Biosafety Considerations for Transgenic Insecticidal Plants: Non-Target Herbivores, Detritivores, and Pollinators

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INTRODUCTION

Transgenic insecticidal plants produce proteins that are toxic to particular groups of insects. In addition to killing pest insects feeding directly on the crop, they may affect non-target organisms that feed on litter from the crop or on nectar or pollen that expresses the toxins. Detrimental effects on non-target species have been documented, but no immediate catastrophic impacts have been identified. Although protocols have been developed to quantify impacts on non-target biodiversity, complete assessment of non-target effects will necessitate determination of changes in the ability of non-target organisms to perform ecological functions such as weed suppression, decomposition, and pollination. Losey et al. (this volume) focus on predators or parasitoids, and in this entry we discuss effects on direct consumers.

BACKGROUND

A wide variety of crops have been modified to produce insecticidal proteins derived from genes transferred from the bacterium *Bacillus thuringiensis* (Bt). Transformed corn, known as ''Bt corn,'' is the most widely planted transgenic insecticidal crop in the world. Genetic material from different strains of *B. thuringiensis* produces toxins effective against different groups of insects. Currently, the only commercially available hybrids are derived from the Bt *kurtstakii* strain (Bt*k* corn) and were developed for selected lepidopteran species that feed on aboveground portions of the corn plant.[1] By 1999 over 6 million hectares of Bt corn was planted, and adoption reached at least this level in 2000 and 2001.[2] Transgenic Bt corn is now the most common management tactic for the European corn borer, *Ostrinia nubilalis*, throughout the United States. Corn hybrids transformed with genetic material from the Bt *tenebrionis* strain, which is active against coleoptera (beetles), are in the final stages of registration. If the new hybrids are approved, they appear destined for widespread use because corn rootworms (*Diabrotica* spp.) cause more damage than European corn borer and are the target of considerably more total kilograms of insecticide.

The potential benefits of transgenic insecticidal corn include reduction of resources devoted to scouting for pests, reduction in broad-spectrum insecticide applications, increased or protected yields due to season-long control of *O. nubilalis*,[3] protection of stored corn from lepidopteran pests,[4] and lower mycotoxin levels due to a reduction in fungal plant pathogens associated with *O. nubilalis* feeding.[5] The varying magnitude of these benefits is discussed in.[6]

Balanced against these potential benefits are possible negative aspects of growing these crops.[6] In general, negative effects of genetically modified crops could include selection for resistance among populations of the target pest, exchange of genetic material between the transgenic crop and related plant species, and impact on non-target species. The negative impact on non-target species can be separated into direct effects on organisms that feed on living or dead corn tissue (e.g., herbivores, pollenivores, detritivores) and indirect effects on organisms that primarily prey upon those direct consumers (predators). The existence of these four functional groups illustrates the often underestimated complexity of the many agroecosystems and the multiple mechanisms for potential impact (Fig. 1). It is important to note that direct consumers provide invaluable ecological services including weed suppression, pollination, and decomposition, while indirect feeders contribute greatly to suppression of insect pests. Interference with these processes could lead to

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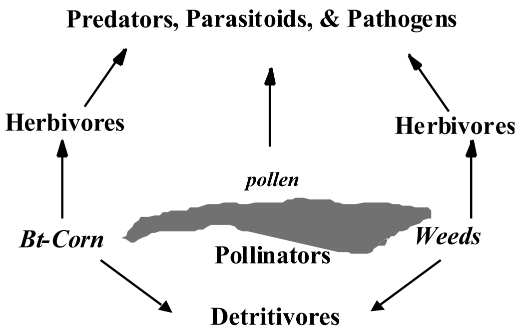

Fig. 1 Functional groups to be considered for assessment of risk from Bt corn and their relationship to Bt corn. (Adapted from Ref. 6.)

increased competition from weeds or delayed breakdown in plant material, both of which could lead to lower yields.

INSECT HERBIVORES

Currently commercialized hybrids of Bt corn and cotton express toxins that are active against lepidoptera, and many herbivorous non-target species are likely to be directly susceptible. There are two major crops that express a beetle toxin: Bt*t* corn and Bt*t* potatoes. Since Bt*t* corn is not commercially available and Bt*t* potatoes have not been widely adopted as of this writing, we will focus primarily on effects on lepidopteran non-targets. However, it is important to note that many families of beetles contain species that may directly consume living or dead corn tissue. Many of the same protocols used to identify which species of butterflies and moths are most at risk may be effective for beetles as well.

Since lepidopteran herbivores that feed on corn plant tissue within the cornfield are considered ''target pests,'' we will consider non-target herbivores to be those species that may contact corn pollen on weedy plant species within fields or on plants outside of fields. Since cotton is not wind-pollinated, the small amount and low mobility of the pollen produced minimizes the potential of impact through pollen drift. It is important to note that different events of Bt corn produce variable amounts of toxin in their pollen, with some events expressing very little. The lepidopteran species most likely affected by Bt corn pollen can be determined by examining their distribution and phenology.[7] Factors that will determine which lepidopteran species are most likely to be affected by pollen from Bt*k* corn include the following: 1) which plant species grow in and around corn; 2) which lepidopteran species feed on those plant species; 3) temporal overlap of corn pollen shed and larval feeding by the non-target lepidoptera; and 4) susceptibility of potentially affected species to the Bt toxin.[7] Integrating distribution, phenology, and susceptibility can allow a ranking of the risk to specific lepidopteran species. Species that may be at particularly high risk could then be identified for further testing.

The monarch butterfly, *Danaus plexippus*, provides one example of how a species might be evaluated after it has been determined to be potentially at risk from Bt*k* corn pollen. Observations that the monarchs' host plant, milkweed, was common in cornfields and often exposed to pollen led to initial studies that confirmed the toxicity of the pollen to monarch larvae.[8,9] Further studies determined that the risk of a short-term catastrophic impact on monarch populations was negligible based on the level of pollen on milkweed leaves and the temporal overlap of pollen shed and larval feeding.[10] However, this study also points out the importance of assessing longer-term, more subtle impacts on monarch populations.[10] Using a ''coarse filter'' such as the four-point one we propose above, other species that warrant more detailed ecological studies like those done for the monarch butterfly could be identified.

POLLINATORS

An assessment of the impact of each Bt corn hybrid on pollinators is required for EPA registration.[1] Although the toxins expressed in Bt corn pollen are specific for lepidoptera, several studies raise questions about its effects on pollinators, i.e., domesticated and wild bees. In the registration documentation, pollen from Bt corn is reported to have no effect on survival of either larval or adult domesticated bees.[1] However, unexpected effects of transgenic plants on domesticated bees have been reported. For example, a preparation of Bt*t*, reported to be specific for coleoptera, caused significant mortality in domesticated bees.[11] Proteins, other than Bt, produced in transgenic rapeseed pollen, targeted for coleoptera and lepidoptera, interfered with learning by domesticated bees.[12] When toxins are not expressed in the pollen, the process of transforming a plant may reduce pollen output, lowering availability of an important food source to pollinators.[13] These studies raise concerns about the precision of genetic transformations and unintended side effects of genetic transfers. In addition, although wild bees provide a substantial amount of the pollination in many systems, they apparently were not tested for Bt corn registration, and we are not aware of any studies that examined the impact of Bt pollen on wild bees.

DECOMPOSERS

The insecticidal toxin (CryIA(b)) found in one type of transgenic corn (event 176) caused significant mortality and reduced reproduction in the soil-dwelling collembolan, *Folsomia candida*.[1] A previous study had shown no effects of feeding on transgenic cotton leaves by *F. candida*.[14] The same insecticidal protein was present in both transgenic crops (cotton and corn); however, the higher dose in the corn appears to have caused the adverse effects. Even though the EPA reports this adverse non-target effect, they conclude that there is a 200-fold safety factor in the levels of toxin that would occur in the field.[1] In addition, because no buildup of corn stalk residues have been observed following use of soil insecticides in cornfields, which presumably would have a negative effect on collembola, ''an observable deleterious effect on the soil ecosystem is not expected to result from the growing of CryIA(b)-endotoxin-containing corn plants.''[1] This conclusion may need to be reconsidered, because there are potentially important differences in the seasonal occurrence of soil insecticides (at planting) to that of transgenic Bt toxins (from roots,[15] pollen deposition, and stalk residues at harvest) relative to the seasonal life cycles of collembola.

CONCLUSIONS

The results of studies on the direct effects of Bt crops on organisms that feed on crop tissues (e.g., living tissue, litter, pollen) are mixed. While no short-term catastrophic impacts have been identified, several impacts that warrant further study have been documented. Research up to this point has focused solely on relatively simple measures of the biodiversity of non-target organisms. The rapid adoption of Bt*k* corn and the predicted equally rapid adoption rate for Bt*t* corn as well as other transgenic plants make a complete assessment of both positive and negative impacts on non-target organisms imperative. A complete assessment of non-target impacts needs to include measures of how ecological functions (e.g., weed suppression, pollination, decomposition) are impacted by transgenic crops in comparison to how they are impacted by conventional pest management tactics.

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