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Genetic-Control of Mosquitoes

David O'Brochta

Frightening reports of how the seemingly innocuous Zika virus is closely associated with birth defects and occasionally severe neurological symptoms have been alarming.

A great deal of attention is focused on the Zika virus' main insect vector, *Aedes aegypti*, also known as the Yellow Fever mosquito because of its role in transmitting the Zika-related flavivirus that causes Yellow Fever, a virus made famous by its devastating toll on workers engaged in constructing the Panama Canal early in the twentieth century.

Calls for action to deal with the transmission and spread of Zika virus include stepping up efforts to control *Aedes aegypti* with considerable attention, at least in the popular media, on 'high tech' solutions involving the use of genetics-based approaches in which mosquitoes whose genomes have been altered in the laboratory by the insertion of specific transgenes are released into the environment to act as smart bombs that deliver some lethal genetic load to the mosquitoes transmitting Zika.

Entomologists have increasingly sophisticated capabilities to manipulate insect genomes using "genetic engineering" approaches and consequently these technologies are being considered as a means to control and even locally eradicate unwanted insects including Zika-infected *Aedes aegypti*. While they will not play a major role in the current crisis, with continued research and development, they will be widely available within the next decade.

Mosquito control needed

Mosquito control will undoubtedly figure prominently in the responses to the expanding threat of Zika infection, but effective mosquito control has been achieved in the past without using particularly new or complicated technologies. Experts on the ground where the threat is highest will be in a position to make the necessary assessment of needs and how best to meet them. Of primary concern is the reduction in infections and the incidence of disease, and while reducing mosquito populations is expected to be part of this effort, how much populations of mosquitoes must be reduced before Zika virus transmission is impacted needs to be determined so that organized efforts of mosquito control have the intended effects. Generally speaking, mosquito control efforts, to have lasting effects, need to be systematic, thorough, and sustainable. Insecticide fogging operations, applying residual insecticides as well as 'source reduction' efforts involving the elimination of standing water are the mainstays of most *Aedes aegypti* control programs and when resourced and managed adequately, can be effective.

Genetics-based insect control strategies, whether they rely on transgenic technologies or not, have features that make them attractive under some conditions, and research and development into these strategies should be a priority. The idea of using genetics as a tool for insect control originated in the middle of the 20th century, and operational programs

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ISB welcomes your comments and encourages article submissions. If you have a suitable article relevant to our coverage of agricultural and environmental applications of genetic engineering, please email it to the Editor for consideration.

designed to control and locally eradicate specific species followed soon after with a notable example being the eradication of the cattle pest *Cochliomyia hominivorax* (the New World screw-worm) from the United States, Mexico, and much of Central America. In this case radiation was used to heavily mutagenize the germ cells of large numbers of *C. hominivorax* mass-reared under factory-like production conditions. These genetically modified insects (now carrying an abundance of chromosomal mutations) were then released into target populations. The mutagenized males were sterile because of their radiation-damaged sperm and when they mated with fertile wild insects left no offspring, resulting in a decline in the population. This so-called Sterile Insect Technique remains a powerful and effective tool, notable for its species specificity and chemical-free ecological footprint.

Insect genetic-control strategies seek to alter the phenotype of a target species in a particular environment by altering their genotypes. Phenotypes of interest might be sterility (as in the case of the Sterile Insect Technique), death, sex, and susceptibility to pathogens and parasites, to name a few. Insect scientists can develop insects with desirable phenotypes using different strategies and technologies. They can mutate existing genes using non-specific approaches such as radiation, as is done in conventional Sterile Insect Technique programs, or use contemporary gene editing technologies involving *CRISPR/Cas9*, that permit precise changes in known sequences of a genome. Alternatively, insect scientists can insert genes into insect genomes using any number of gene integration technologies including those based on transposons (*piggyBac, mariner, Hermes, Minos*), site-specific recombination (*phiC31,Cre/lox, Flp/FRT*) and homology-directed repair using engineered endonucleases (*CRISPR/Cas9, TALENs, ZFNs*). (Criscione et al. 2015)

Two Novel Genetic Approaches

Currently there are two novel genetic approaches to combat the threat posed by *Aedes aegypti* as a vector of human pathogenic viruses that are in similar phases of development and are being deployed in a limited number of locations.

The first is a population reduction strategy with some similarities to the Sterile Insect Technique and involves the production and release of large numbers of transgenic male *Aedes aegypti* that carry two novel transgenes in their genome, one of which will kill the progeny inheriting this transgene.

The gene integration technology used to create these mosquitoes is based on gene vectors constructed from the *piggyBac* transposable element to insert transgenes into the genome of *Aedes aegypti* in such a way that insures the transmission of the transgenes to subsequent generations. The germ-line transformation technology used in this case is fairly mature and was first used in mosquitoes twenty years ago. Although requiring some technical skills and specialized equipment in order to precisely deliver by micro-injection various genetic technologies (DNA, RNA and/or proteins) into very young developing insect embryos, the creation of transgenic or otherwise genetically modified *Aedes aegypti* is within reach of most researchers either through their own efforts or those of specialized service providers such as the *Insect Transformation Facility* in the Institute of Bioscience and Biotechnology Research at the University of Maryland College Park (https://www.ibbr.umd.edu/facilities/itf).

Males carrying the progeny-killing transgene are released into a target population where they will mate with wild females. The progeny-killing transgene encodes a protein that stimulates and controls its own expression. Once this gene is expressed, its expression rapidly accelerates resulting in the massive overexpression of this protein in most of the cells of the developing mosquito, and this eventually kills the mosquito sometime in the larval stage. In the laboratory or mass-rearing facility the progeny-killing transgene can be kept inactive by the presence of tetracycline in the water used to rear larvae — thereby avoiding unwanted lethality. The second transgene encodes for a fluorescent protein useful for distinguishing transgenic from non-transgenic insects in the laboratory and field. Oxitec Ltd. (http://www.oxitec.com/) developed this progeny-killing technology and is releasing mosquitoes containing this technology (OX153 mosquitoes) at two locations in Brazil. This progeny-killing technology, like the Sterile Insect Technique, is self-limiting and requires the regular release of male insects carrying the transgene to achieve the desired reduction in population. Because the progeny of OX153 mosquitoes die as larva, the transgene responsible for reducing the population will not persist in the environment.

...this strategy aims not to kill mosquitoes but to render them harmless by making them incapable of harboring and transmitting Zika, Dengue, and Yellow Fever viruses.

The second genetics-based strategy for combating *Aedes aegypti* that is under development and being tested in a small number of locations around the world including Brazil aims not to kill mosquitoes but to render them incapable of becoming infected by human pathogenic viruses. *Aedes aegypti* when infected with the non-pathogenic, intracellular bacteria *Wolbachia* display a remarkable resistance to infection by flaviviruses, including Zika, Dengue, and Yellow Fever viruses. *Aedes aegypti* are not naturally infected with *Wolbachia* although many other species of insects are, but *Aedes aegypti* can be artificially infected in the laboratory. Artificially infected *Aedes aegypti* pass the intracellular *Wolbachia* on to the next generation and do so in a way that insures that all progeny arising from the mating of *Wolbachia*-infected and uninfected mosquitoes will be *Wolbachia*-infected. The release of a small number of *Wolbachia*-infected *Aedes aegypti* into an uninfected population will quickly result in the entire population becoming infected with *Wolbachia*, and along with the *Wolbachia* infection comes resistance to flaviviruses. So, this strategy aims not to kill mosquitoes but to render them harmless by making them incapable of harboring and transmitting Zika, Dengue, and Yellow Fever viruses. Unlike the progeny-killing strategy of Oxitec, the *Wolbachia* infection strategy is self-sustaining with *Wolbachia*-infected mosquitoes quickly replacing uninfected mosquitoes and with the infection expected to persist in the population without any further introductions of *Wolbachia*-infected mosquitoes. Technically, *Wolbachia*-infected *Aedes aegypti* are not transgenic mosquitoes but their phenotypes have been manipulated by the introduction of *Wolbachia* and this can be considered a genetics-based strategy. The Eliminate Dengue Program is a not-for-profit international team of collaborators (http://www.eliminatedengue.com) that is developing and testing this strategy.

Future Strategies – Gene Drive

Strategies such as those of the Eliminate Dengue Program require low initial inputs and sustain themselves because of *Wolbachia's* unique ability to prevent the development of mosquitoes without *Wolbachia*. This preferential survival of *Wolbachia*-infected individuals insures that the entire mosquito population will become *Wolbachia*-infected. Using contemporary gene editing technology such as the *CRISPR/Cas9* system, transgenes can be assembled and inserted into mosquito genomes that have a propensity to disseminate throughout populations of mosquitoes in ways similar to Wolbachia (Champer et al. 2016). That is, a unique combination of transgenes including Cas9- and guide-RNA expressing transgenes can be inserted into any gene within the mosquito genome and the transgenes will efficiently copy and insert themselves into the corresponding site on the homologous chromosome. Therefore, any mosquito that develops from a zygote heterozygous for the transgene will contain a germ-line with cells that are homozygous for the target gene and inserted transgenes. This conversion of heterozygous cells to cells that are homozygous is a result of efficient copying and inserting of the transgenes from their

original location to the corresponding location on the homologous chromosome resulting in the production of only transgene-containing gametes, all of which is triggered by the transgenes themselves. The frequency of the transgenes in the population will increase rapidly, and such a system is often referred to as a "gene drive" system, since the observed increase results in the transgenes "spreading" or "driving" through the population at rates that greatly exceed those of genes that are not copied and inserted as described. Gene drive systems are being considered to reduce or eliminate mosquito populations and to render them resistant to virus or parasite infections.

To use gene drive systems to eliminate mosquito populations, the gene drive system is inserted into a location in the mosquito genome that will cause mutations resulting in fitness-reducing phenotypes such as female sterility or severe distortions in the ratio of males and females. Over time, both of these phenotypes can result in a reduction or elimination of mosquito populations.

Gene drive systems can also be used to convert populations of mosquitoes into flavivirus-resistant insects by attaching additional transgenes to the gene drive system that confer a virus-resistant phenotype. As with *Wolbachia* infection, the spread of the virus resistance transgene is expected to be rapid.

Two examples of *CRISPR/Cas9*-based gene drive systems in *Anopheles* mosquitoes were recently published and represent proof-of-principal demonstrations of the general feasibility of these mosquito elimination and conversion strategies. Analogous systems for use in *Aedes aegypti* for the purposes of combating the ongoing Zika problem do not exist, and their development and testing are expected to take years before their performance characteristics and safety are adequately documented.

Conclusions

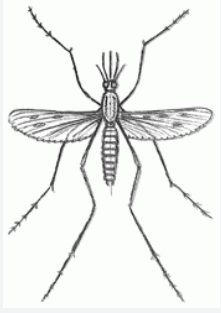
Mosquito control is clearly an option during the current Zika outbreak that, if done properly, can result in significant public health outcomes. Conventional approaches involving the use of insecticides and source reduction are known to be effective when the infrastructure and resources are available to initiate, implement, and maintain these programs.

The currently available 'next generation' genetics-based mosquito control strategies, while promising and important, are still very much in the development stages. OX153 mosquitoes from Oxitec when released in sufficient numbers can reduce the size of *Aedes aegypti* populations; but whether this comes with a public health benefit – reduced Dengue or Zika transmission and disease – remains an open question.

Clearly a benefit of this type of genetic approach is the potential to reduce the need for widespread insecticide applications; however, the release of mosquitoes genetically altered in the laboratory to contain unique transgenes poses new questions concerning environmental and public health risks that need to be resolved before this approach gains widespread acceptance.

Wolbachia-infected, flavivirus-resistant *Aedes aegypti* are an exciting alternative approach that is attractive because of the low inputs needed to initiate a local population conversion and the promise of this conversion to *Wolbachia*-infected and flavivirus-resistance being self-sustaining.

These and newer genetic strategies involving the use of gene drive systems could play real and meaningful roles in the near future, but in the short term, insecticides and source reduction are likely to be our best option.



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US Regulation of GE Plants and Animals: Changes and Approvals

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For more than seven years, the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) has struggled to update its regulations for genetically engineered (GE) plants. Current regulations date to the 1980s. At this time, APHIS became part of the federal government's Coordinated Framework for the Regulation of Biotechnology. Advancements in technology and the agency's experience in implementing current rules drive APHIS' attempts to modernize its procedures.

During February 2015, APHIS withdrew a 2008 proposed rule that would have amended regulations covering GE crops. The agency started a new effort to update the rules three months later. Before this gathered momentum, White House officials announced a plan to revise the Coordinated Framework in light of advances in science and technology. Now part of a much larger modernization project, APHIS still strives to renovate its rules.

At a November 18 stakeholder meeting, John Turner, director of APHS' Biotechnology Regulatory Services (BRS) Biotechnology Risk Analysis Program, stressed that new rules should reflect the agency's extensive experience. "[W]e spent a lot of time reviewing some of the same things over and over, very familiar traits and very familiar crops," Turner said. "And then you pose the question: Is there a way to leverage all of this previous work so that we can expedite the review of similar things when they come in, align with the previous work that's been done?"

Michael J. Firko, deputy administrator of BRS, said that they are considering a new way to trigger regulation. Currently, certain facts trigger APHIS' designation of a product as a regulated article: if the organism has been altered by genetic engineering *and* there is a possibility that the GE organism could be a plant pest. The possibility that a GE organism is a plant pest arises when the GE organism was produced using plant pests (such as the use of a vector derived from plant pest DNA) *or* APHIS scientists have another reason to believe that the GE organism is a plant pest.

Firko suggested how the new approach to regulation might work. In the future, he said, "when we're regulating only those things that represent a risk, we would not be regulating products, plants, whatever, until such time that we have completed either a plant pest risk assessment

for plant pests or a weed risk assessment for plants and be able to document a reasonable risk hypothesis with scientific support about why we think this needs to be regulated. So regulate only with documented risk." Firko's "analyze first and regulate only when needed" strategy would include some form of "science and risk-based trigger."

The vague description of a key aspect of the new approach failed to reassure attendees of the November meeting. Val Giddings, senior fellow with think-tank Information Technology & Innovation Foundation (Washington, DC), pressed for a clearer definition of the new regulatory trigger. "What you're talking about, if I understand it," Giddings said, "is supplanting the current plant pest DNA risk trigger with something else, but I haven't heard any articulation of what the something else [is] in terms of a trigger that would bring something in to be reviewed." Firko could not provide a clarification of the new trigger.

The timeline for revising regulations also caused concern among attendees. Firko said that the best-case scenario for completing the revisions would be about three years. The predicted delay is due the complexity of federal rulemaking and the upcoming change in presidential administration. Such a delay in overhauling regulations – with the attendant uncertainty – creates serious problems for companies, warned Daphne Preuss, president and chief executive officer of Chicago-based Chromatin, Inc. As one example, her seed technology company shut down programs while waiting to see if new regulations justify the investment. "Three to four years of uncertainty," Preuss said, "takes us well beyond . . . a small company's ability to risk capital."

APHIS No Longer Regulates Monsanto's GE Corn, MON 87411

In APHIS' view, the GE corn, as well as progeny derived from the GE corn, are not likely to pose a plant pest risk. Monsanto no longer requires APHIS' authorization for importation, environmental release, or interstate movement of MON 87411 corn plants. APHIS based the approval upon analyses of field and laboratory data about MON 87411 corn as a potential On October 23, Michael Firko approved Monsanto Company's petition for determination of nonregulated status of MON 87411 corn, the company's rootworm-resistant and glyphosate-tolerant GE corn. plant pest risk.

Firko's announcement outlined seven reasons why APHIS decided that MON 87411 corn is unlikely to pose a plant pest risk.

1. Researchers did not find a plant pest risk due to insertion or expression of foreign DNA, the transformation process, or due to changes in the metabolism of the GE corn.

2. Compared with conventional corn, the GE corn does not have an increase in disease or pest incidence.

3. Exposure to or consumption of the GE corn is unlikely to adversely impact nontarget organisms beneficial to agriculture.

4. The GE corn is no more likely to be difficult to control as a weed than conventional corn.

5. The GE corn is not likely to increase the weed risk potential of other plant species with which it can interbreed in the United States or its territories.

6. Growing the GE corn should not require significant changes to agricultural or cultivation practices.

7. Horizontal gene transfer of the new DNA carried by the GE corn to other organisms is highly unlikely. In the unlikely event that gene transfer did occur, then it is not expected to cause disease, damage, injury or harm to plants.

APHIS used the comprehensive analysis to support its Plant Pest Risk Assessment. Before approving Monsanto's petition, however, the agency completed an Environmental Assessment and Finding of No Significant Impact for conferring the nonregulated status. As John Tuner said in the November 18 stakeholder meeting discussed above, APHIS officials want to find a way to leverage experience with these types of plant pest risk analyses to expedite the review of similar GE products.

FDA Sanction of GE Salmon Spawns New Industry and Protests

On November 19, the US Food and Drug Administration approved a GE salmon as fit for human consumption. About two decades had passed since AquaBounty Technologies (Maynard, Massachusetts) filed its first

application to the agency. Laura Epstein, a senior policy analyst for the FDA's Center for Veterinary Medicine, explained that approval took so long because the salmon would be the first GE animal sold as food for humans in the United States. "With most products that are the first of its kind, we are very careful," she told *Nature*.

AquAdvantage Salmon mature to market size in 18 months, compared with three years for conventional farm-raised Atlantic salmon. The rapid growth occurs because the GE fish have a recombinant DNA construct that includes a Chinook salmon growth hormone gene under the control of a promoter from an ocean pout gene. The promoter keeps the growth hormone gene active; the normal growth hormone gene of Atlantic salmon is only active during the summer.

The FDA regulates the GE salmon under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act because the recombinant DNA introduced into the fish met the definition of a drug. To earn FDA approval, AquaBounty had to prove that food from the GE salmon is safe to eat, and that the recombinant DNA does not harm the fish.

Thumbs-up from the FDA has a restriction: The GE salmon cannot be raised in the United States. Currently, the salmon are raised in land-based, contained tanks located in Canada and Panama. The breeding stock is maintained in Canada, and fish will be grown in Panama using eggs shipped from the Canadian facility. In both facilities, the tanks have redundant levels of physical barriers and special plumbing to ensure that salmon eggs and fish do not escape into the environment. Even if GE salmon evaded these measures, the fish could not interbreed or start a population of GE salmon in the wild; the GE fish are reproductively sterile. Because of these precautions, the FDA determined that its approval of GE salmon would not have a significant environmental impact.

The FDA also decided that food from GE salmon is as nutritious as food from conventional Atlantic salmon, and that there are no biologically relevant differences in the nutritional profile of GE salmon compared to that of other farm-raised Atlantic salmon. Consequently, the agency will not require food from GE salmon to be labeled as such. Anticipating an outcry about this decision, the FDA issued a draft guidance document on voluntary labeling to indicate if food has or has not been derived from GE salmon.

Consumer groups were not pleased by the FDA's decisions, voicing concerns about the lack of required GE labels and speculative effects of the GE salmon on human health and the environment. Costco, Whole Foods, Trader Joe's, Target, and Safeway number among companies that promise never to sell food from GE salmon.

It should be easy for the protesting businesses to keep their promise. At full capacity, the Panamanian facility will produce about 100 tons of GE salmon per year, whereas the United States imports more than 200,000 tons of Atlantic salmon each year. The amount of GE salmon will be "a drop in the bucket," Greg Jaffe told *Nature*. Jaffe, director of biotechnology at the Center for Science in the Public Interest (Washington, DC), observed that "Consumers would have to hunt to find salmon that are genetically engineered, as opposed to avoiding them."

FDA-authorized GE Chicken is Not Destined for a Fast Food Bucket

The FDA approved a GE chicken on December 8. The chicken will never be fried, barbecued, baked, broiled, or roasted, and its eggs will never find their way into an omelet.

The GE chickens – approved by the FDA's Center for Veterinary Medicine – were engineered to produce in their egg whites a recombinant form of human lysosomal acid lipase (LAL). The FDA's Center for Drug Evaluation and Research approved recombinant human LAL, which is purified from the egg whites, and will be used to treat people with LAL deficiency. Alexion Pharmaceuticals Inc. (Cheshire, Connecticut) produces the recombinant enzyme, which is known as Kanuma™.

A person who is deficient in LAL builds up fats within cells of various tissues; this causes liver disease, cardiovascular disease, and other disorders. Also known as Wolman disease, LAL deficiency usually proves fatal in the first year of life. A milder form of LAL deficiency can appear in early childhood or later in life, and can also affect life expectancy. Therapy with Kanuma provides a recombinant LAL protein to replace missing or inactive enzyme in a patient. Kanuma administration is the first therapy approved in the US for treatment of people who have LAL deficiency.

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Animal and Plant Health Inspection Service, USDA Announcements

Environmental Impact Statement; Introduction of the Products of Biotechnology

Notice of intent to prepare an environmental impact statement

Federal Register Docket No. APHIS–2014–0054

The U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) announced in the Federal Register on February 5, 2016, its notice of intent (NOI) that it is developing a draft programmatic environmental impact statement (EIS), required under the National Environmental Policy Act, that will evaluate a range of alternatives that the Agency can take as it works to update its biotechnology regulations. The notice also invites the public to comment on the range of alternatives that APHIS will study in the draft EIS, along with definitions that APHIS plans to use in the draft EIS.

Dates: APHIS will consider all comments that received on or before March 7, 2016.

Addresses: You may submit comments by either of the following methods:

- *Federal eRulemaking Portal*: Go to *http://www.regulations.gov/#!docketDetail;D=APHIS-2014-0054*.
- *Postal Mail/Commercial Delivery*: Send your comments to Docket No. APHIS-2014-0054, Regulatory Analysis and Development, PPD, APHIS, Station 3A-03.8, 4700 River Road Unit 118, Riverdale, MD 20737-1238.

Supporting documents and any comments received on this docket may be viewed at *http://www.regulations.gov/#!docketDetail;D=APHIS-2014-0054* or in APHIS' reading room, which is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 799-7039 before coming.

For Further Information Contact

Sidney W. Abel, Assistant Deputy Administrator, Biotechnology Regulatory Services, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 851-3896.

Syngenta Seeds Inc.; Availability of a Preliminary Finding of No Significant Impact and Preliminary Decision for an Extension of a Determination of Nonregulated Status of Corn Genetically Engineered for Insect and Glufosinate-Ammonium Resistance

Federal Register Docket No. APHIS–2016–0002

The Animal and Plant Health Inspection Service has reached a preliminary decision to extend the determination of nonregulated status of Pioneer corn event DP–004114–3 (hereinafter Pioneer 4114 corn) to Syngenta's corn event MZIR098 in response to a request from Syngenta Seeds Inc. MZIR098 corn has been genetically engineered for resistance to insects and to the herbicide glufosinateammonium using the same mechanism of action as Pioneer 4114. APHIS is making available for public comment our preliminary regulatory determination, preliminary finding of no significant impact, and plant pest risk similarity assessment for the proposed determination of nonregulated status.

Dates: APHIS will consider all comments received on or before March 18, 2016.

Addresses: You may submit comments by either of the following methods:

- Federal eRulemaking Portal: Go to http://www.regulations.gov/#!docket Detail;D=APHIS-2016-0002.
- Postal Mail/Commercial Delivery: Send your comment to Docket No. APHIS–2016–0002, Regulatory Analysis and Development, PPD, APHIS, Station 3A–03.8, 4700 River Road Unit 118, Riverdale, MD 20737–1238.

The Syngenta Seeds Inc. extension request, APHIS' preliminary finding of no significant impact and preliminary determination, and any comments received on this docket may be viewed at http://www.regulations.gov/#!docket Detail;D=APHIS-2016-0002 or in our reading room, which is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 799–7039 before coming. Supporting documents and any comments we received regarding our determination of nonregulated status of the antecedent organism, Pioneer 4114 corn, can be found at http://www. regulations.gov/#!docketDetail;D= APHIS-2012-0026. Supporting documents may also be found on the APHIS Web site for MZIR098 corn (the organism under evaluation) under APHIS Petition Number 15–218–01p, and the antecedent organism Pioneer 4114 corn under APHIS Petition Number 11–244–01p.

Contact:

Dr. John Turner, Director, Biotechnology Risk Analysis Programs, Biotechnology Regulatory Services, APHIS, 4700 River Road Unit 147 Riverdale, MD 20737–1236; (301) 851–3954, email: john.t.turner@aphis.usda.gov. To obtain copies of the supporting documents, contact Ms. Cindy Eck at (301) 851– 3892, email: cynthia.a.eck@ aphis.usda.gov.

Monsanto Co.; Availability of a Preliminary Plant Pest Risk Assessment, Draft Environmental Assessment, Preliminary Finding of No Significant Impact, and Preliminary Determination of Nonregulated Status for Maize Genetically Engineered for Resistance to Dicamba and Glufosinate

Federal Register Docket No. APHIS–2015–0048

The Animal and Plant Health Inspection Service has prepared a preliminary determination regarding a request from Monsanto Co. seeking a determination of nonregulated status for maize designated as event MON 87419, which has been genetically engineered for resistance to the herbicides dicamba and glufosinate. We are also making available for public review and comment our preliminary plant pest risk assessment, draft environmental assessment, and preliminary finding of no significant impact for the preliminary determination of nonregulated status.

Dates: They will consider all comments received on or before March 18, 2016.

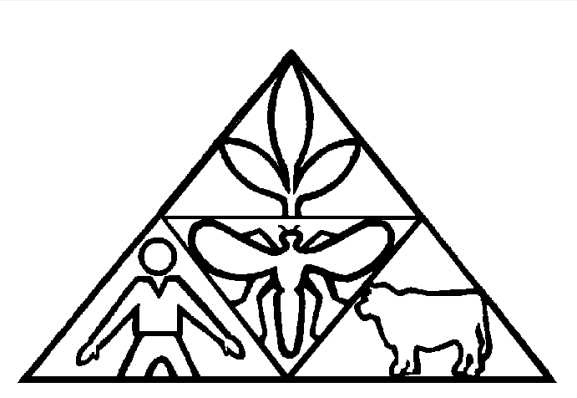
Addresses: You may submit comments by either of the following methods:

- Federal eRulemaking Portal: Go to http://www.regulations.gov/ #!docketDetail;D=APHIS-2015-0048.
- Postal Mail/Commercial Delivery: Send your comment to Docket No. APHIS–2015–0048, Regulatory Analysis and Development, PPD, APHIS, Station 3A–03.8, 4700 River Road Unit 118, Riverdale, MD 20737–1238.

Supporting documents for this petition and any comments received on this docket may be viewed at http:// www.regulations.gov/#!docketDetail;D= APHIS-2015-0048 or in the reading room, which is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 7997039 before coming. Supporting documents for this petition are also available on the APHIS Web site at http://www.aphis.usda.gov/ biotechnology/petitions_table_pending.shtml under APHIS Petition Number 15–113–01p.

Contact:

Dr. John Turner, Director, Biotechnology Risk Analysis Programs, Biotechnology Regulatory Services, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737– 1236; (301) 851–3954, email: john.t.turner@aphis.usda.gov. To obtain copies of the petition, contact Ms. Cindy Eck at (301) 851–3892, email: cynthia.a.eck@aphis.usda.gov.



The Global Pipeline of GM Crops out to 2020

A recent publication by Parisi, Tillie & Rodriguez-Cerezo in *Nature Biotechnology* (2016) provides a description of the situation regarding GM crops development in the world. The study, which is an update of a previous one performed by JRC-IPTS in 2008, describes the technical evolutions in the global pipeline of GM crops, including all events available on the market and those at the pre-commercial, regulatory, and most advanced R&D stages. From 2008 to 2014, the number of GM events at the commercial cultivation, pre-commercial or regulatory stages has more than doubled. Although a few arable crops and certain agronomic traits are still dominant in the pipeline out to 2020 (usually for feed or industrial use), quality traits are slowly emerging, notably for biofortified food and industrial applications. The combination of several traits by commercial stacking is another strong trend in the pipeline.

Another aspect described in the paper is the emergence of new technology developers from developing countries, such as India, China, and Brazil, as well as, although still less advanced, from Africa. Developers from those countries tend to focus on a more diversified set of crops, which could bring more specialty crops into the global pipeline, although for the moment, most of these crops are intended for domestic markets.

Finally, the paper also assesses the likelihood of future incidents of low-level presence (LLP) of unapproved GM material. Because the number of GM events is growing and the authorization processes of different countries remain highly asymmetric, the number of LLP issues in crop shipments will probably increase in the near future and involve a growing number of countries. This will likely soon become a world concern and this situation calls for an international dialogue on the issue, together with other important topics related with GM regulation, such as the expiry of patents or the regulation of new plant breeding technologies.

Source

Parisi C, Tillie P, Rodríguez-Cerezo E. The global pipeline of GM crops out to 2020. *Nature Biotechnology* 34, 31–36. (2016) doi:10.1038/nbt.3449