

Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape

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Widespread planting of maize throughout the agricultural Midwest may result in detritus entering adjacent stream ecosystems, and 63% of the 2009 US maize crop was genetically modified to express insecticidal Cry proteins derived from *Bacillus thuringiensis*. Six months after harvest, we conducted a synoptic survey of 217 stream sites in Indiana to determine the extent of maize detritus and presence of Cry1Ab protein in the stream network. We found that 86% of stream sites contained maize leaves, cobs, husks, and/or stalks in the active stream channel. We also detected Cry1Ab protein in stream-channel maize at 13% of sites and in the water column at 23% of sites. We found that 82% of stream sites were adjacent to maize fields, and Geographical Information Systems analyses indicated that 100% of sites containing Cry1Ab-positive detritus in the active stream channel had maize planted within 500 m during the previous crop year. Maize detritus likely enters streams throughout the Corn Belt; using US Department of Agriculture land cover data, we estimate that 91% of the 256,446 km of streams/rivers in Iowa, Illinois, and Indiana are located within 500 m of a maize field. Maize detritus is common in low-gradient stream channels in northwestern Indiana, and Cry1Ab proteins persist in maize leaves and can be measured in the water column even 6 mo after harvest. Hence, maize detritus, and associated Cry1Ab proteins, are widely distributed and persistent in the headwater streams of a Corn Belt landscape.

genetically modified crops | crop detritus | ELISA | rivers

Throughout the midwestern United States, agriculture has transformed the landscape and maize is a dominant crop. Approximately 35.2 million ha of maize are planted annually in the United States, with 85% of the maize crop now consisting of insect-resistant and herbicide-resistant transgenic varieties (1). A commonly planted transgenic maize variety has been engineered to express the insecticidal Cry1Ab protein from *Bacillus thuringiensis* (Bt) to resist crop damage by the European corn borer (*Ostrinia nubilalis*); planting of insect-resistant transgenic maize represented an estimated 63% of the 2009 maize crop in the United States (1). Cry1Ab protein is expressed throughout the tissues of Bt maize (2, 3), and after maize is harvested, the protein is detectable in terrestrial detritus left on agricultural fields for at least 7 mo (4, 5). Recent work established that maize detritus enters, is processed, and can be transported within streams (6, 7), and laboratory trials suggested that consumption of Bt maize detritus may affect stream-dwelling invertebrates (7, 8) but some studies have shown little or no effect if detritus is leached prior to consumption (9). However, the fate of Cry1Ab protein in senescent and decaying maize detritus in aquatic environments is largely unknown.

The upper Midwest is a productive agroecosystem that is engineered with ditches and underfield drains (i.e., tile drains) that facilitate drainage of water from the croplands into headwater streams to prevent flooding (10, 11). Most agricultural

watersheds in the Midwest consist of channelized stream networks that rapidly accumulate and transport water, along with associated solutes and detritus (7, 12–14). These watersheds typically have a high drainage density (15), with numerous headwater streams ultimately draining into the Mississippi River or the Great Lakes.

The altered hydrology of the midwestern landscape results in stream networks with flashy hydrographs that can vary by 100-fold or more over a period of hours (e.g., 16, 17), resulting in the entrainment and downstream movement of terrestrial detritus, including crop byproducts (7, 17–19). For example, using stable isotope analysis, Dalzell et al. (18) found that the contribution of C4 carbon (attributed to maize) to the transported particulate organic carbon pool in west-central Indiana streams was 17% at base flow and increased to 22% during flood conditions. Although these simplified channels contain few natural retention structures, maize detritus is retained within the channels when discharge is low (7), and this material is rapidly decomposed by microorganisms (6) and may be consumed by aquatic invertebrates (6, 8, 20). Given the widespread planting of Bt maize, some portion of the maize detritus within stream channels may contain the Cry1Ab protein, and fluvial networks could distribute this material away from fields and across the larger landscape.

The concentration of Cry1Ab protein in Bt maize has been quantified in detritus from the terrestrial environment (4, 5, 21); however, to date, no similar measurements have been attempted on detritus collected within stream channels. Cry1Ab protein dissolved in water has also been detected (22), but the occurrence of the Cry1Ab protein has not been measured across multiple streams, including headwater channels, which are tightly linked to the terrestrial environment. A common agricultural practice of leaving crop detritus on the fields postharvest (i.e., conservation tillage) likely facilitates overland transport of maize detritus to stream channels via wind and entrainment in surface runoff from heavy precipitation. We have observed large accumulations of maize detritus along the riparian zone and within stream channels in numerous streams several months after harvest, suggesting the potential for Cry1Ab protein to occur in entrained detritus and to be dissolved in stream water.

Widespread planting of Bt maize and our observation of the large detrital accumulations in streams long after harvest promp-

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spatial pattern across the landscape (Fig. 2), and, in fact, GIS analysis showed that all 50 sites with detectable Cry1Ab protein were located within 500 m of a field planted with maize (Fig. 3). There was overlap between sites that contained stream water samples that tested positive for Cry1Ab and Cry1Ab-positive maize detritus [21 of 28 sites, respectively (i.e., 75% overlap)]. The mean concentration (\pm SD) in stream water samples that were positive for Cry1Ab protein was 14 ± 5 ng/L, with a maximum concentration of 32 ng/L.

We looked for evidence of accumulation or dilution in the spatial distribution of Cry1Ab-positive stream water throughout the study area. Of the 50 positive Cry1Ab water samples, 12 were located downstream of another Cry1Ab-positive sampling site, 20 were not, and 18 represented an uppermost sampling site and had no upstream site for comparison (Fig. 2). Water from two tile drains was sampled during the survey. One water sample was negative for Cry1Ab, and the other was positive for Cry1Ab with a concentration of 21 ng/L, which was the third highest concentration we measured in water samples, suggesting that drainage water from maize fields could be a source of dissolved Cry1Ab to streams.

Discussion

Maize Detritus in Streams. Maize detritus is a common allochthonous input to streams that drain agricultural fields, and previous stable isotope studies have shown that maize detritus comprises up to 17% of the transported particulate organic carbon budget at base flow in midwestern agricultural streams (18), with greater contributions occurring during storm flows (18, 19). In this study, we found that maize detritus was common in low-gradient stream channels in northwestern Indiana, even 6 mo after harvest. The close proximity of streams to maize fields suggests that this material may be present in streams across the midwestern Corn Belt. For example, GIS analysis showed that 91% of the 50,634 km of stream/river length in Indiana is located within 500 m of a maize field. Similar patterns occur throughout the Corn Belt states of Iowa, Illinois, and Indiana combined, where, again, 91% of the 256,446 km of stream length is located within 500 m of a maize field. Given the predominance of agricultural land use in the midwestern United States and the frequency of headwater streams draining this landscape, maize is an understudied and previously unrecognized source of allochthonous carbon to these ecosystems.

Our synoptic survey represents a snapshot in time and reflects the stochastic nature of maize inputs to streams and the role of precipitation events in delivery of crop byproducts to adjacent stream ecosystems. The dried detritus left on fields after harvest, as part of conservation tillage, enters headwater streams as a result of surface runoff and/or wind events occurring throughout the year (Fig. 3). During heavy precipitation, overland flow is the likely mechanism transporting this material to stream channels. The stochastic nature of precipitation results in maize material entering headwater streams at any point in the year and not just after harvest, and regional differences in tillage practices and precipitation patterns result in differential delivery of maize detritus to adjacent stream channels. For example, there were eight large discharge events between maize harvest and our sampling date, with the last being on May 1, 2007 (24), and the associated precipitation events likely delivered maize detritus into the streams. Despite the stochasticity in inputs of maize detritus and the lack of retentive structures in these managed simplified channels, we found maize detritus retained in more than half of the sites we sampled, which were distributed widely across the landscape (Fig. 1).

Once maize detritus enters stream channels, this carbon source degrades rapidly via a combination of microbial decomposition, physical breakdown, and invertebrate consumption (6, 8, 20), and that energy may fuel stream food webs. Maize detritus in agricultural streams decomposes in ~ 66 d ($k = -0.015$ d⁻¹) (6). Therefore, the material that we found during our synoptic survey had entered these streams relatively recently. Maize detritus is rapidly colonized by stream-dwelling invertebrates, and growth rates of invertebrates feeding on nontransgenic decomposing maize are comparable to those feeding on the deciduous leaf litter commonly found in forested streams (8).

Prevalence of Cry1Ab Protein in Maize in Streams. We found that maize detritus with transgenic proteins (i.e., Cry1Ab) was common in streams draining an agricultural landscape where transgenic maize had been planted. Our data demonstrate that maize material is transported throughout a stream network away from the field of origin. Regardless of where transgenic crops are planted, the stream network can expand the distribution of this material in the landscape (Fig. 1). When deposited on the stream bank or retained within the stream channel, transgenic detritus is available as a food resource for both terrestrial and aquatic nontarget detritivores. Detritivores (e.g., caddisflies, earthworms,

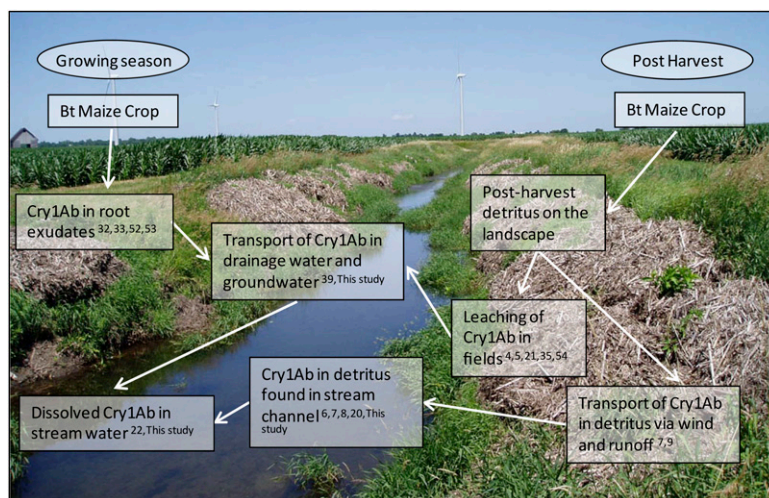


Fig. 3. Conceptual diagram illustrating pathways (and associated literature) of maize and Cry1Ab proteins entering stream ecosystems. The photograph depicts maize accumulation in the riparian zone and active stream channels observed at our study sites. Superscripted numbers cite references listed in the references section (52–54).

isopods) are key bioindicators for examining the impacts of Bt maize detritus on nontarget organisms because of their low mobility, diversity, and functional feeding roles (7, 8, 25–29). Despite its prevalence in the stream network, the effects of transgenic maize on nontarget organisms will depend on input rates, retention, decomposition, persistence, and concentration of Cry1Ab protein in maize detritus.

Our data demonstrate that long after harvest, Cry1Ab is present in submerged Bt maize detritus; thus, stream organisms may be exposed to Cry1Ab for several months. Previous research on aquatic leaching rates of Cry1Ab from maize detritus collected from streams (6), combined with work on detrital leaching rates on agricultural fields (5), suggests that the concentrations of Cry1Ab in detrital maize in streams would have been higher if we had sampled closer to maize harvest. Our data emphasize that the interaction between terrestrial and aquatic leaching of Cry1Ab from maize detritus must be recognized to understand fully the distribution of Cry1Ab in time and space.

Prevalence of Cry1Ab Protein in Stream Water. In an agricultural landscape designed to drain fields efficiently, solutes enter streams via tile drainage and/or overland flow (30, 31). We suggest that Cry1Ab proteins should behave like other solutes, and this study demonstrates that dissolved Cry1Ab proteins can be detected in stream water (however, note ref. 22). We found dissolved Cry1Ab protein across the landscape (Fig. 2), and it occurred more frequently than Cry1Ab in maize detritus retained in the stream channel. Although stream networks are connected by the unidirectional flow of water, there was no longitudinal pattern of Cry1Ab in stream water. The frequency of dissolved Cry1Ab in stream water suggests that streams are integrating the patchy distribution of Cry1Ab-containing detritus. It is unknown if there are ecological consequences for stream-dwelling organisms that are exposed to the dissolved Cry1Ab concentrations we observed.

Although Cry1Ab-positive maize detritus co-occurred with Cry1Ab in stream water 75% of the time, there are multiple pathways through which Cry1Ab protein can enter streams (Fig. 3). Cry1Ab can be introduced into agricultural soils through root exudates (32, 33) and from maize biomass (34), with the exuded and leached protein persisting for up to 180 d and 3 y, respectively (34, 35). Cry1Ab protein binds strongly to surface soils containing clay minerals, humic acids, and organomineral complexes (36–42), and it has the potential to enter adjacent streams through surface runoff and erosion. Our results from tile drains indicate that tiles may be a mechanism by which Cry1Ab leached from detritus on fields or from soils can be transported to streams. Cry1Ab released from root exudates or decaying maize detritus moves vertically through soils and can be detected at the base of 15-cm-long soil profiles for up to 9 h (39). On agricultural fields, Cry1Ab may move through the soil profile to tile drains, which are generally placed 1 m under the soil surface, thus potentially facilitating the movement of Cry1Ab to surface waters. In areas that are not drained by tiles, it is possible that shallow groundwater could transport dissolved Cry1Ab to streams. Fully characterizing the persistence and prevalence of dissolved Cry1Ab protein in the environment remains a challenge, and research to date indicates a need for temporal sampling regimens that encompass all seasons and hydrological conditions.

Method Development and Use for Detection of Cry1Ab. This study represents a unique application of optimized detection methods and quantifies the spatial distribution of Cry1Ab protein in both detritus and the water column of streams in a Corn Belt landscape. The methods are sensitive enough for detecting dissolved Cry1Ab concentrations that are diluted in stream water as well as “composite” environmental samples, such as the decomposing maize material (both transgenic and traditional maize) found in stream channels. With these refined techniques, we demonstrate

the ubiquity of Cry1Ab protein in maize byproducts and in the water column in a region with extensive row-crop agriculture. In addition, our survey was conducted in the spring and confirms that the Cry1Ab protein persists in the environment at low concentrations relative to concentrations in fresh maize material.

Conclusions

Since its adoption in the United States in 1996, the use of Bt maize has increased steadily, and 63% of the 2009 maize crop was genetically modified to express Cry1Ab protein, representing ≈ 23 million ha. This study demonstrates that maize detritus can be dispersed throughout a stream network and that compounds associated with Bt maize, such as Cry1Ab proteins, may be a more common occurrence in watersheds draining maize-growing regions than previously recognized. In addition, tile drains, which drain row-crop agriculture in much of the midwestern United States, are a likely source of dissolved Cry1Ab to streams. Cry1Ab proteins are distributed beyond field boundaries and persist after initial crop harvest. The question of whether the concentrations of Cry1Ab protein we report in this study have any effects on nontarget organisms merits further study.

Methods

Field Synoptic Survey. We conducted a 1-d synoptic survey (May 16, 2007) by sampling 217 stream sites that occurred at road crossings in a northwestern Indiana county. This 1,053-km² area is intensively cultivated and lies within the Midwest Corn Belt, with $\approx 97\%$ of land area planted in a maize-soybean rotation (43). Before intensive agricultural cultivation, streams in this area drained wetlands, tall grass prairie, and mixed oak and beech-maple forests (44, 45). Active management of streams for rapid drainage has resulted in simplified stream channels with few retentive structures (e.g., woody debris). The 217 sites are typical of streams in this geographic region and range from small headwaters to larger middle-order systems.

At each stream site, we recorded the presence or absence of maize detritus in the active channel. If present, we collected samples of maize detritus (i.e., leaves, cobs, stalks) from multiple points within the active channel. Maize detritus from the riparian zone above the high-water mark was also sampled. We placed maize detritus samples into individually labeled bags. We collected a stream water sample from each site (except from two sites that were dry, $n = 215$) and filtered it in the field through a 0.7- μm GF/F glass-fiber filter (Whatman) into an acid-washed 60-mL bottle. Detritus and water samples were frozen at -30°C until Cry1Ab analysis. For spatial analysis, we recorded the latitude and longitude coordinates of each stream site using a handheld global positioning system (GPS) device.

Analysis of Cry1Ab Protein in Maize Detritus. We determined the concentration of Cry1Ab protein in maize detritus (leaves and husks) using a commercial double-antibody sandwich ELISA. Maize detritus was thawed and oven-dried at 60°C for 48 h and then ground into powder using an electric grinder. To extract Cry1Ab protein, we added 1 \times PBS Tween-20 (PBST) to ≈ 0.5 g of ground maize leaves in a ratio of 1 g to 75 mL and to 0.5 g of husks, stalks and/or cobs in a ratio of 1 g to 50 mL. We then homogenized maize detrital samples using a handheld tissue homogenizer (BioSpec Products, Inc.), centrifuged samples at $7,546 \times g$ for 10 min, and used the supernatant for the ELISA analysis. We determined the concentration of Cry1Ab in maize detrital samples based on an eight-point standard curve, ranging from 0.5 to 100 ng/mL, which was created from the serial dilution of purified Cry1Ab protein (Abraxis) dissolved in PBST. We included five buffer blanks to account for matrix effects associated with the PBST. We aliquoted 100 μL of each sample in triplicate into a 96-well ELISA plate (Strategic Diagnostics, Inc.) and read the absorbance at 450 and 650 nm using a SpectraMax M2 microplate reader (Molecular Devices Corporation). We subtracted the absorbance at 450 nm from the absorbance at 650 nm to correct for turbidity and then subtracted the mean buffer absorbance to account for PBST matrix effects. We expressed the concentration of Cry1Ab protein as nanograms of Cry1Ab per gram of dry mass. We determined the MDL of 0.56 ng/mL (28 ng/g of dry mass for husks, stalks, and/or cobs; 42 ng/g of dry mass for leaves) by multiplying the SD of a low standard (0.72 ng/mL) run seven times by 3.14 (46). We used a Wilcoxon signed rank test to examine the differences in Cry1Ab concentrations between Bt maize detritus collected in-stream and Bt maize detritus collected in the associated riparian zone.

Analysis of Cry1Ab Protein in Stream Water. We used Millipore Amicon Ultra-15 centrifugal ultrafilter devices (30,000-dalton nominal molecular weight limit) to concentrate Cry1Ab protein from stream water samples. After water samples were thawed, we filled centrifugal filter devices with 15 mL of stream water and spun the samples using a swinging-bucket centrifuge at $873 \times g$ for 30 min. After centrifuging, we weighed the retentate (concentrated Cry1Ab) in an Eppendorf tube to determine retentate recovery. We determined the concentration of Cry1Ab in stream water samples based on an eight-point calibration curve, ranging from 0 (PBST only) to 400 ng/L, that was created from the serial dilution of purified Cry1Ab protein dissolved in PBST. We also included three negative controls (distilled water only) to identify any potential contamination among samples. All standards and negative controls went through the Cry1Ab extraction procedure along with the water samples (i.e., centrifugal devices). We pipetted 100 μ L of retentate into a 96-well ELISA plate and read the absorbance at 450 and 650 nm using a SpectraMax M2 microplate reader, and we subtracted the absorbance at 450 nm from the absorbance at 650 nm to correct for turbidity. We expressed the concentration of Cry1Ab protein as nanograms of Cry1Ab/L stream water. We calculated the MDL of Cry1Ab protein in water using the concentrations of the three lowest standards (2.6, 5.23, and 10.43 ng/L). For each standard, we measured seven samples from a stock solution and then multiplied the SD of those measurements by 3.14 (46). We then took the average of the three MDLs to determine our overall MDL of 6 ng/L.

Spatial Analysis. We downloaded the 2006 Cropland Data Layer (CDL) from the Natural Resources Conservation Service Geospatial Data Gateway (47) or the US Department of Agriculture, National Agricultural Statistics Service, Research and Development Division (48) for the states of Indiana, Illinois, and Iowa. We acquired detailed stream line data from either the High Resolution National Hydrology Dataset (49) or the Environmental Systems

Research Institute (50). Detailed state and county boundary files were also acquired from the Environmental Systems Research Institute (50). These layers were composed in ArcMap (Environmental Systems Research Institute) (51), and the GPS coordinates recorded at each of the 217 sampling sites were imported. The points were mapped according to their latitude and longitude on the North American Datum 1983.

We buffered sample sites to create circular zones of proximity at 200 and 500 m. The CDLs for Indiana, Illinois, and Iowa were converted from their native tagged image format raster format to a vector polygon, reclassifying the value for maize and eliminating all other land cover and crop types. Proximity zones for the various result values were selected based on an intersection with maize fields. We tabulated results to find the total number of each result value (maize detritus: no maize, + maize, + Cry1Ab maize; stream water: – Cry1Ab, + Cry1Ab) that was within the respective distance of a field under maize cultivation in 2006.

To find the length (in km) of streams/rivers throughout the Midwest that were within 500 m of maize fields, we buffered statewide maize polygons at 500 m to create unique polygon layers. This result was used to clip the detailed stream lines, producing line features only where a stream was intersected by the maize proximity zone. The resulting line file had the length of each feature calculated, and the total was reported for the three states and northwestern Indiana county separately.

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