Bt or not *Bt*: Is that the question?

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Recent public concerns over the transgenic (genetically modified) plants and nontarget impacts such as those from *Bt*-toxin expressing corn pollen on the monarch butterfly populations have escalated, despite good pest management intentions and good science (1–9). Plant resistance to insect pests has evolved naturally over many millions of years and involves (*i*) both constitutive and inducible phytochemical and morphological mechanisms in plants, (*ii*) counteradaptations to plant defenses by the herbivores, and (*iii*) biotic interactions of the multitrophic level communities of insect pathogens, parasites, and predators (10–13). The outcomes of such complex biotic interactions are sometimes determined by local mosaics of abiotic environmental conditions or regional climate changes that directly influence the component participants and their behavioral, physiological, and genetic adaptations (14–16). The intentional selection and breeding of insect and/or pathogen-resistant plant genotypes such as corn (*Zea mays)* has resulted in slow, but steady, progress against stalk-boring larvae such as the European corn borer and other such species, and host plant resistance in general has significantly reduced the need for broad-spectrum insecticides across agroecosystems and forests (17, 18).

The use of fairly specific natural microbial insecticides such as the Cry1Ab or Cry 1Ac endotoxins from *Bacillus thuringiensis* var. *kurstaki* (*Btk*) have been hailed as a new jump-shift in targeted pest control methods, with putatively minimal impacts on nontarget organisms in millions of acres of forests for gypsy moth control and cornfields for stalk-boring Lepidoptera control (19, 20). The specificity of *Btk* (for Lepidoptera) and *Bti* (for Diptera) has not always been accepted in the public mind. For example, in the 1980s mosquito control sprays with *Bti* were suspected to be killing nontarget Lepidoptera (including the endangered Schaus swallowtail butterfly) in Munroe County of the Florida Keys (P. Eliazar, personal communication) potentially caused by bacterial spore or other microbial contamination of the formulation. Contamination of *Btk* during production with other microbes and different types of *Bt* toxins that could poison other animals and humans has reportedly been reduced since 1989 (21).

It has recently been possible to genetically engineer plants, such as *Z. mays*, to avoid problems with insecticidal spray drift and more efficiently express toxins in particular plant tissues against lepidopterous pests. Such efficient, effective, and relatively safe tools for pest management have eluded classical plant breeders and integrated pest management practitioners for many decades (20). However, even though safe for most other taxonomic groups of insects other than Lepidoptera, the nontarget impacts of *Btk* require careful and thorough evaluation combined with extensive publicizing. If not done, the real risks, which may be minimal, can be significantly amplified in the public minds by perceived risks and distrust of biotech corporations and the regulatory federal agencies and similar goal-affiliated agricultural research institutions and universities (22, 23). Additional risks for crop pest management such as the rapid development of insect resistance to the *Bt* insecticides also need serious attention and consideration in long-term agroecosystem design as well (24, 25).

The use of aerial applications of *Btk* toxins (and live spores) across millions of acres of forests and urban areas against the gypsy moth, *Lymantria dispar,* resulted in heated debates across the United States as the moth defoliation damage steadily spread. This largely emotional public reaction to gypsy moth damage and the pesticides sprayed for control illustrates the importance of careful studies of nontarget impacts and open disclosure of these potential impacts to the public (21). Despite the assurances about short duration field toxicity of *Btk*, it turned out that the purported window of “vulnerability” for hundreds of nontarget Lepidoptera species was not simply 2–4 days postspray as reported for the gypsy moth larvae, but instead could extend for 4–6 weeks or more, even under natural field conditions with several heavy rains, direct sunlight (and UV radiation), and opportunities for microbial degradation (26, 27).

The mechanisms permitting this extended toxicity of aerially applied Btk sprays to nontarget lepidopteran species so exposed under natural conditions are still not clearly identified, but may include naturally lower toxic dosages than for gypsy moths (28, 29), differential interactions with natural phytochemicals (such as tannins and phenolics) in different plant species (30), vegetative growth of the live spores and subsequent protein crystallization and concentration on the nutrient-rich leaf phylloplanes (21, 31), and/or other leaf phylloplane microbial synergisms of *Btk* toxicity inside the insect gut (32, 33). With these concerns publicized, and the potential of ecosystem-wide effects (21, 26, 27) there was eventually a need recognized (federally) to officially investigate nontarget effects of *Btk* sprays for gypsy moths, which resulted in large funding through the Forest Service to a single state.

A similar political need to assess the extent of nontarget risks of *Bt* corn pollen surfaced shortly after the 1999 *Nature* publication on monarch butterflies (1, 7). It seems clear, for various reasons described in the six articles that recently appeared in PNAS (1–6), that the risks to nontarget lepidopterans of *Bt* toxins in corn pollen are significantly less than those involved with aerial application of *Bt* sprays for gypsy moths. It may be that much of the concern about *Btk* corn pollen deals more with perceived risks than with realized and potential risks (34). However, even with studies by federal research and enforcement agencies (19, 21), the public and some of the scientific community remain understandably cautious about accepting the stated “safety” assurances associated with new technologies (22, 23).

The potential dangers of movement of transgenic pollen from the corn plant into the agroecosystem and adjacent hedge-rows and forest edges has been demonstrated to be of minor and rather localized concern (4, 6). However, escape of crop transgenes into wild relatives via hybridization (22) or virus-infected transgenic plants producing more virulent (pathogenic) genotypes with recombined mRNA of the transgene should not be lightly dismissed (35). These articles addressing Bt-corn pollen impacts on the migratory “conservation flagship species” of monarch butterfly (1–5) and one swallowtail butterfly (6) strongly suggest that the

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Table 1. Penultimate instar larval performances in bioassays of *P. glaucus* and *P. troilus* on leaves of their favorite host species that had been dusted with *Btk* corn pollen and non-*Btk* pollen at 10% fresh leaf weight concentrations (2,700–3,600 grains per square cm on tulip tree and 1,350–1,800 grains per square cm on spicebush, respectively) compared with untreated control leaves

	P. glaucus on TT				*P. troilus* on SB			
Treatment	*n*	RGR	RCR	ECI (%)	*n*	RGR	RCR	ECI (%)
Control leaf	9	0.297	2.55	11.7	5	0.304	3.82	9.9
		0.056	0.31	1.7		0.070	0.49	4.3
Btk pollen	17	0.152**	2.12	7.2**	11	0.148*	4.38	5.8
		0.027	0.40	1.1		0.034	0.98	2.1
Non-Btk pollen	16	0.150**	2.99	4.9**	12	0.136**	3.87	4.0*
		0.036	0.28	1.0		0.024	0.75	0.8

Significant differences between treatment means and control are indicated (**, $P < 0.05$; *, $P < 0.10$; *t* test). TT, tulip tree (Magnoliaceae); SB, spicebush (Lauraceae); RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiencies of conversion of ingested food. Data are presented as mean ± SE.

large heavy pollen grains of corn almost always fall within a few meters of the plant (2, 6) with little potential for lethal dosages accumulating on butterfly host plants (1, 5, 6). Even with the "hottest" pollen (engineered for *Bt* toxin expression at the highest levels; event 176), the critical lethal dosages are confined to areas inside and very near the field (within a few meters). Plant anther parts also may reach nontarget host plants with the pollen, but have 100 times the toxic impact of pollen itself (5). There were no studies of the persistence of toxicity of Btk pollen in the soil or aquatic systems after it was washed off by rain.

The sublethal impacts on the monarch butterfly and other Lepidoptera are also still not completely understood at this time. For example, it is still not clear what consumption of Bt pollen at sublethal levels will do to susceptibility to natural enemies and disease, nor to interactions affecting migratory or reproductive success of the monarch butterfly populations. The results in one study (6) suggest that natural enemies can be major mortality factors in and around these cornfields. Similarly, Bt sprays can slow larval growth and enhance the mortality to Lepidoptera caused by predatory and parasitic activity (21).

Because native nontarget Lepidoptera that inhabit hedge rows and forest edges immediately adjacent to corn fields were not included in the monarch butterfly *Btk* corn pollen studies, a small study was undertaken (Table 1) by using *Papilio glaucus* (the Eastern tiger swallowtail butterfly on its favorite host, tulip tree) and *Papilio troilus* (the spicebush swallowtail butterfly on *Lindera benzoin*). These insect species are distributed throughout the eastern half of the United States and into Canada and are common throughout the entire midwestern corn belt of North America where the most field corn is produced (20). When fresh Bonus variety sweet corn *Btk* pollen and non-*Btk* corn pollen (from adjacent plots) were multiply sifted (for anther and other plant parts) and applied to the leaves of favorite host tree species at the very high concentration of 10% pollen per fresh leaf weights, no differences were detected in effects of *Btk* and non-*Btk* pollen for either *P. glaucus* or *P. troilus* (Table 1). However, the average growth rates were suppressed (when fed leaves dusted with both pollen types) to about half of that observed on control leaves (no pollen). These suppressed relative growth rates were the result of lower overall efficiencies of conversion of ingested food into larval biomass, with no reduction in relative consumption rates (Table 1). Similar results were obtained for 1% pollen by leaf fresh weight (data not shown). In a parallel study, the leaves were dusted on only half at 10% levels and selective avoidance of both *Btk* and non-*Btk* pollen types by *P. glaucus* was indicated by leaf area consumed in 24 h (*t* tests; $P = 0.002$ for Bt pollen and $P = 0.031$ for non-Bt pollen versus controls). However, *P. troilus* ate both sides of the leaf with no apparent deterrence by pollen of either type compared with control sides ($P = 0.42$, $P = 0.47$, respectively).

The implications of these results are that even unrealistically large amounts of *Btk* sweet corn pollen (estimated by 10% weights to range from 2,700 to 3,600 grains per square cm for *P. glaucus* on tulip tree and 1,350 to 1,800 grains per square cm for *P. troilus* on spicebush leaves) does not have significantly different effects than non-*Bt* pollen. In any case, at distances of 1–2 m of the cornfield edge, chances of 10% (by leaf fresh weight) concentrations accumulating on tree leaves are highly unlikely (and even 1% levels would be improbable), based on these studies of natural pollen dispersal in the field (1, 4, 6).

Although the biotech industries and some researchers are aware that the event 176 corn pollen will reportedly be phased out of commercial production soon (1), the general public may be skeptical that self-regulation by the companies is sufficient or appropriate. The level of regulation and nontarget concerns espoused by the Environmental Protection Agency and university agricultural researchers has been questioned by the environmentally concerned public and other researchers. Premature press releases by the biotech consortia such as the Agricultural Biotechnology Stewardship Working Group (ABSWG) have been interpreted as "eager interest groups (that) appear to spin even preliminary and debated results" (22, 23). Also, on August 24, 2001, stating "confidential business information restrictions" on some data, the Environmental Protection Agency posted the "data call-in" with the executive summary from the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) that "extensive empirical lab and field studies on the potential hazard and actual exposure indicate that *Bt* corn pollen poses negligible risk to monarch butterflies."

Hopefully these recent six PNAS studies, carefully conducted by many excellent scientists at various locations, will clarify the biological risks associated with different types of corn pollen for some nontarget lepidopterans. However, these studies also illustrate the variability (uncertainty) of predicting realized risks in different regions of North America. For example, it has been shown that in Iowa, Minnesota, and Wisconsin that cornfields and soybean fields produce 73–78 times more monarchs (on their milkweed hosts) than nonagricultural habitats. However, in Ontario, Canada, in the same year, milkweeds in cornfields were not a significant contributor to monarch production (2). It seems that the more significant risks to monarch butterflies may be the use of very clean tillage practices, extensive herbicides, and other crop choices that affect milkweed populations (as well as other insecticides; refs. 2, 3, and 36). These PNAS studies also illustrate that different *Bt* events will have different toxicity against different instars (3, 5) and that phenological differences between pollen shed and monarch migration will vary considerably with latitude and localities (1, 2, 4).

The management of pests in field and forest systems is based on numerous biotic, abiotic, and economic factors. The decision to use any pest management option will almost certainly have some impact on nontarget organisms (6, 37). The decision not to use Btk sprays for gypsy moth management programs can have more severe impacts on nontargets than heavy spraying. For example, generalized parasites and predators and pathogens associated with outbreak populations of the herbivorous pest can affect nontargets severely, either directly or indirectly (38, 39). Broad-spectrum insecticides needed for sweet corn production will likely continue to be much more severe for the monarch butterfly larval survival than the *Bt* corn pollen (3). The relative risks of various pest management options need to be clearly, openly, patiently, and extensively explained to the public and the general scientific community to reduce the intensely emotional reaction that frequently can be observed if perceived as one-sided, rushed in their safety evaluations, or not in alignment with long-term sustainable management (22, 23, 34, 40).

With all of the promise of various microbial pesticides from 40,000 *Bacillus thuringiensis* isolates (41, 42), it is surprising how little is known about its long-term survival and natural field ecology (31, 43–46). This knowledge seems important in assessing the potential for genetic exchanges (including transgenes) by different bacteria "species" or transmission of pathogens from insects to other species (47, 48). Pathogenicity of Bt isolates have been described against five insect orders and mites, nematodes, flukes, molluscs, and protozoans (49). Effects on these and related nontargets remain basically of unknown significance in ecosystem functions globally (50, 51). It is interesting that the first scientific description of *B. thuringiensis* in 1915 by a German (Berliner) came from the Thuringia area of that country (21) not far from the newly founded Max Planck Institute for Chemical Ecology in Jena. Here, and with international collaborators, natural and genetically engineered plants are under intense examination by several research teams to elucidate chemical mechanisms of plant resistance to herbivores directly and also indirectly via plant-to-plant volatile communication and multitrophic level signaling of the herbivore's natural enemies in relation to herbivore attacks (52). Potentially exciting new tools for pest management involving the larger part of the insect and plant community may be possible provided that "risks" are carefully evaluated from ecological as well as economic perspectives.

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