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Risk Assessment of GM Crops on Aquatic Environments: From Idea to Implementation

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Abstract

Genetically modified (GM) plants, expressing traits such as insect resistance or herbicide tolerance, are widely adopted in agriculture. GM maize, soybean, cotton and calona have the largest acreage worldwide. Environmental risk assessment of GM crops is a science based process to assess the likelihood of adverse effects on the environment. Numerous studies have assessed the nontarget effects on terrestrial species, while the potential effects on aquatic organisms do not draw much attention. In this review, we provide an overview on environmental risk assessments of GM crops on aquatic ecosystem published in past 5 years, including our recent works on Bt rice risk assessment on paddy zooplanktons. The assessment processes mainly focus on the ways of GM crop materials entering into aquatic ecosystem and the effects on aquatic organisms. Some of the assessments indicate that aquatic organisms such as caddisflies (trichopteran) and a water flea (*Daphnia magna*) were adversely impacted by byproduct of GM maize expressing *Bacillus thuringiensis* (Bt) endotoxin. We suggest that special emphasis should be placed on aquatic ecosystem in risk assessment of GM crops in the future.

Keywords: Aquatic ecosystem; GM crop; Soil microorganisms; Biodiversity

Introduction

GM crops have been deliberately developed for introducing new traits which does not occur naturally in the species. These traits include resistance to certain insect pests, plant pathogens, environmental stresses, or resistance to chemical treatments (e.g. resistance to herbicide), or improving shelf life or the nutrient profile. A record 181.5 million hectares of GM crops were grown globally in 2014, increased more than 100-fold during the 19-year period from 1996 to 2014. Herbicide tolerance occupied 57% of the total global GM crop area, insect-resistant Bt crops occupied 15% and stacked traits occupied 28% [1].

There are concerns that the commercial cultivation of GM crops could result in adverse effects on the environment, such as GM crops becoming agricultural weeds, outcrossing with other species and increasing their competitiveness, contributing to horizontal gene transfer, leading to super pests and super diseases, and impacting non-target organisms and biodiversity [2,3]. The impact of GM crops on non-target organisms has always been a hot research topic in environmental risk assessment of GM crops. Non-target organisms investigated mainly focused on terrestrial dwellers, such as earthworm, woodlouse, pillbug, sowbug, collembola, mite nematodes and soil microorganisms [4].

Aquatic environments not only execute a wide range of ecological functions such as cycling nutrients and carbon, but also provide habitat and food for aquatic and terrestrial organisms [5]. The surrounding terrestrial landscape connects with aquatic ecosystem by material inputting from fields planted with GM crops to streams or water ponds nearby [6]. GM crop biomasses or foreign proteins may enter into aquatic ecosystem by erosion of soil-bound proteins, surface runoff of freely-soluble proteins, aerial movement of pollen and crop dust, and spreading of senescent crop residues [7]. Consequently, aquatic organisms are probably under the exposure to foreign proteins from GM crops. Thus, aquatic ecosystems need to be considered in the environmental risk assessment of GM crops that have insecticidal or other traits [7]. However, few studies have assessed the potential impacts of GM crops on aquatic organisms and these studies were limited to maize contain genes derived from the common soil bacterium, *Bacillus thuringiensis* (Bt) [8-11].

In this review, we summarized the results of studies on risk assessment of GM crops on aquatic ecosystem. Those studies, mainly focusing on crops with insect resistant trait (Bt) or stacked traits, were conducted to determine the ways and amount of GM crop materials and foreign proteins entering into aquatic ecosystem as well as the fate and the effects of those proteins on aquatic organisms.

Ways for GM Crop Materials or Foreign Proteins Entering into Aquatic Ecosystem

GM crops expressing Bt toxins can enter into the soil ecosystem by root exudates, plant residues remaining in the field after harvest, or by pollen shedding during flowering [12-16]. Bt corn exudes Cry1Ab protein to the rhizosphere soil up to a concentration of about 95,000 ng g^{-1} soil, and the activity was verified in a bioassay using tobacco hornworm larvae (*Manduca sexta*) [14]. The bound toxins to soil particles retain its insecticidal properties and are protected against microbial degradation largely, resulting in persistent larvicidal activity in various soils for up to 234 days [17]. Those Bt proteins entered into soil are easy to enter into adjacent aquatic ecosystems by surface runoff as freely-soluble proteins or soil-bound proteins [7].

After autumn harvest, GM crop detritus, including leaves and stalks, remain in fields and can enter streams during storms through movements by wind and water. Stream-side litter traps were used to quantify Bt maize litter inputs into streams, and it was found that the input of crop byproducts ranged from 0.1 to 7.9 g of ash-free dry mass m^{-2} of stream channel; it was also found that corn pollen was aerially deposited into streams, and annual inputs ranged from 0.1 to 1.0 g m^{-2} [10]. Residues of Bt maize in streams can travels distance ranged from 0.38 to 138 m depending on water velocities [10].

Tank et al. surveyed 217 stream sites in Indiana to determine the extent of maize detritus and presence of Cry1Ab protein in the stream network. It was found that 86% of stream sites contained maize leaves, cobs, husks, and/or stalks. Cry1Ab protein was detected in stream-channel maize at 13% of sites and in the water column at 23% of sites. Hence, maize detritus, and associated Cry1Ab proteins, are widely distributed and persistent in the headwater streams of a Corn Belt landscape [18].

We conducted a 3-year study to determine the amount, persistence and movement of Cry1Ab/1Ac protein released from Bt rice. Bt rice was planted in fields upstream to non-Bt rice field. The Cry1Ab/1Ac protein was detected in the water of Bt rice fields during the growth stage, and it was not detected in the water of non-Bt fields which received water from Bt fields. In the surface and 10 cm deep soil, Cry1Ab/1Ac protein was detected in Bt fields, but not in non-Bt fields during harvest period. Based on the above results, we was conclude that Bt-MH63 and Bt-SY63 rice can release detectable amounts of Cry1Ab/1Ac protein into soil and water in the growth period and the Bt protein does not move into adjacent paddies along with the irrigating water [19].

Effect of GM Crop Materials on Aquatic Organisms

Plenty of researches studied the effects of Bt crops on nontarget terrestrial organisms. Headwater streams in agriculture regions can receive GM crop detritus after the fall harvest, which is then consumed by a diverse community of stream invertebrates. However, the effects of GM crop detritus on nontarget aquatic organisms were not been sufficiently studied.

Rosi-Marshall et al. examined the impact of Bt crop byproducts on nontarget stream insect caddisflies (trichopteran), which are common in streams and consume decomposing pollen and leaf litter in agricultural streams. They 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. Another trial measured mortality of *Helicopsyche borealis*, an algal-scraping trichopteran, reared in chambers with maize pollen, and result showed that H. borealis mortality was not significantly different at low pollen concentration. However, at high pollen concentration the mortality was higher in the Bt treatment (43%) than in the non-Bt treatment (18%).

Those results suggest that stream dwelling trichopterans can be harmed by the Bt δ-endotoxin in Bt maize byproducts.10 Chambers et al. also conducted laboratory feeding trials and found that the leaf-shredding trichopteran, *Lepidostoma liba*, grew significantly slower when fed Bt maize compared to non-Bt maize, while other invertebrate taxa that we examined showed no negative effects. They used field studies to assess the influence of Bt maize detritus on benthic macro invertebrate abundance, diversity, biomass, and functional structure in situ in 12 streams adjacent to Bt maize or non-Bt maize fields and found no significant differences in total abundance or biomass between Bt and non-Bt streams [20]. Jensen et al. investigated four nontarget invertebrate species fed Bt near isolines; the growth of two closely related trichopterans was not negatively affected, whereas a tipulid crane exhibited reduced growth rates, and an isopod exhibited reduced growth and survivorship [9].

Bøhn's study demonstrated that *Daphnia magna* fed on Bt maize (cry1Ab) showed significantly reduced fitness performance compared to the non-Bt control, and it concluded a toxic effect rather than a lower nutritional value of Bt maize accounting for the lower fitness of *D. magna* [8,21]. Swan's group investigated the breakdown rates of Bt corn tissue and its impact on invertebrate abundance and invertebrate community composition in streams in two years. In one year, they found that Bt leaf litter degraded slower (67-68%) than corresponding near isolines, and this was associated with significantly fewer individuals of *Pycnopsyche* sp., a leaf-chewing caddisfly. In another year, they did not find the those differences between Bt and non-Bt treatment, and concluded that corn tissue breakdown is unlikely to be altered by Bt, but more so by hybrid- and site-specific factors such as nutrients [22].

Axelsson et al. conducted decomposition experiments under natural stream conditions using leaf litter from GM (Bt) populus trees to examine the hypothesis that GM trees would affect aquatic arthropod community. GM trees did not differ in nutrients and decomposition rate compared to no-GM trees, but changed the composition of aquatic insects (ephemeroptera, plecoptera and trichoptera) colonizing the leaf litter, ultimately manifested in a 25% and 33% increases in average insect abundance. They suggest that forest management using GM trees may affect adjacent waterways in unanticipated ways, which should be considered in future commercial applications of GM trees [23]. A recent study tested non-target effects of Bt corn tissue on rusty crayfish (*Orconectes rusticus*), a common invertebrate detritivore in streams near corn field. After 8 weeks of exposure, there was no statistically significant difference in growth between crayfish in Bt and isogenic treatments. However, survival rate was 31% lower in the Bt treatment compared with the non-Bt treatment. They concluded Bt corn and isogenic corn were of equivalent nutritional value, but Bt corn does have a toxic effect on rusty crayfish during long-term exposure [24].

Rice growth differs from dry-land crops such as corn and cotton in that it requires water layer in paddy during most of the developmental stages. In tropic or subtropic zones, paddy water teems with zooplanktons. We conducted a two-year study and found that the population of rotifers, cladocerans and copepods in paddy field varied significantly between years and rice developmental stages, but did not differ significantly between Bt and non-Bt rice treatments. Under open-air conditions, we used Bt rice straw as a food source for the water flea *Daphnia hyalina*. After one and two months of culture, the density of *D. hyalina* did not differ between Bt rice treatments and non-Bt rice treatments [25].

On the basis of this study, we assessed the realized effects of Bt rice under normal pest management practices which means using pesticides when required. Non-Bt rice was sprayed 5 times while Bt rice was sprayed 2 times, which ensured both rice types achieved a normal yield. Field investigations showed that rice type (Bt and non-Bt) significantly influenced zooplankton abundance and diversity, which were up to 95% and 80% lower in non-Bt rice fields than Bt rice fields. Laboratory culturing experiments showed that water from non-Bt rice fields was significantly less suitable for the survival and reproduction of *D. magna* and *Paramecium caudatum* in comparison with water from Bt rice fields. Higher pesticide residues were detected in the water from non-Bt than Bt rice fields. Those results demonstrated that Bt rice is safer to aquatic ecosystems than non-Bt rice, and its commercialization will be beneficial for biodiversity restoration in rice-based ecosystems [26].

Conclusion

Worldwide, the adoption of GM crops has kept growing for 19 years and will continue to increase in the future.1 Environmental risk assessments are compulsory for regulatory decisions for the commercial release of GM crops. Aquatic environments are as important as terrestrial environments in providing basic ecological functions and ecosystem services. Some of aquatic organisms are sensitive to GM crop materials or foreign proteins. Thus, it is necessary to assess the risks of GM crops on aquatic organisms. We suggest that both the potential for exposure and toxic effects on aquatic organisms should be considered. First, it is required to quantify the inputs of GM crop materials, such as senesced leaves and stalks, after harvest to agricultural streams, and to measure the transport distances of these materials.

The transport distance may vary depending on type of agro ecosystems, cropping habit of farmers and climate. Second, it is required to compare the decomposition rate and nutrient change between GM and non-GM crop materials under submerged conditions since the decomposition of organic matter is a key ecosystem process; at the same time, the bio-activity and persistence of the foreign proteins are required to be surveyed in situ or/and laboratory. Third, to assess the toxic effects, suitable surrogate aquatic species need be selected according to local dominant aquatic species, such as paramecia, cladocera, rotifer, trichopterans, diptera larva, decapoda and fish etc., to conduct laboratory feeding experiments; in addition, long term toxic effects on aquatic organisms should be assessed in field experiments at population or community level.

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