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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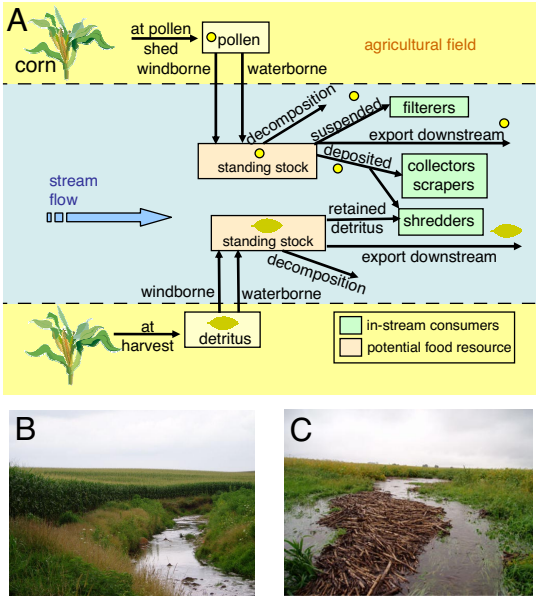

Fig. 1. Potential fate of corn byproducts in streams adjacent to corn fields. (*A*) Filterers are aquatic invertebrates that consume suspended materials, collectors and scrapers consume deposited materials, and shredders consume decomposing detritus. (*B*) Illustration of a typical headwater agricultural stream during pollen shed with a buffer strip of grass and adjacent corn fields. (*C*) Illustration of accumulations of corn byproducts after harvest.

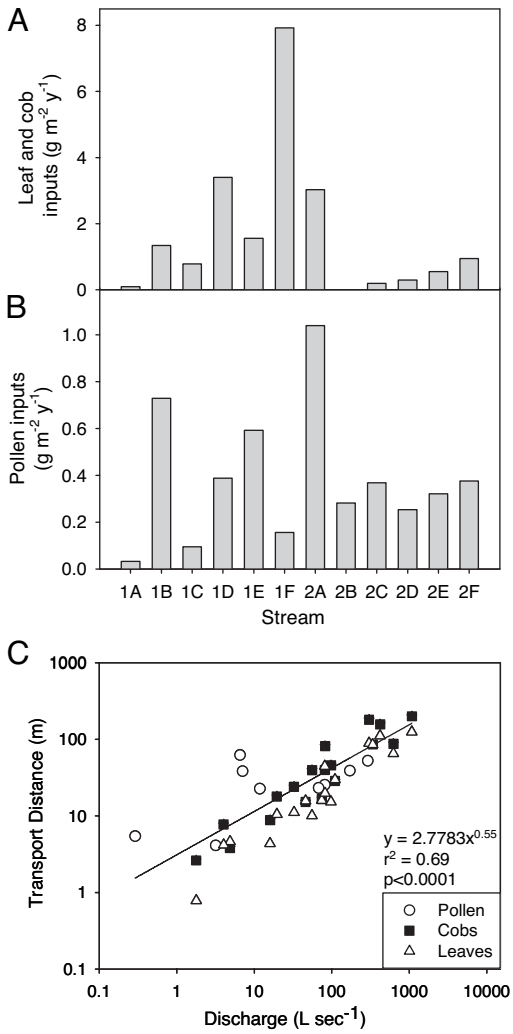

Fig. 2. Input rates and transport distances of corn byproducts in 12 streams (1A–1F and 2A–2F) in northern Indiana. (*A* and *B*) Annual leaf litter and cob (*A*) and corn pollen (*B*) input rates in g m^{-2} y^{-1}. (*C*) Relationship between transport distances of pollen, leaves, and cobs across a range of discharges for 12 streams ($r^2 = 0.69$, $P < 0.0001$, $y = 2.78x^{0.55}$).

transgenic and nontransgenic corn byproducts are processed in the same manner. Corn byproducts retained during baseflow, if not decomposed or consumed, are subject to transport to downstream reaches during high discharge (e.g., storms), which can occur throughout the year in our study area (12). Storm flows are a primary driver of particle export from headwater channels (13), and we predict that transgenic crop byproducts in these headwaters are likely transported to downstream systems during high discharge. We quantified inputs of corn byproducts to headwater streams and the potential for downstream transport, but the ultimate impact of these materials on downstream ecosystems is currently unknown and in need of further study.

Although the Bt δ-endotoxin in corn byproducts entering streams did not appear to affect microbial processes associated with litter decomposition, the presence of the toxin is potentially significant to consumers inhabiting these systems. The Bt δ-endotoxin targets lepidopteran (butterflies and moths), dipteran (true flies), and coleopteran (beetles) pests. Previous research demonstrated that the effects on nontarget terrestrial organisms depended on exposure concentration and that terrestrial insects, specifically lepidopteran larvae, typically do not consume enough Bt corn pollen in the field to be negatively affected (4). Research on the effects of *B. thuringiensis* var. *israelensis* (Bti), applied directly into aquatic habitats for mosquito and blackfly control, found conflicting results (14), with some studies demonstrating negative effects on nontarget aquatic insects (15, 16) and some studies showing no deleterious effects of Bt toxins on nontarget aquatic taxa (17). Bti application consists of the direct application of Bti bacteria and differs from incorporation of Bt into a plant's genome with the subsequent presence of Bt δ-endotoxin throughout the plant. No studies to our knowledge have examined the impact of Bt crop byproducts on nontarget stream insects, such as trichopterans (caddisflies), which are common in streams (18) and are closely related to lepidopterans, the group targeted by the Cry1Ab protein in Bt corn.

Trichopterans have diverse feeding strategies (18) and will potentially consume crop byproducts when they are available as a food resource. Filter-feeding trichopterans build nets to filter particles from the water column, and corn pollen is in the size range of particles captured by their nets (19). Trichopteran taxa that feed by scraping biofilms off submerged surfaces may consume deposited pollen. Detritivorous trichopterans that feed on leaf litter are also common in streams and may consume decomposing corn leaves when present in agricultural streams. We hypothesized that when trichopterans consume Bt corn byproducts, they could be negatively affected by the Cry1Ab protein.

In the field, we found that 50% of filtering trichopterans collected from our study streams during peak pollen shed had pollen grains in their guts and that detritivorous trichopterans were located in accumulations of decomposing corn litter in the streams after harvest. In laboratory feeding trials, we found that the leaf-shredding trichopteran, *Lepidostoma liba*, had >50% lower growth rates when they were fed Bt corn litter compared with non-Bt corn litter ($P = 0.008$; Fig. 3*A*), although mortality of *L. liba* among litter types did not differ. Rates of *L. liba* fed non-Bt corn litter were comparable to rates of *L. liba* fed maple leaves, a common food resource consumed in nonagricultural streams (C. C., unpublished data). In streams, leaf-shredding trichopterans that consume Bt corn litter may experience reduced growth, which can negatively

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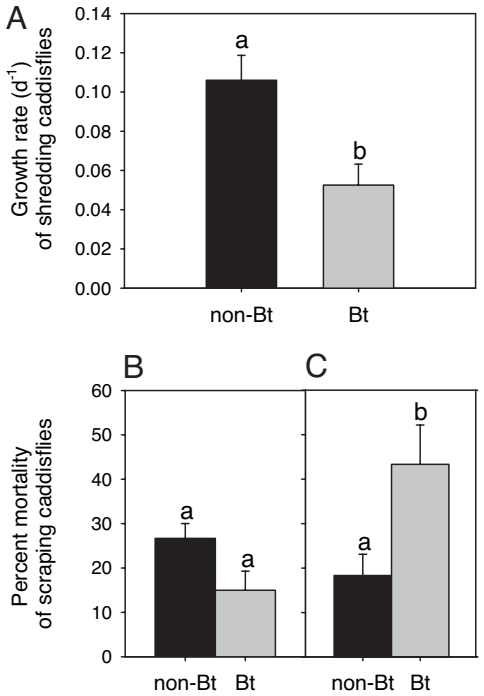

Fig. 3. Potential ecological effects of Bt corn. (*A*) Growth rates of the shredding caddisfly *L. liba* fed non-Bt and Bt corn leaves ($P = 0.008$, Student's *t* test). (*B* and *C*) Mortality rates of the scraping caddisfly *H. borealis* when fed non-Bt and Bt corn pollen at mean ambient concentrations (0.055 g m^{-2}) ($P = 0.42$, Tukey's post hoc test) (*B*) and high concentrations (2.75 g m^{-2}) ($P = 0.059$; Tukey's post hoc test) (*C*). Error bars represent standard errors, and significant differences are indicated by different letters.

influence fitness, because adult size of aquatic insects is directly related to fecundity (20).

We also measured mortality rates of *Helicopsyche borealis*, an algal-scraping trichopteran, reared in chambers with algal biofilms and corn pollen at concentrations based on the mean daily aerial input rates (0.055 g m^{-2}) we measured at our study sites. Results showed that *H. borealis* mortality was not significantly different between Bt and non-Bt treatments ($P = 0.486$; Fig. 3*B*). However, at a pollen concentration of 2.75 g m^{-2}, which was between two and three times higher than maximum aerial input rates observed at our sites (1 g m^{-2}), mortality was higher in the Bt treatment (43%) than in the non-Bt treatment (18%) ($P = 0.027$; Fig. 3*B*). These results suggest that pollen adhering to algal biofilms can be consumed by scraping trichopterans and that, at high concentrations, stream-dwelling trichopterans can be harmed by the Bt δ-endotoxin in crop byproducts. Lower growth rates and higher mortality of stream caddisflies, as measured in our laboratory feeding studies, could potentially reduce secondary production (21) and consequently the prey biomass available to stream and riparian predators, such as fishes, amphibians, and birds. We predict that the effects will be most evident with caddisflies because of their close relationship to the lepidopteron target species, but how the effect would extend to other aquatic invertebrates is currently unknown.

Discussion

Results of our research on the influences of Bt crop byproducts on headwater streams have several broad implications. First, previous research assumed that transgenic crop byproducts would remain on fields (3), which overlooks the potential for these materials to enter and be dispersed by headwater streams. Surface waters can transport transgenic DNA (22), and we now show that toxin-containing crop byproducts are likewise dispersed through the landscape by streams. Secondly, stream insects have not been a focus for examination of the ecological effects on nontarget organisms despite their proximity to agricultural fields and, in the case of trichopterans, their close phylogenetic relationships to target species.

Our results indicate that Bt corn byproducts may have negative effects on the biota of streams in agricultural areas. Based on these findings, we suggest that the assessment of potential nontarget effects from transgenic crops should be expanded to include relevant aquatic organisms, such as stream insects. Lastly, headwater streams in the midwestern United States are already impaired by nutrient enrichment and extensive habitat degradation (23, 24); Bt crop byproducts could represent an additional stressor to these systems, which has implications for stream restoration and riparian management in agricultural landscapes.

Materials and Methods

Study Sites. During 2005 and 2006, we quantified corn byproduct inputs to 12 headwater streams (200-m reaches) in northern Indiana, an intensively cropped region with ≈90% of land area in crops (study sites: 1A–1F in 2005 and 2A–2F in 2006). We selected study reaches with corn on both sides to document the maximum input of corn byproducts that might occur in theses systems. Riparian vegetation consisted of grass buffers between fields and streams (Fig. 1*B*). Streams ranged in width, discharge, water velocity, distance from corn fields, and bank slope (Table 1).

Table 1. Average discharge, velocity, buffer width, channel width, stream width, and channel slope for each stream study site

Site	Discharge, liters sec^{-1}	Velocity, m sec^{-1}	Average grass buffer width, m	Average channel width, m	Average distance from corn field, m	Average stream width, m	Average bank slope, %
1A	1.0	0.004	36.3	5.1	41.4	1.3	0.33
1B*	4.9	0.005	12.9	3.8	16.7	2.2	0.69
1C	191.7	0.19	14.8	6.7	21.5	3.9	0.41
1D	46.6	0.04	1.7	4.4	6.2	4.2	0.57
1E	56.7	0.07	2.5	4.2	6.7	3.0	0.43
1F	31.1	0.09	26.7	4.6	31.3	1.9	0.47
2A	597.0	0.30	15.3	8.2	24.0	4.5	0.39
2B	141.7	0.15	5.2	6.1	11.3	3.3	0.41
2C	31.8	0.10	31.6	3.7	38.2	2.0	0.60
2D	292.7	0.14	22.3	5.6	27.9	5.3	0.44
2E	23.0	0.08	25.2	5.4	37.5	1.6	0.49
2F	112.7	0.16	2.5	3.8	6.9	3.7	0.53

*Site 1B was not flowing in summer and contained standing water similar to a long wetland.

Inputs of Corn Byproducts and Pollen. After corn was harvested, until the following growing season, we measured inputs of leaves, stalks, and cobs to the streams, using 10 litter traps per stream (25). Contents of litter traps were retrieved approximately monthly, material was sorted, and all corn material was processed and weighed to estimate AFDM entering streams. In 2006, we used a corer to quantitatively sample stream sediments and estimate AFDM of corn byproducts present in sediments (26). During July, we measured pollen inputs to the 12 study streams, using pollen traps deployed daily throughout the 5- to 10-day pollen shed (27). In each reach, we deployed traps every 20 m ($n = 10$), 35 cm above the stream surface to collect pollen; microscope slides with exposed double-sided tape were anchored on each trap and deployed for 24 h. We collected two slides from each trap per day during pollen shed and stored slides in the dark until pollen counts were made by using fluorescence microscopy (×40) (28). We calculated pollen input rates as grams of corn pollen deposited per meter squared streambed per day ($\approx 3.5 \times 10^6$ corn pollen grains in 1 g).

Transport Distance and Decomposition of Corn Byproducts. We measured transport distances of corn pollen, leaves, and cobs during baseflow conditions at all streams in 2005 and 2006 and also measured transport distances at higher discharge in 2006. After harvest, we measured transport distances by releasing painted corn leaves and cobs and measuring distance traveled before retention (24). We measured pollen transport during pollen shed by releasing stained corn pollen and calculating the average distance a grain traveled before settling (particle transport distance, S_w) (19, 30). We estimated water transit time in the reach before pollen release by adding a solution of dissolved NaCl and following it with a conductivity meter. We added 0.5 g of basic fuschin-stained corn pollen (to differentiate released pollen from *in situ* pollen) suspended in 4 liters of water as a pulse to a well mixed area of the stream over ≈1 min. We collected depth-integrated water samples every 15–30 sec at multiple stations spaced downstream of the release point. We measured the concentration of pollen grains by filtering the water samples onto 0.45-μm filters and counted pollen grains using fluorescence microscopy. We estimated the total pollen passing over each station using discharge, time, and particle concentration. We calculated S_w with an exponential decay model: $\ln N_x = \ln N_0 - kx$, where N_x is the distance at x meters downstream of the injection point, N_0 is the total pollen passing over the most upstream station, and k is the exponential decay rate. Linear regression was then used to calculate the decay rate (k) and test for significance of the relationship. Transport distance (S_w) was calculated as the inverse of the decay rate: S_w (m) $= k^{-1}$.

After harvest, we measured decomposition rates for both Bt and non-Bt corn leaves using standard litterbag methods. Dried corn leaves were collected from fields directly after harvest in late October. Thirteen grams of air-dried corn leaves were placed into each litterbag (10-mm mesh). Twenty-one Bt and 21 non-Bt litterbags were anchored to the streambed with rebar ($n = 3$ streams). Types of Bt and non-Bt corn placed in each stream were selected to closely match lignin and C/N content. Three replicate litterbags were retrieved on days 0, 3, 7, 13, 25, 37, and 70, from November 7, 2005, to January 13, 2006. Contents were processed to obtain AFDM remaining on each date; relationships between percent remaining and time elapsed were used to obtain decay coefficients ($-k$) (31). We used analysis of covariance with corn type (Bt/non-Bt) nested within stream to test for differences between Bt and non-Bt decomposition rates.

Effects of Caddisflies Consuming Bt Corn Byproducts. During pollen shed 2005, we examined guts of filter-feeding caddisflies (*Hydropsyche* spp.) to assess whether they consumed corn pollen. We measured the effects of caddisflies consuming corn pollen with laboratory feeding trials in which we fed scraping caddisflies, *H. borealis*, algae (their typical food resource), and pollen at concentrations measured in the field (average ambient as measured by aerial inputs = 0.055 g m^{-2}) and at 2.75 g m^{-2} (two to three times higher than maximum observed input rate). *H. borealis* were collected from a stream in Illinois that did not have corn planted in its drainage. We used six replicates of each treatment, and in each replicate, 10 marked individuals were monitored for 18 days. Each replicate consisted of 125 ml of aerated groundwater inoculated with algae. We measured mortality in replicate chambers as the response variable and Tukey's method was used to assess differences in mortality among treatments.

We measured effects on leaf-shredding caddisflies, using *L. liba*, a common inhabitant of headwater streams and an important processor of leaf litter (32, 33). Eight replicate aquaria containing *L. liba* were fed senesced Bt leaves, and eight were fed senesced non-Bt leaves collected from our study sites. In *L. liba* growth studies, aquaria were filled with 1 liter of water and 500 ml of sand substrate and stocked with four individuals of *L. liba* averaging 3.5 mm in length. Leaves were cut into 14-mm-diameter portions and preconditioned for 3 days in stream water to allow for natural microbial colonization. Photoperiod was held constant at 10:14 light:dark, and temperature was maintained at 12°C to mimic natural conditions during early spring when the experiments were performed. Total length of each individual was measured before and after feeding trials from digital pictures taken with Image J software. Feeding trials lasted 29 days; leaves were added to aquaria as needed. Leaves were selected to minimize potential differences in nutritional quality. A previous study (34) was criticized for not using isogenic varieties of corn materials in feeding trials (4). However, Bt corn detritus has 33–97% higher lignin concentrations than respective non-Bt isolines (35), meaning that the presence or absence of Bt in a given isoline is confounded with nutritional quality. Therefore, we matched Bt and non-Bt detritus with similar lignin content and C/N to standardize nutritional value of the detritus. Initial and final biomass of each individual was estimated by using length–mass relationships, and daily growth rates were estimated by following procedures described in ref. 36. Tukey's method was used to assess differences in growth rates and mortality among treatments.

We thank Drs. Gene Likens and David Lodge and two anonymous reviewers for valuable comments on the manuscript and A. Aubeneau, A. Burtner, M. Fernandes, T. Frauendorf, P. Hoppe, S. Khan, S. Lee, D. Lee, M. Payan, S. Peterson, K. Pfeifer, A. Stojak, J. Tabel, T. Warrner, B. Welch, and H. Wellard for field and laboratory assistance. This work was supported by U.S. National Science Foundation Grant DEB-0415984.

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