ISB NEWS REPORT
AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY

August 2014

INSECT RESEARCH

CONTENTS

Conserving Important Natural Enemies by Using Bt Crops: An Example from India

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Introduction

Transgenic cotton producing insecticidal crystal (Cry) proteins from *Bacillus thuringiensis* (Bt) for control of Lepidoptera was planted on 23.9 million ha in 15 countries in 2013, with India having the largest area of 11.0 million ha[1]. Cotton is attacked by a large complex of insect pests, but the most damaging is the bollworm complex, primarily *Helicoverpa zea* (Boddie), *H. armigera* (Hübner), *H. punctigera* (Wallengren), *Heliothis virescens* (Fabricius) and *Pectinophora gossypiella* Saunders.

Adoption of Bt cotton has reduced the use of traditional broad-spectrum insecticides and increased grower profit, but it has also changed the traditional pest complex in adopting countries such as India. With the effectiveness of the Cry proteins in Bt cotton, there has been a dramatic decline in the pest status of bollworms. However, because Cry proteins do not control sap feeders, including aphids, leafhoppers, mirids, and mealybugs, they are emerging as serious pests[2]. Additionally, a traditionally minor pest, the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), has become a serious pest on Bt cotton in India[3]. With its rapid life cycle and high reproductive capacity, it has become a perennial and serious pest of seedling to mid-season cotton in many cotton regions in India. *T. tabaci* has a unique feeding method in which it rasps leaf surface cells and consumes their liquid contents, thus reducing the photosynthetic capacity of the plant. Previous reports have confirmed that thrips species acquire Cry proteins when feeding on Bt plants[4]. This phenomenon has raised concern that predators of *T. tabaci* may ingest Cry proteins and be harmed.

In India, most of the predators of *T. tabaci* are generalists that rely on *T. tabaci* as a food source early in the season, the time when Cry protein expression in the cotton plant is high. Cotton supports large and diverse arthropod natural enemy communities and there is ample evidence to suggest that these natural enemies can have a significant impact on cotton pest population dynamics[5]. One genus in particular, *Orius* spp. (Hemiptera: Anthocoridae), contains well-known omnivorous and generalist predators that feed on various arthropods including thrips, spider mites, leafhoppers, aphids, whiteflies, and small caterpillars. Members of this genus are important biological control agents in many crop ecosystems including cotton. *Orius insidiosus* (Say) is a widely distributed species that has proven to be an important biological control agent in greenhouse and field situations and which also feeds on plant tissues (e.g., pollen grains and young leaves) to supplement its diet. Previous studies have confirmed that it can acquire Cry proteins expressed in Bt plants[4]. Thus, *O. insidiosus* has the capacity to acquire Cry proteins from feeding on hosts

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that have consumed Bt plant tissue, as well as feeding on Bt plant tissues directly.

Objectives

In the present study, we investigated if Cry proteins expressed in Bt cotton move from plants to *T. tabaci* and subsequently to *O. insidiosus* and cause harm to this important predator. Specifically, we 1) documented the amount of Cry1Ac and Cry2Ab protein in Bollgard-II® cotton; 2) determined the uptake of Cry1Ac and Cry 2Ab by *T. tabaci*; 3) determined whether *O. insidiosus* could acquire Cry1Ac and Cry 2Ab when it fed on *T. tabaci* that had fed on cotton expressing Cry1Ac and Cry 2Ab; and 4) determined if the survival, development and reproduction of *O. insidiosus* were affected by consuming *T. tabaci* that had fed on cotton expressing Cry1Ac and Cry 2Ab.

Materials and Methods

Plants. Seeds of Bollgard-II® (Event 15895) producing Cry1Ac and Cry2Ab, and the corresponding non-transformed near-isoline Stoneville 474, were obtained from Monsanto Company (St. Louis, MO). The two cotton varieties were grown simultaneously in the same growth chambers. Plants were individually grown in 6 L plastic pots filled with Cornell mix soil. Small leaves from the upper canopy were used to conduct the bioassays with *T. tabaci* and *O. insidiosus*.

Prey. *T. tabaci* were maintained on onion plants in a climatic chamber and fresh cabbage leaves were provided to adult thrips for egg laying. Upon hatching, first instar *T. tabaci* were transferred to either Bollgard-II® cotton or non-Bt cotton leaves. After feeding for 2-4 d, *T. tabaci* larvae were supplied to *O. insidiosus* as food. Before initiation of the bioassay, the protein uptake by different stages of *T. tabaci* was confirmed (see below).

Predator. In the bioassays, *O. insidiosus* were fed either larvae (mixed stages) of *T. tabaci* reared on non-Bt cotton leaves, or larvae reared on BG-II cotton. Nymphs of *O. insidiosus* were observed daily and their survival and development were recorded. When the adults emerged, they were weighed and their gender was determined. A male and a female from the same treatment were kept in a 30-ml plastic cup and allowed to mate. All pairs of adult *O. insidiosus* from both treatments were fed 50 to 60 of their respective *T. tabaci* larvae reared on BG-II or non-Bt cotton per day. Leaves and prey were replaced daily. The duration of pre- and post-oviposition periods as well as fecundity and longevity were measured. Progeny from these adults were then used to examine nymphal survival and development for a second generation using the methods described above.

Concentrations of Cry1Ac and Cry2Ab in the cotton plant, prey and predator. Leaf samples (leaf discs of 5 mm dia.) were collected from five different Bt and non-Bt cotton plants at regular intervals during the assays. Each sample was obtained from a leaf in the upper third of a BG-II or control plant. All samples were weighed and placed into 1.5-ml centrifuge tubes, respectively, and stored at -20 ºC until Cry protein measurements using enzyme-

PUBLISHED BY

Information Systems for Biotechnology

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linked immunosorbent assays (ELISA) (see below) were made.

T. tabaci larvae, used as prey for *O. insidiosus* after feeding on BG-II or non-Bt plants, were collected in microcentrifuge tubes. They were grouped and weighed in a batch of ca 20 mg per replication, and then stored at -20 ºC until the Cry level could be determined. Similarly, *O. insidiosus* adults were collected at the end of a bioassay and stored in microcentrifuge tubes. They were also grouped and weighed in a batch of ca 20 mg per replication, and then stored at -20 ºC until assayed using ELISA. For all ELISA samples, 5 replications were used.

ELISA procedures. Bt protein concentrations in plants and insects, including the non-Bt treatments, were measured using sandwich ELISA using Cry1Ab/Cry1Ac and Cry2Ab detection kits from EnvironLogix (Portland, ME). ELISA was performed according to the manufacturer's instructions. Spectrophotometric measurements were taken using a UV-visible recording spectrophotometer (UV160U, Shimaszu, Columbia, MD). We ran the negative controls (samples from non-Bt treatment) and absorbance readings, and values double the negative control were considered positive.

Statistical analysis. Data on survival of *O. insidiosus* were analyzed using the Wilcoxon test for homogeneity. Data on other life history parameters of *O. insidiosus* reared on *T. tabaci* fed on BG-II and non-Bt cotton were compared using Student's paired t-test. Before analysis, all percentage data were arcsine or square root transformed, as necessary. All statistical analyses were performed with SAS version 9.1 (SAS Institute 2001). For all tests, $\alpha = 0.05$.

Results

Cry1Ac and Cry2Ab in plants, prey and predators. In the Bt treatment, Cry1Ac and Cry2Ab proteins were detected in all three trophic levels. In contrast, no Bt protein was detected at any trophic level in the non-Bt treatment. The average Bt protein titers from BG-II cotton leaves used for rearing the prey and predators were 1,256 and 43,633 ng Cry1Ac and Cry2Ab per g of fresh leaf tissue, respectively. From this quantity of Cry1Ac and Cry2Ab proteins expressed in leaves of BG-II cotton, 277 and 916 ng (22.1 and 2.1% of the total expressed in BG-II cotton leaves) per g fresh weight (FW), respectively, were detected in *T. tabaci* larvae reared on BG-II cotton leaves for 2 to 4 d at the second trophic level. This confirmed the flow of the protein from BG-II cotton to the non-target herbivore. Subsequently, at the third trophic level, 55 and 119 ng per g FW of Cry1Ac and Cry2Ab, respectively, were detected (4.4 and 0.3 % of the total expressed in BG-II cotton leaves) in *O. insidiosus*-fed *T. tabaci* larvae reared on BG-II cotton leaves for 2 to 4 d. This confirms the acquisition of Cry1Ac and Cry2Ab by *T. tabaci* larvae from BG-II cotton and its further uptake by *O. insidiosus* after feeding on *T. tabaci* larvae.

Development and reproduction of O. insidiosus-fed T. tabaci larvae reared on BG-II and non-Bt cotton plants. There were no significant differences ($P>0.05$) in survival and nymphal development for the first or second generation when *O. insidiosus* fed on *T. tabaci* larvae reared on leaves of BG-II compared to non-Bt cotton. Furthermore, there were no significant differences ($P>0.05$) in pre-ovipositon, oviposition periods, fecundity, fertility, and adult longevity of adult *O. insidiosus* that had fed as both nymphs and adults on larvae of T. tabaci in the two treatments. This confirms the lack of harm to *O. insidiosus* when it fed on *T. tabaci* that had consumed Cry1Ac and Cry2Ab expressed in Bollgard-II® cotton.

Discussion

Genetically modified crops producing Bt proteins may pose a risk to important non-target organisms, including important biological control agents, if they are susceptible to the protein and if the organism is exposed. The hazard posed by the protein can be determined in Tier 1 laboratory studies in which the organism is subjected to a high dose by feeding directly on the protein in a diet or on a plant producing the proteins[6]. Determining the level of exposure of a non-target, natural enemy to a Bt protein is more complex. It must be shown that its prey has acquired the protein from the plant and that the natural enemy can in turn acquire it from the prey. Thus, the concentration of the Cry protein contained in the food source has to be determined. Leaves of BG-II are known to have the highest concentrations of Cry proteins compared

to other plant parts and in this study we found high levels of both Cry1Ac and Cry2Ab proteins in plant leaves.

The uptake of Cry1Ac and Cry2Ab by the pest, *T. tabaci*, and predator, *Orius insidiosus*, was confirmed in the present study. In this study, *T. tabaci* reared on BG-II cotton contained 24 and 2.2 % of the amount of Cry1Ac and Cry2Ab protein, respectively, expressed in BG-II cotton plant leaves, while lower amounts (4.4 and 0.3 %) were found in *O. insidiosus* when it fed on larvae of *T. tabaci* reared on BG-II cotton. However, the life history parameters of *O. insidiosus* were not affected in this tri-trophic interaction.

Based on a realistic exposure route with confirmed transfer of Bt proteins through the food chain, our results demonstrate no biological effects of BG-II cotton containing Cry1Ac and Cry2Ab on an important predator. This indicates that the Bt proteins can control the bollworm complex while allowing natural enemies, such as *O. insidiosus*, to flourish and help control secondary pests such as *T. tabaci*.

This study in context

Over the last decade we have performed multiple studies on the potential effects of commercialized Cry proteins on natural enemies and **Table 1** illustrates our results. In these 16 tests, we have not seen any deleterious effects on natural enemies that consume prey that have fed on Bt plants. These data confirm the safety of Bt crops to important natural enemies[7].

Table 1. List of natural enemies not harmed by Bt proteins when they are exposed to proteins through hosts (Shelton lab)

Bt protein (crop)	Enemy type	Order	Scientific name	Common name	Pest species (host)
Cry1Ac/ Cry2Ab (cotton)	Predator	Coleoptera	*Coleomegilla maculata*	Lady beetle	Cabbage looper (*Trichoplusia ni*)
		Hemiptera	*Geocoris punctipes*	Big-eyed bug	Cabbage looper (*Trichoplusia ni*)
		Hemiptera	*Orius insidiosus*	Minute pirate bug	Cabbage looper (*Trichoplusia ni*)
		Hemiptera	*Orius insidiosus*	Minute pirate bug	Onion thrips (*Thrips tabaci*)
		Hemiptera	*Zelus renardii*	Assassin bug	Cabbage looper (*Trichoplusia ni*)
		Neuroptera	*Chrysoperla rufilabris*	Lacewing	Cabbage looper (*Trichoplusia ni*)
	Parasitoid	Hymenoptera	*Cotesia marginiventris*	-	Cabbage looper (*Trichoplusia ni*)
		Hymenoptera	*Copidosoma floridanum*	-	Cabbage looper (*Trichoplusia ni*)
Cry1F (corn)	Parasitoid	Hymenoptera	*Cotesia marginiventris*	-	Fall armyworm (*Spodoptera frugiperda*)
	Predator	Coleoptera	*Coleomegilla maculata*	Lady beetle	Fall armyworm (*Spodoptera frugiperda*)
		Hemiptera	*Zelus renardii*	Assassin bug	Fall armyworm (*Spodoptera frugiperda*)
		Hemiptera	*Geocoris punctipes*	Big-eyed bug	Fall armyworm (*Spodoptera frugiperda*)
		Hemiptera	*Orius insidiosus*	Minute pirate bug	Fall armyworm (*Spodoptera frugiperda*)
		Neuroptera	*Chrysoperla rufilabris*	Lacewing	Fall armyworm (*Spodoptera frugiperda*)
Cry1C (broccoli)	Parasitoid	Hymenoptera	*Diadegma insulare*	-	Diamondback moth (*Plutella xylostella*)
Cry1Ac (broccoli)	Predator	Rhabditida	*Heterorhabditis Bacteriophona*	Entomopathogenic nematode	Diamondback moth (*Plutella xylostella*)

Acknowledgments

This study was published earlier this year and should be cited as: Kumar, R., J. Tian, S. Naranjo and A. M. Shelton. 2014. Effects of Bt cotton on *Thrips tabaci* and its predator, *Orius insidiosus*. J. Econ. Entomol.107: 927-932. We are grateful for the support of the Fulbright Commission of India and the United States that allowed R. J. Kumar, the lead author, to conduct this study in our laboratory. Additional support was provided by the Biotechnology Risk Assessment Program Competitive Grant 2010-33522-21772 from the USDA, National Institute of Food and Agriculture. The data provided in Table 1 are summarized from many different studies, including those supported by the same grant.

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Ethylene Production via Engineered Microbes

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Background

Ethylene is a versatile hydrocarbon and its derivatives are abundant. Principally used in the production of polyethylene for plastic bags and trashcan liners, ethylene can also be converted to a range of other chemicals and polymers, including ethylene oxide used in the production of detergents and surfactants, and polystyrene used for packaging and insulation. Ethylene can also be catalytically converted to gasoline-like (C5-10) molecules[1], which exemplifies its versatility. Given this utility, it is not surprising that ethylene is the most widely used chemical in the world, with the global demand met via production by steam cracking of fossil fuels. This process represents one of the most energy intensive and highest CO_2-emitting processes in the chemical industry. By current state of the art technology, 2 MJ of energy are invested per pound of ethylene made and accounts for 1.5% of United States' carbon footprint[2]. Given the ethylene industry's massive market and the increasing demand, this footprint will continue to expand without new and innovative methods to produce this multipurpose molecule. As is the case with most petroleum-derived chemicals, both the environmental and financial costs make it clear that routes to renewable ethylene production via engineered microbes (herein referred to as bioethylene) are of great interest to the chemical industry. For bioethylene to become an economically viable alternative, bioethylene yields produced by engineered microorganisms must improve considerably. Furthermore, the feedstock from which these engineered microbes can produce ethylene must be as plentiful as the demand. While such systems do not currently exist, the promise of synthetic biology-driven metabolic engineering makes them more feasible today than was once imagined. Here we briefly describe routes to bioethylene production, their advantages, and current bioengineering strategies to improve yield.

Bioethylene Production and the Ethylene-forming Enzyme

Biologically, ethylene serves as a critical plant hormone,

modulating growth and development in higher plants. It is produced in most parts of the plant and has been implicated in the defense response to both biotic and abiotic stresses. Given its ubiquity, it is perhaps unsurprising that a variety of routes to bioethylene production exist in higher plants, fungi, and microbes. Routes to ethylene production in nature are nearly as diverse as its chemical uses; however, not all routes to ethylene are amenable to integration into engineered systems for bioethylene production. For example, in higher plants, ethylene is produced via a two-step reaction from methionine via S-adenosyl methionine[3]. This reaction ultimately results in the generation of cyanide as a byproduct, thus limiting the incorporation of such systems into microbes without the necessary means to alleviate CN toxicity. In some microbes and fungi such as *Escherichia coli* and *Cryptococcus albidus*, ethylene is produced spontaneously via oxidation of 2-keto-4-methylthiobutyric acid, a transaminated derivative of L-methionine[4]. In these L-methionine-dependent systems, only trace amounts of ethylene are produced. Furthermore, the produced ethylene is result of a non-physiological reaction (i.e., not directly produced as an enzyme product). Consequently, the engineering of systems exploiting this pathway to generate bioethylene is a significant challenge.

Due to the role of ethylene in both plant development and stress response, a variety of plant associated pathogens and symbionts have evolved the ability to produce ethylene. While the precise roles ethylene plays in disease progression and symbiosis are unclear, evidence does suggest that plants infected with certain pathovars, such as *Pseudomonas syringae* and *Penicillium digitatum*, are indeed affected by the production of ethylene from its microbial attackers[5]. The production of ethylene from these microbes differs from those of higher plants and fungi. In these organisms, a single ethylene-forming enzyme (EFE) can catalyze the formation of ethylene in a single step using cellularly-produced alpha ketoglutarate (AKG) and arginine as substrates along with O_2. In addition to producing ethylene, the proposed reaction also generates succinate, L-delta-1-pyrroline-5-carboxylate, guanidine, and CO_2[6]. Current understanding of the EFE reaction supports a dual-circuit mechanism where EFE catalyzes two separate reactions; however it remains difficult to discern if these reactions are coupled or if EFE acts promiscuously to catalyze two separate reactions. If the reactions are eventually revealed to be separable, further engineering of EFE could improve the efficiency of carbon utilization. While further analysis is needed to determine the precise details of the EFE catalyzed reaction, the demonstration of ethylene production via single enzyme conversion of native metabolites marks a significant milestone as it provides a straightforward means to produce bioethylene in engineered hosts.

Advantages of Bioethylene

Bioethylene production as a chemical or biofuel precursor offers a number of advantages when compared to other strategies involving the direct production of alcohol- or fatty acid-derived chemicals or fuels. As a gaseous product, only very small amounts of ethylene accumulate in the media, thus eliminating many of the challenges associated with product toxicity to the host organism. Harvesting ethylene from the off-gas of growing cultures can be accomplished via several methods, including cryogenic distillation, solvent extraction, and membrane separation. While the product streams are likely to be free of many contaminants, it is important to note that the harvesting of ethylene from biological systems presents some unique challenges as well. For example, the engineering of photosynthetic systems for the production of bioethylene results in the coproduction of O_2. Given the flammability hazards associated with ethylene/O_2 mixtures, recovery of bioethylene requires special design considerations to mitigate such risks.

In addition to the production capabilities of engineered organisms, the extent to which bioethylene will supplant petroleum-derived ethylene will depend largely on the cost and availability of feedstocks. With the necessary substrates – namely AKG and arginine – for bioethylene production using EFE originating from central metabolism, a diverse set of renewable feedstocks ranging from biomass to CO_2 and sunlight can be utilized for production, depending on the host organism. Heterologous expression of EFE and ethylene production have been demonstrated in a variety of engineered organisms including *E. coli* and *Saccharomyces cerevisiae* as well as photosynthetic hosts such as *Synechococcus elongatus* PCC7942 and *Synechocystis* sp. PCC68037. Despite the advantages offered by the many potential hosts for bioethylene production, many suffer from the lack of "tools" available for engineering such systems. For example, production directly from CO_2 and sunlight in photosynthetic hosts is ideal, as they represent the most abundant feedstocks available;

however, engineering such hosts to improve yield remains difficult due to a relative lack of well-established genome engineering methods for these organisms compared to more tractable hosts such as *E. coli*. Similarly, some of the highest bioethylene yields have been observed from engineered *Pseudomonas putida*. Further improvement in yields from this organism will remain a major challenge without an efficient means to manipulate the *P. putida* genome. Motivated by the likely requirement of heavily engineered strains for optimized bioethylene production, many have attempted to port EFEs into more genetically tractable systems, thus opening the door to improving production via both traditional and synthetic biology-driven metabolic engineering strategies.

Bioengineering Strategies to Improve Bioethylene Production

The expression of EFE in commonly engineered hosts can produce ethylene levels that exceed the production of ethylene from unmodified strains. Our lab and others have demonstrated that ethylene titers are significantly affected by a number of interconnected parameters ranging from EFE gene copy number, protein solubility, substrate availability, and growth media composition. For example, ethylene production in *E. coli* from the plasmid-based expression of EFE can vary dramatically, depending on promoter strength, copy number, and the *E. coli* strain used. Together, these data suggest a significant need for more systematic investigations into the extent to which each variable affects production, including the effect of integrating genes encoding EFE directly onto the chromosome.

The most commonly used and well-studied EFE for bioethylene production was originally discovered in *P. syringae*; however, its heterologous expression is problematic in many engineered hosts. In *E. coli*, no enzyme activity is measured when expressed at 37 ºC, and western blot analysis has revealed a significant fraction of the protein remains insoluble regardless of the temperature at which it is produced[7]. These considerations make EFE an ideal target for protein engineering to both improve solubility and function at higher temperatures. Recent phylogenetic analysis of EFE and EFE-like enzymes performed at University of Louisiana Lafayette has revealed a great diversity amongst these proteins[7]. As sequencing capabilities continue to expand at dramatic rates, it is conceivable that EFE variants with improved function and or solubility will be identified in the metagenome and their activities accessed via DNA synthesis and expression in heterologous hosts.

In vitro assays of EFE enzymes have indicated substrate availability as the most likely limit to bioethylene production from microbial hosts. Therefore, metabolic engineering strategies to improve AKG and arginine levels in hosts expressing EFE, such as the knockout of competing pathways and the overexpression of enzymes catalyzing rate-limiting steps of substrate formation, will likely significantly improve ethylene yields. Indeed, early works by our group in these areas has yielded encouraging results; however, since the concentrations of metabolites such as AKG, which sits at a metabolic node connecting carbon and nitrogen metabolism, are tightly regulated within the cell, it is often difficult to predict which genetic changes will ultimately lead to increases in substrate availability. As such, recent work from our groups at National Renewable Energy Laboratory and University of Colorado has sought to improve bioethylene production from *E. coli* using evolutionary approaches involving the high-throughput integration and screening of genome-scale libraries to identify strains with improved production. Specifically, we are currently exploring the use of methods such as trackable multiplex recombineering (TRMR) that take advantage of parallel DNA synthesis and lambda red recombineering to integrate mutations originating from barcoded synthetic DNA cassettes upstream of each gene in the *E. coli* genome[8]. With this method, each integrated DNA cassette creates a single designed mutation with a known or at least anticipated effect on the expression of the downstream gene. The molecular barcode associated with each cassette enables the rapid identification of the genetic modification giving rise to an observed phenotype (e.g., improved ethylene production).

Like most approaches employing directed evolution, the use of genome scale libraries or protein engineering to boost ethylene production is currently limited by the availability of high throughput screens and selections for increased ethylene or EFE substrate production. Given ethylene's role as a common effector molecule, there is an abundance of protein receptors capable of binding ethylene, thus making engineering a system that could link the presence of ethylene to a detectable change in gene expression very feasible. Though no such tools currently exist, we anticipate this to be an exciting area of research. Likewise, genetic tools that link an increase in EFE substrates, such as AKG, will be extremely useful. One such tool using a FRET-based strategy to monitor

intracellular AKG levels has been described and is currently being explored as a means by which one can identify alleles from genome-scale libraries, giving rise to elevated AKG levels in *E. coli*[9].

Conclusion

The benefits of bioethylene are clear, and as yields improve, potentially significant. With the multitude of routes to bioethylene production accessible from a diverse set of engineered hosts, the notion of supplanting a small share the petroleum-derived ethylene supply are no longer out of the question. While it remains unlikely that current heterotrophic microbes will be able to efficiently supplant the demand for ethylene, lessons learned from improving ethylene production in genetically tractable hosts such as *E. coli* (e.g., strategies for improving substrate yield) can readily be extended to improve ethylene production in hosts utilizing biomass-derived feedstocks as well as from more desirable photosynthetic hosts (e.g., *Synechocystis*) utilizing CO_2 and sunlight. Optimization of such pathways in heterologous hosts will require synthetic biology-enabled tools for genome engineering as well as innovative methods for detecting ethylene and its biological precursors. Successful outcomes in these areas can reduce our dependence on fossil fuels, and provide a viable feedstock for bio-based chemicals and fuels.

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Inorganic Farmer Trumps Organic Farmer in Australia & Other News Briefs

Phill Jones

Hailed as a landmark Australian court case that could have consequences for genetically engineered (GE) crop cultivation in the country, *Mash v Baxter* marked the first time that one Australian farmer sued another for negligence over GE crop contamination. In his 150-page decision of May 28, 2014, Justice Kenneth Martin focused on the simple legal issues at hand.

On their Western Australia property called Eagle Rest, Stephen and Susan Marsh grew oats and raised sheep. The National Association for Sustainable Agriculture Australia (NASAA) certified their oats and sheep as organic. Michael Baxter's farm, Sevenoaks, is located west of Eagle Rest; a road reserve of about 20 meters separates the two farms. Baxter grew conventional crops and Roundup Ready® canola.

During November 2010, Baxter cut GE canola, stacked the plants in windrows, and left them to dry before harvesting canola seeds. The harvest technique is known as "swathing," and the cut canola plant with attached seed pods is called a "swathe." While the canola plants were drying, wind blew some of the GE canola onto Eagle Rest land.

The discovery GE canola swathes and seed pods on Eagle Rest property led to NASAA's decertification of about 70% of Eagle Rest land. The group concluded that the airborne swathe incursion and the GE canola seeds scattered across the soil of Eagle Rest posed an "unacceptable risk" of "contamination." During the following year, the Marshes found eight GE canola plants that were growing as self-sown volunteer plants on Eagle Rest. They pulled the plants from the ground.

In 2012, the Marshes sued Baxter for damages under common law negligence and the tort of private nuisance. Justice Kenneth Martin heard evidence during a three-week trial in February 2014 at the Western Australia Supreme Court. The judge dismissed both of the Marshes' causes of action.

With regard to the private nuisance claim, Justice Martin decided that the Marshes had failed to show that there had been any unreasonable interference by Baxter in the Marshes' use and enjoyment of Eagle Rest. "Mr. Baxter was not to be held responsible," the judge said, "merely for growing a lawful GM crop and choosing to adopt a harvest methodology (swathing) which was entirely orthodox in its implementation." In the judge's view, the decertification arose from an erroneous application of NASAA's own standards, and that Baxter could not be held legally responsible for NASAA's "unjustifiable reaction." Justice Martin rejected the cause of action in common law negligence, because Baxter had not been shown to have acted negligently by growing the GE canola or by swathing the lawfully grown GE canola crop.

Before the decision, agribusiness experts predicted that the case would probably lead to regulations outlining boundaries between organic farms and farms producing GE crops. Yet the judge turned the spotlight on NASAA's decertification decision. "The legal cause of the economic loss," the judge wrote, "was the work of NCO [NASAA's certification body] in unreasonably (erroneously, it presents) applying NAASA Standard 3.2.9." That standard states that "certification shall be withdrawn where NASAA considers there is an unacceptable risk of contamination from GMOs or their derivatives." The judge agreed with Baxter that no contamination occurred because the Marshes' sheep would not be affected by eating GE canola seeds, and crops such as the Marshes' oats cannot cross-breed with canola seeds.

While European Countries Ban GE Crops, a Farmer Fights Back

Baxter cultivated GE canola in accordance with local laws. Some members of the European Union forbid the cultivation of any GE crops. In March, France adopted a decree to stop the sowing of Monsanto's insect-resistant MON810 maize, the only GE crop commercially cultivated in the EU. On May 5, the French parliament gave final approval to a law that prohibits the cultivation of any variety of GE maize. The ban would block the planting of Pioneer Hi-Bred's GE corn, TC1507, which is expected to win EU approval this year.

Italy is another European Union member that has declared the cultivation of any GE crop to be illegal. Yet one farmer protests the ban. Giorgio Fidenato has been spreading smuggled Monsanto MON810 GE corn seeds

across his fields at the base of the Italian Alps.

"This stuff, with the same light, water and fertilizer, produces ten times more than the other kind," Fidenato told Public Radio International in June. "So, what more could you want?!"

Fidenato has had a tough time with his pro-GE crop position. In 2010, anti-GMO activists crept onto Fidenato's fields before harvest time and clipped stalks. Before the country-wide ban on GE crops, government officials took the farmer to court twice. Both times, the EU overruled Italian judges, because the EU approved the cultivation of MON810.

In July, Italy will lead the European Council. The minister of agriculture has said that regulation of genetically engineered organisms will be a priority.

United Kingdom Science Council Urges the Advancement of AgBiotech

During March, the UK's Council for Science and Technology issued a report promoting GE crop technology. Sir Mark Walport, the government's chief scientist, and Dame Nancy Rothwell, vice-chancellor of Manchester University, jointly chair the council. "The longer the EU continues to oppose GM whilst the rest of the world adopts it," they told the prime minister in a letter, "the greater the risk that EU agriculture will become uncompetitive, especially as more GM crops and traits are commercialised successfully elsewhere."

According to the council report, the European Academies Science Advisory Council (EASAC) has stated that there is no rational basis for the EU's current stringent regulatory process. Stringent regulation of GE crop technology "would be justified if it offered no benefits, if it was associated with inherent risks to the health of humans or animals or the environment, and if the technology was so poorly understood there was a high probability of unforeseen consequences." Yet more than 19 years of study have failed to uncover these risks. "Notably, even in the highly litigious USA," the council said, "there have been no successful lawsuits, no product recalls, no substantiated ill effects, and no other evidence of risk from a GM crop product intended for human consumption since the technology was first deployed commercially in 1994."

The council endorses EASAC's proposal that a future regulatory framework should be product-based, rather than process-based. In other words, the council suggests shifting to a regulatory system like that used by the US Food and Drug Administration. Anticipating that the approval process at the European level would remain an impediment even with a change to a new product-based system, the council suggested that the commercial cultivation of new GE crops should be approved at a national level.

The European Union Considers Mechanism for Banning AgBiotech

European Union officials are mulling over a change in the regulation of GE crops at the national level. On May 28, the EU supported a compromise pact – proposed about five years ago – that would enable member states to opt-out of any approval of a GE crop. Britain's farming and environment ministry issued a statement approving the measure as a means to "help unblock the dysfunctional EU process for approving GM crops for cultivation." Representatives from France and Germany approved the measure as way to ban GE crops.

Early reports indicate that the opt-out process would be anything but straightforward. Apparently, a member state that disagrees with an EU-approved GE plant would have to ask the European Commission to ask the relevant company to exclude the member state from requests for authorization of the new GE crop. If the company declines the request, then a member state has two paths to opt out of the GE crop. As Food & Water Watch's Eve Mitchell says, these paths are "all dreadfully unclear legally." So, the opt-out measure may provide neither a clear path for advancing GE crop technology nor a clear path for banning the technology.

On June 12, the Environment Council approved the basic proposal. The meeting report included a list of possible grounds that a member state can use to restrict or prohibit authorization of a GE crop. The list includes – but is not limited to – environmental reasons, socioeconomic reasons, land use and town planning, agricultural policy objectives, and public policy issues. In the next step, the Italian Presidency should start negotiations with the newly-elected European Parliament in early autumn 2014.

US Survey Reveals Views about Food Made from GE Crops

During May, the International Food Information Council (IFIC) released its latest "Consumer Perceptions of Food Technology" survey, which was based upon 1,000 interviews with US adults in the spring. The results

indicate that 63 percent of US adults approve the FDA's policy for labeling foods produced using biotechnology. The FDA requires labelling if the technology substantially changed a food's nutritional content or composition, or when the agency identifies a potential safety issue. IFIC also found a slight increase in opposition to FDA policy: 19 percent disapproved the FDA policy, compared with 14 percent in 2012.

"Years of legislation, ballot measures, and mischaracterization of food biotechnology have not affected overall support of FDA's biotech labeling policy," IFIC President and CEO David Schmidt said in a press release. "However, they have likely played a role in the modest increase we're seeing in those who oppose it."

Using biotechnology to enhance food may lead to greater acceptance of GE crops. Seventy-two percent of those surveyed said that they would probably purchase food products made with oils modified by biotechnology to provide more healthful fat. Greater than 66 percent said that they would be likely to purchase biotech-improved foods if the technology reduced the potential for carcinogens, decreased the number of crop pesticide applications, enhanced nutritional benefits, and eliminated the trans-fat content in foods.

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