [illegible] root exudates in the field. *Bt* toxin in the rhizosphere might improve the control of insect pests, or it might promote the selection of toxin-resistant target insects. [illegible] as well as target insects, so there may be a risk that non-target insects and organisms in higher trophic levels could be affected by the toxin. Further investigations will be necessary to shed light on what might happen underground.

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1. Tapp, H., Calamai, L. & Stotzky, G. *Soil Biol. Biochem.* **26,** 663–679 (1994).
2. Crecchio, C. & Stotzky, G. *Soil Biol. Biochem.* **30,** 463–470 (1998).
3. Tapp, H. & Stotzky, G. *Appl. Environ. Microbiol.* **61,** 1786–1790 (1995).
4. Koskella, J. & Stotzky, G. *Appl. Environ. Microbiol.* **63,** 3561–3568 (1997).
5. Tapp, H. & Stotzky, G. *Soil Biol. Biochem.* **30,** 471–476 (1998).
6. Losey, J. E., Raynor, L. S. & Carter, M. E. *Nature* **399,** 214 (1999).
7. Hilbeck, A., Baumgartner, M., Fried, P. M. & Bigler, F. *Environ. Entomol.* **27,** 480–487 (1998).
8. Benizri, E., Courtade, A. & Guckert, A. *Soil Biol. Biochem.* **27,** 71–77 (1995).
9. Lowry, O. H., Rosebough, N. R., Farr, A. L. & Randall, R. J. *J. Biol. Chem.* **193,** 265–275 (1951).
10. Wadman, M. *Nature* **397,** 636 (1999).

Palaeobiology

Herbivorous diet in an ornithomimid dinosaur

In 1997, twelve well-articulated skeletons of an ornithomimid dinosaur[1] from the Upper Cretaceous Ulansuhai Formation[2] in China were discovered. Each skeleton contained a preserved gastrolith mass inside the ribcage that was attached on the medial surface of the articulated dorsal ribs and gastralia. The occurrence and characteristics of gastrolith masses in this ornithomimid indicate that these non-avian toothless theropods may have had gizzards and been herbivores, like modern herbivorous birds that use grit to grind up plant matter.

The gastroliths found in these dinosaurs (Fig. 1) are mainly composed of grains of silicate, with no bony elements (energy dispersive X-ray analysis detected hardly any phosphorus pentoxide in the matrix) or insect remains. The gastroliths are found in the same region of each individual, generally close to the middle dorsal vertebrae. One of the isolated gastrolith masses belongs to a [illegible] by the size of its rib impressions, and [illegible] that observed in crocodilians[3].

III – Experimentos necessários para a biosegurança de qualquer variedade de milho transgenico tipo Bt

1- faltou um experimento para elucidar a presença ou não de fragmentos de recombinante DNA em grastrointestinal em animais por eles alimentados (veja em anexo o doc. nº 3)

Veja
Chowdhury et al. (2003). J. animal Science 81: 2546-2551

p. 4

Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11 [1]

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Received October 16, 2002.
Accepted June 12, 2003.

III 2- faltou experimento que avalia o efeito do pólen da variedade em avaliação, para biosegurança, sobre larvas de diferentes insetos da fauna brasileira (veja o trabalho em anexo)

Veja Losey et al. Nature 1999
mostraram larvas de diferentes fauna
tiverem suas sobrevivenca caidos
a 56 /. do natural apos 4 dias
de alimentar em pollen Bt

p. 6
N/

Transgenic pollen harms monarch larvae

Although plants transformed with genetic material from the bacterium *Bacillus thuringiensis* (*Bt*) are generally thought to have negligible impact on non-target organisms[1], *Bt* corn plants might represent a risk because most hybrids express the *Bt* toxin in pollen[2], and corn pollen is dispersed over at least 60 metres by wind[3]. Corn pollen is deposited on other plants near corn fields and can be ingested by the non-target organisms that consume these plants. In a laboratory assay we found that larvae of the monarch butterfly, *Danaus plexippus*, reared on milkweed leaves dusted with pollen from *Bt* corn, ate less, grew more slowly and suffered higher mortality than larvae reared on leaves dusted with untransformed corn pollen or on leaves without pollen.

Pollen for our assay was collected from N4640-*Bt* corn and an unrelated, untransformed hybrid, and was applied by gently tapping a spatula of pollen over milkweed (*Asclepias curassavica*) leaves that had been lightly misted with water. Pollen density was set to visually match densities on milkweed leaves collected from corn fields. Petioles of individual leaves were placed in water-filled tubes that were taped into plastic boxes. Five three-day-old monarch larvae from our captive colony were placed on each leaf, and each treatment was replicated five times. Milkweed leaf consumption, monarch larval survival and final larval weight were recorded over four days.

Larval survival (56%) after four days of feeding on leaves dusted with *Bt* pollen was significantly lower than survival either on leaves dusted with untransformed pollen or on control leaves with no pollen (both 100%, $P=0.008$) (Fig. 1a). Because there was no mortality on leaves dusted with untransformed pollen, all of the mortality on leaves dusted with *Bt* pollen seems to be due to the effects of the *Bt* toxin.

There was a significant effect of corn pollen on monarch feeding behaviour ($P=0.0001$) (Fig. 1b). The mean cumulative proportion of leaves consumed per larva was significantly lower on leaves dusted with *Bt* pollen (0.57 ± 0.14, $P=0.001$) and on leaves dusted with untransformed pollen (1.12 ± 0.09, $P=0.007$) compared with consumption on control leaves without pollen (1.61 ± 0.09). The reduced rates of larval feeding on pollen-dusted leaves might represent a gustatory response of this highly specific herbivore to the presence of a 'non-host' stimulus. However, such a putative feeding deterrence alone could not explain the nearly twofold decrease in consumption rate on leaves with *Bt* pollen compared with leaves with untransformed pollen ($P=0.004$).

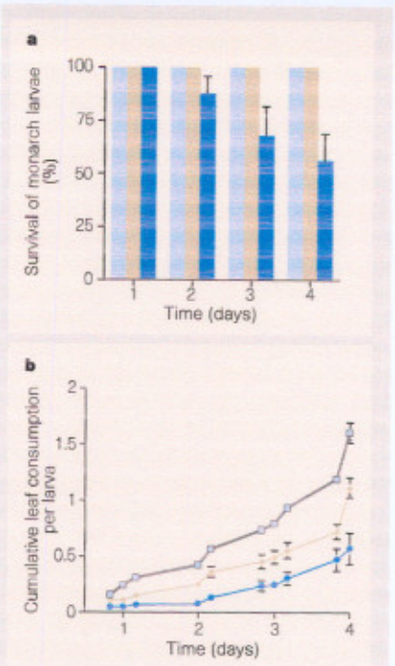

Figure 1 Survival and leaf consumption of second- to third-instar monarch larvae on each of three milkweed leaf treatments: leaves with no pollen (light blue), leaves treated with untransformed corn pollen (green) and leaves dusted with pollen from *Bt* corn (dark blue). **a**, Mean (±s.e.m.) survival based on the proportion of larvae surviving in five replicates of each treatment. **b**, Mean (±s.e.m.) cumulative leaf consumption based on the total amount of leaf area consumed per larva in five replicates of each treatment. The amount of leaf area consumed per larva in each experimental unit was calculated for each time interval by dividing the amount of leaf area consumed in that interval by the number of larvae alive during the time interval. Cumulative consumption was calculated by summing the leaf area consumed per larva at each interval. Colours of lines correspond to those of the bars in **a**.

These results have potentially profound implications for the conservation of monarch butterflies. Monarch larvae feed exclusively on milkweed leaves[4]; the common milkweed, *A. syriaca*, is the primary host plant of monarch butterflies in the northern United States and southern Canada[5]. Milkweed frequently occurs in and around the edges of corn fields, where it is fed on by monarch larvae[6]. Corn fields shed pollen for 8–10 days between late June and mid-August, which is during the time when monarch larvae are feeding[7]. Although the northern range of monarchs is vast, 50% of the summer monarch population is concentrated within the mid-western United States, a region referred to as the 'corn belt' because of the intensity of field corn production[8]. The large land area covered by corn in this region suggests that a substantial portion of available milkweeds may be within range of corn pollen deposition.

With the amount of *Bt* corn planted in the United States projected to increase markedly over the next few years[9], it is imperative that we gather the data necessary to evaluate the risks associated with this new agrotechnology and to compare these risks with those posed by pesticides and other pest-control tactics.

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1. Ostlie, K. R., Hutchison, W. D. & Hellmich, R. L. *Bt Corn and European Corn Borer* (NCR publ. 602, Univ. of Minnesota, St Paul, 1997).
2. Fearing, P. L., Brown, D., Vlachos, D., Meghji, M. & Privalle, L. *Mol. Breed.* **3,** 169–176 (1997).
3. Raynor, G. S., Ogden, E. C. & Hayes, J. V. *Agron. J.* **64,** 420–427 (1972).
4. Malcolm, S. B., Cockrell, B. J. & Brower, L. P. in *Biology and Conservation of the Monarch Butterfly* (eds Malcolm, S. B. & Zalucki, M. P.) 253–267 (Natural History Museum of Los Angeles County, Los Angeles, 1993).
5. Malcolm, S. B., Cockrell, B. J. & Brower, L. P. *J. Chem. Ecol.* **15,** 819–853 (1989).
6. Yenish, J. P., Fry, T. A., Durgan, B. R. & Wyse, D. L. *Weed Sci.* **45,** 44–53 (1997).
7. Brower, L. P. *J. Exp. Biol.* **199,** 93–103 (1996).
8. Wassenaar, L. I. & Hobson, K. A. *Proc. Natl Acad. Sci. USA* **95,** 15436–15439 (1998).
9. Andow, D. A. & Hutchison, W. D. in *Now or Never: Serious New Plans to Save a Natural Pest Control* (eds Mellon, M. & Rissler, J.) 19–65 (Union of Concerned Scientists, Cambridge, Massachusetts, 1998).

The mystery of female beauty

Yu and Shepard[1] have reported a preference for heavy women with high waist-to-hip ratios (WHR) in a culturally isolated population in southeast Peru. Their findings are interesting because a preference for low WHR is widespread in westernized populations[2–5]. However, we disagree with their argument that cultural invariance is necessary for an adaptionist interpretation of WHR preference.

WHR and waist circumference are positively correlated with testosterone and negatively associated with oestrogen[6]. Women with low WHR have better health and fertility than women with high WHR[5]. However, women in England and Texas with high

III – 3 – dentro da avaliação de biosegurança de milho tranagenico faltou trabalho que avalia o produto dessa variedade sobre a água em diferentes ecosistemas (veja o trabalho em anexo- doc. nº. 4)

Marshall et al. (2007) mostraram que produto do Milho Bt (pollen e outros) prejudicou fauna que sobrevive em agua alcancada pelos produtos de milho Bt. reduzeu significativamente seus crescimento

PNAS vol. 14 no. 41 em anexo

Toxins in transgenic crop byproducts may affect headwater stream ecosystems

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Communicated by Gene E. Likens, Institute of Ecosystem Studies, Millbrook, NY, July 31, 2007 (received for review March 5, 2007)

Corn (*Zea mays* L.) that has been genetically engineered to produce the Cry1Ab protein (Bt corn) is resistant to lepidopteran pests. Bt corn is widely planted in the midwestern United States, often adjacent to headwater streams. We show that corn byproducts, such as pollen and detritus, enter headwater streams and are subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials showed that consumption of Bt corn byproducts reduced growth and increased mortality of nontarget stream insects. Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystem-scale consequences.

caddisflies | genetically modified crops

Headwater streams are intimately connected with the adjacent terrestrial environment (1, 2). Thus, the proximity of crop fields and stream channels in the agricultural midwestern U.S. suggests that crop byproducts can enter streams. Much of the Midwest is planted in, or influenced by, row crop agriculture. In 2006, 33.1 million hectares of corn were planted in the U.S., and 35% of this was transgenic corn (www.nass.usda.gov/index.asp) modified to express the δ-endotoxin Cry1Ab, derived from *Bacillus thuringiensis* (hereafter "Bt corn"). Crop byproducts from Bt corn contain this toxin (3, 4), but until now the effects of Bt corn byproducts on stream organisms have not been examined. This is in sharp contrast to numerous studies examining potential effects on nontarget organisms in the terrestrial environment (4–8).

Crop byproducts are a component of the benthic detritus pool in agricultural streams (9), but quantitative information on the input, transport, and fate of these materials in the aquatic environment is lacking. During pollen shed, wind can transport corn pollen from 40 to 60 m away from source fields (10), and rain can dislodge and transport pollen away from crops (6). After harvest, crop byproducts remain on fields and may be transported to adjacent streams via wind and water. Once in stream channels, possible fates of crop byproducts include microbial decomposition, consumption by aquatic invertebrates, burial via sedimentation, or downstream transport (Fig. 1*A*).

We quantified inputs of corn byproducts to headwater agricultural streams, measured transport distances of these materials within streams, and examined the effects of these materials on stream-dwelling aquatic insects. We focused on headwater streams because of their dominance in the agricultural landscape, their tight linkage to the terrestrial environment, and their proximity to cornfields in the Midwest. Headwaters are also a logical starting point for assessing potential impacts of crop byproducts on aquatic environments because they serve as an initial conduit for transport to downstream water bodies. We measured inputs of corn byproducts to 12 typical headwater streams (Fig. 1 *B* and *C*) in an intensely agricultural region of northern Indiana in 2005 and 2006. The landscape in this part of Indiana is 90% row crop agriculture, and we believe that the inputs we measured are representative of the large number of streams in the agricultural Midwest. We then quantified downstream transport distances of these materials during baseflow conditions. Lastly, we used laboratory feeding studies to examine the effects of Bt corn byproducts on selected aquatic insect taxa commonly found in headwater streams.

Results

Beginning with autumn harvest and extending through the next growing season, we used stream-side litter traps to quantify litter inputs and found that the input of unharvested crop byproducts ranged from 0.1 to 7.9 g of ash-free dry mass (AFDM) m^{-2} of stream channel (Fig. 2*A*). We also found storage of crop byproducts within stream channels; benthic sediments within streams contained up to 6.4 g of AFDM m^{-2} of particulate corn byproducts. Pollen shed occurred during July and lasted ≈5–10 days at each site. Using pollen sticky traps placed in stream channels near the water surface, we found that corn pollen was aerially deposited into all streams, and annual inputs ranged from 0.1 to 1.0 g m^{-2} (Fig. 2*B*). Inputs of corn byproducts were highly variable among the 12 study streams for both litter and pollen, suggesting that potential impacts of these novel carbon sources could vary depending on the magnitude of the inputs to a given stream.

Using short-term releases of labeled material, we found that mean travel distance for leaves and cobs ranged from 0.38 to 180 m and that pollen traveled from 20 to 60 m (Fig. 2*C*). Despite the large range in size of byproducts, transport distances for all corn byproducts were strongly influenced by stream discharge ($r^2 = 0.69$, $P < 0.0001$; Fig. 2*C*). At site 2F, pollen was estimated to travel >2,000 m because of high water velocities, which contrasted with sites 1B and 1C, where pollen did not move because water velocity was near zero. Mechanisms for crop byproduct retention include deposition onto the streambed and adherence to benthic algal biofilms and macroalgae. Results from our estimates of transport distances for the various corn byproducts indicate that transgenic material entering streams is retained during base flow and thus is available for microbial processing, consumption by aquatic insects, or export during storms.

Decomposition of plant litter by microbes and physical abrasion generates food for local aquatic consumers and also facilitates the transfer of energy and nutrients from upstream to downstream reaches within a river network (11). We measured breakdown rates of Bt and non-Bt corn litter to determine whether the Bt δ-endotoxin influences rates of organic matter processing in our study streams. We found no difference in decomposition rates between Bt ($k = 0.020\ d^{-1} \pm 0.002$ SEM) and non-Bt ($k = 0.015\ d^{-1} \pm 0.003$ SEM) corn litter ($P = 0.95$; analysis of covariance), suggesting that

Author contributions: E.J.R.-M., J.L.T., T.V.R. and M.R.W. contributed equally to this work; E.J.R.-M., J.L.T., T.V.R., and M.R.W. designed research; E.J.R.-M., J.L.T., T.V.R., M.R.W., M.E.-W., C.C., N.A.G., J.P., and M.L.S. performed research; E.J.R.-M., J.L.T., T.V.R., M.R.W., M.E.-W., C.C., N.A.G., J.P., and M.L.S. analyzed data; and E.J.R.-M., J.L.T., T.V.R., and M.R.W. wrote the paper.

The authors declare no conflict of interest.

Abbreviation: AFDM, ash-free dry mass.

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www.pnas.org/cgi/doi/10.1073/pnas.0707177104

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III – 4 – faltou ainda para a avaliação de biosegurança dessa variedade um experimento que avalia o efeito do seu plantio sobre insetos que não sejam caterpillars, como aphis (veja em anexo o doc. nº 5)

Faria et al. (2007) mostraram alta susceptibilidade de Milho Bt ao Aphis e, isso insentiva ainda crescimento de outros insetos parasitoides. Há ainda disequilibrio e disturbio ao ecosistema natural

Plos one 2(7) e500

Comentário final:

Todos os trabalhos de experimentos realizados e apresentados no processo que foram feitos pela empresa produtora mostram falhas comprometedoras e tecnicamente com pouco mérito científico. Eles não alcançaram mérito para serem publicados em jornais científicos especializados, julgados por pares e em revistas indexadas. Isto é contrário aos trabalhos anexados a este parecer, que mostram diferentes falhas de experimentos de avaliação nesse processo.

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PLoS one

High Susceptibility of *Bt* Maize to Aphids Enhances the Performance of Parasitoids of Lepidopteran Pests

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Concerns about possible undesired environmental effects of transgenic crops have prompted numerous evaluations of such crops. So-called Bt crops receive particular attention because they carry bacteria-derived genes coding for insecticidal proteins that might negatively affect non-target arthropods. Here we show a remarkable positive effect of Bt maize on the performance of the corn leaf aphid *Rhopalosiphum maidis*, which in turn enhanced the performance of parasitic wasps that feed on aphid honeydew. Within five out of six pairs that were evaluated, transgenic maize lines were significantly more susceptible to aphids than their near-isogenic equivalents, with the remaining pair being equally susceptible. The aphids feed from the phloem sieve element content and analyses of this sap in selected maize lines revealed marginally, but significantly higher amino acid levels in *Bt* maize, which might partially explain the observed increased aphid performance. Larger colony densities of aphids on Bt plants resulted in an increased production of honeydew that can be used as food by beneficial insects. Indeed, *Cotesia marginiventris*, a parasitoid of lepidopteran pests, lived longer and parasitized more pest caterpillars in the presence of aphid-infested Bt maize than in the presence of aphid-infested isogenic maize. Hence, depending on aphid pest thresholds, the observed increased susceptibility of Bt maize to aphids may be either a welcome or an undesirable side effect.

Citation: Faria CA, Wäckers FL, Pritchard J, Barrett DA, Turlings TCJ (2007) High Susceptibility of *Bt* Maize to Aphids Enhances the Performance of Parasitoids of Lepidopteran Pests. PLoS ONE 2(7): e600. doi:10.1371/journal.pone.0000600

INTRODUCTION

With the rapid expansion of the commercial use of genetically modified (GM) plants, there is an increasing demand for information on their possible impact on non-target organisms. Of particular interests are parasitoids and predators that have an important function in pest regulation. To date several studies on the direct and indirect impact of GM plants on these beneficial insects have been conducted (reviewed by [1–3]), whereby most emphasis has been on so-called Bt plants, which are crops into which a gene has been incorporated from the entomopathogenic bacterium *Bacillus thuringiensis*. The introduced genes encode for the production of specific insecticidal proteins. An impact on entomophagous insects resulting from this transformation could be due to direct effects of the toxin, indirect effects via reduction in host or prey quantity and quality, or through unintended changes in plant characteristics caused by the insertion of the transgene. The first two potential effects have been widely investigated [1–3], but very few studies have specifically looked at the impact of other plant characteristics that may have unintentionally been altered as a result of transformation.

The primary targets of the Bt toxin are insects belonging to the Lepidoptera, Diptera and Coleoptera [4,5]. This, and the fact that the toxin is not transported in the phloem [6–8] makes that aphids are very unlikely to be directly affected by the toxin. Recent reports suggest that aphids actually perform better on Bt maize lines than on their near isogenic counterparts [8–11], but the generality and cause of the differences remain, as yet, unknown. We too found indications that the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), does better on Bt maize (unpubl.). These findings prompted the current study that aims to assess possible effects of the incorporation of the Bt gene into maize on the corn leaf aphid *R. maidis*, and to test if such effects reflect on the performance of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a generalist larval parasitoid of several important lepidopteran pests that can use aphid honeydew as a food source (Video S1) [12]. By including six distinct Bt lines in the study we could rule out that the consistent differences in aphid susceptibility between the transformed and near isogenic lines resulted from accidental changes due to differences in breeding history after transformation.

The six Bt lines, which covered three different transformation events, were indeed found to be significantly more susceptible to *R. maidis*. Subsequent analyses of phloem samples of transgenic and near-isogenic pairs were performed to determine if amino acid composition might explain the observed higher aphid performance on Bt maize.

As a consequence of the higher aphid numbers there were larger quantities of honeydew on Bt maize plants. Honeydew is often exploited as food by animals like honeybees, wasps, insect predators and even vertebrates [13–15]. It can also be a key alternative food source for parasitoids in the absence of plant-provided nectar [16–18], which is often the case in agricultural monocultures. We tested if the parasitic wasp *C. marginiventris* might benefit from enhanced performance of aphids on Bt maize by measuring their longevity and parasitism rates in cages with aphid-infested transgenic maize and in cages with aphid-infested non-transformed isolines. Sugar composition and the intake by the

Academic Editor: Hany El-Shemy, Cairo University, Egypt

Received January 9, 2007; **Accepted** June 9, 2007; **Published** July 11, 2007

Funding: This study was funded by the Swiss National Centre of Competence in Research "Plant Survival", research program of the Swiss National Science Foundation.

Competing Interests: The authors have declared that no competing interests exist.

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