The Future (and the present) of Genetically Modified Viruses in the Environment

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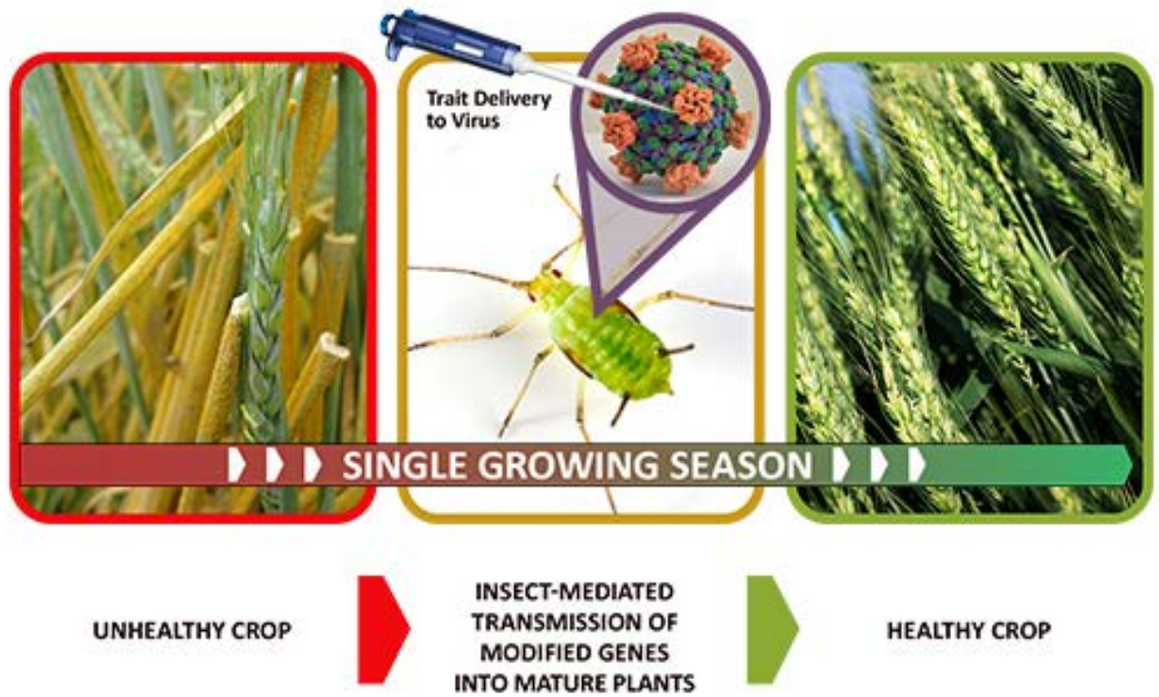
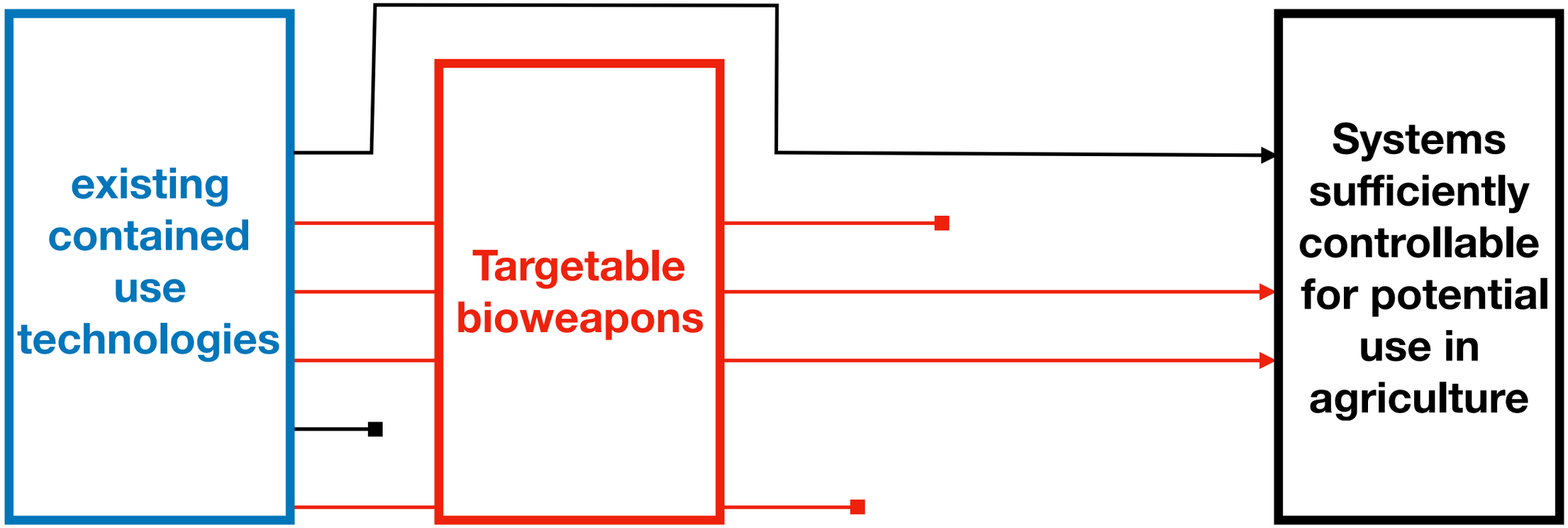
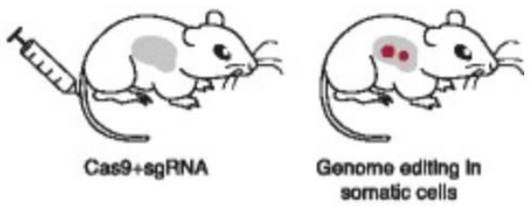
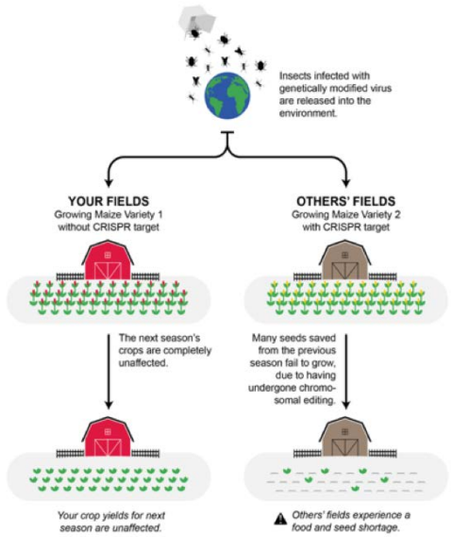


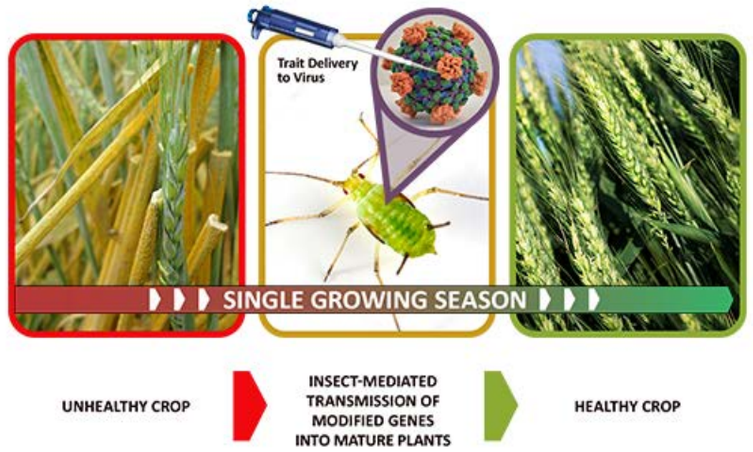
https://www.darpa.mil/news-events/2016-10-19

Genetically Modified Viruses in the Environment









Genetically Modified Viruses in for contained use are extensive and will continue to develop

Study Description Go to

Brief Summary:

This study is to determine whether JX-594 (Pexa-Vec) plus best supportive care is more effective in improving survival than best supportive care in patients with advanced Hepatocellular Carcinoma (HCC) who have failed sorafenib.

Condition or disease	Intervention/treatment	Phase
Hepatocellular Carcinoma Liver **Cancer** HCC	Biological: JX-594 recombinant vaccina GM-CSF Other: Best Supportive Care	Phase 2

Study Design Go to

Study Type :	Interventional (Clinical Trial)
Actual Enrollment :	129 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment
Masking:	None (Open Label)
Primary Purpose:	Treatment
Official Title:	A Phase 2b Randomized Trial of JX-594 (Vaccinia GM-CSF / TK-deactivated **Virus**) Plus Best Supportive Care Versus Best Supportive Care in Patients With Advanced Hepatocellular Carcinoma Who Have Failed Sorafenib Treatment
Study Start Date :	December 2008
Actual Primary Completion Date :	December 2011
Actual Study Completion Date :	December 2011

my local Hospital

Genetically Modified Viruses in for contained use are extensive and will continue to develop

inject GM virus (CRISPR enabled) into tail and one week later 40% of the liver cells are edited at the target gene

ARTICLE

doi:10.1038/nature14299

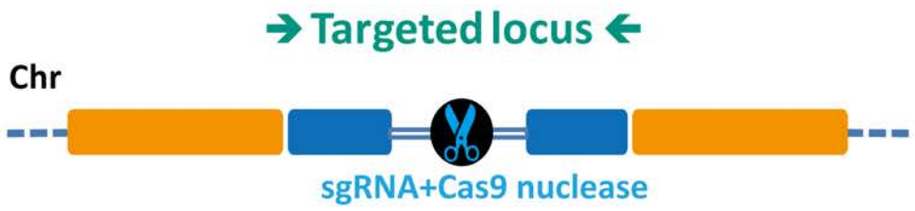
In vivo genome editing using *Staphylococcus aureus* Cas9

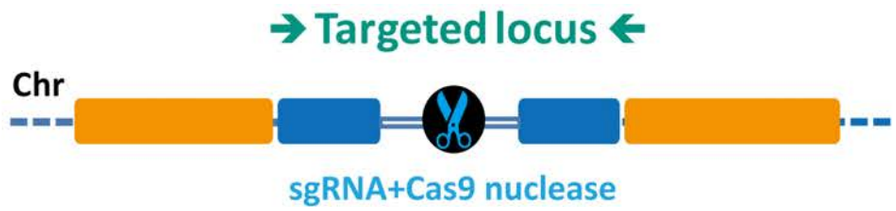
2015

F. Ann Ran[1,2]*, Le Cong[1,3]*, Winston X. Yan[1,4,5]*, David A. Scott[1,6,7], Jonathan S. Gootenberg[1,8], Andrea J. Kriz[3], Bernd Zetsche[1], Ophir Shalem[1], Xuebing Wu[9,10], Kira S. Makarova[11], Eugene V. Koonin[11], Phillip A. Sharp[3,9] & Feng Zhang[1,6,7,12]

What is CRISPR

or ZFNS, TALENs





https://www.genecopoeia.com/wp-content/uploads/2013/11/Targeted-locus-cs9-small.jpg

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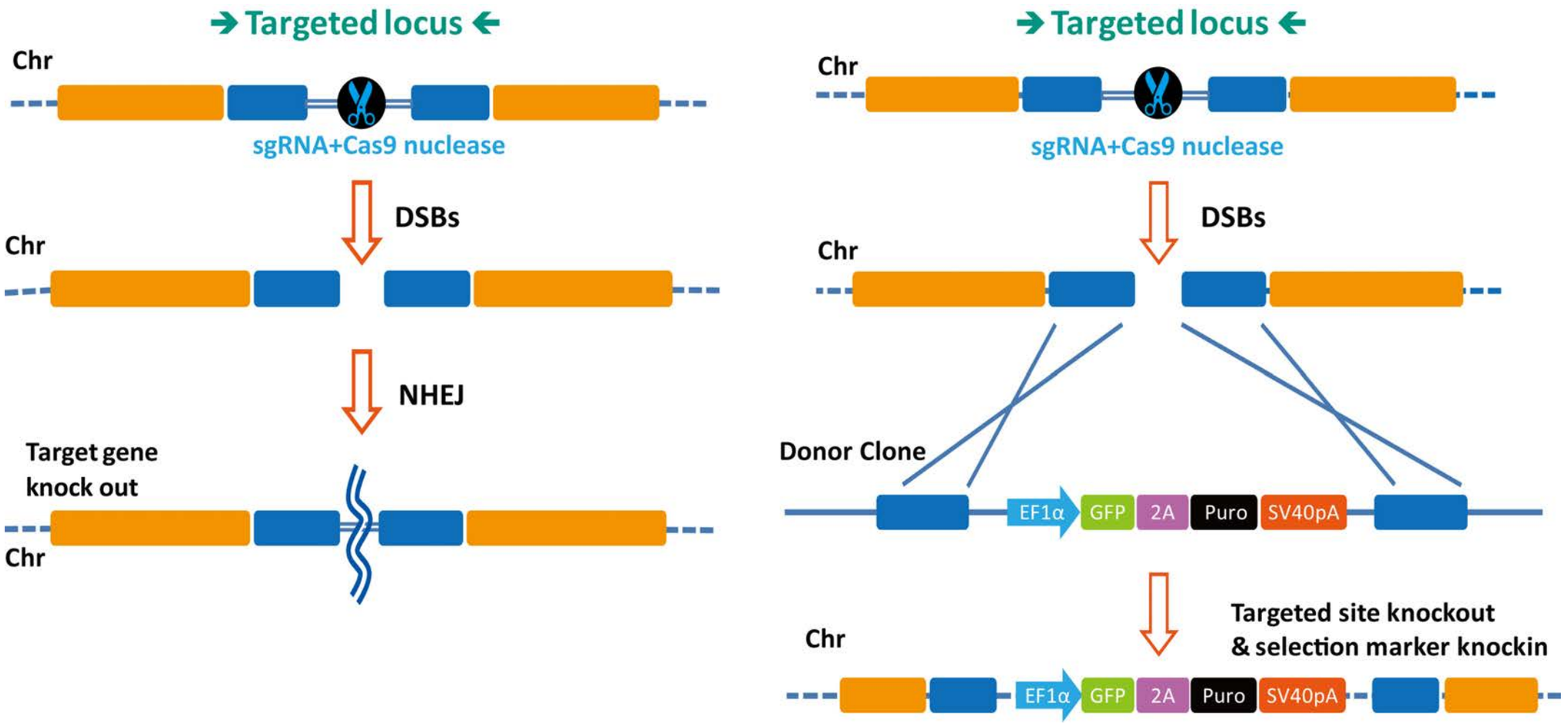

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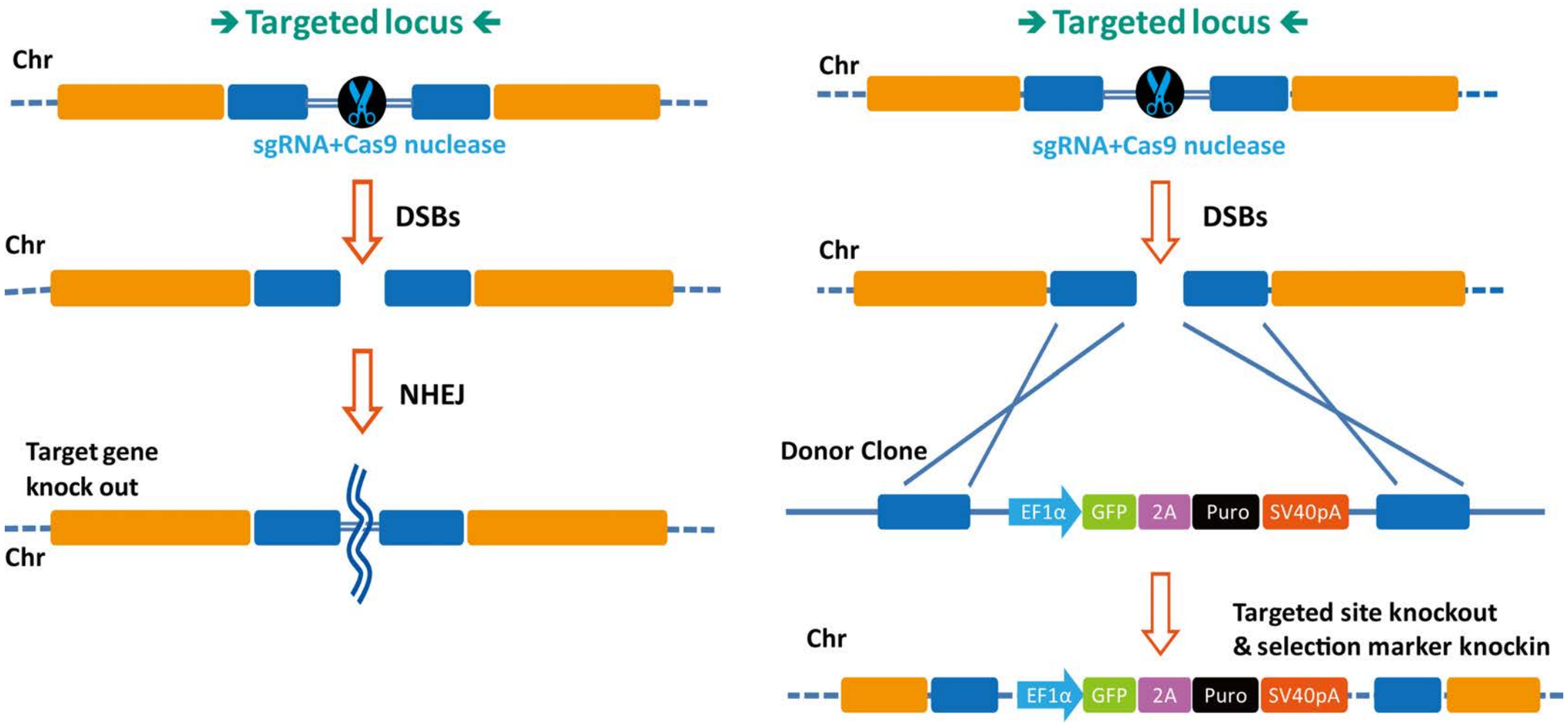


x1000s more efficient

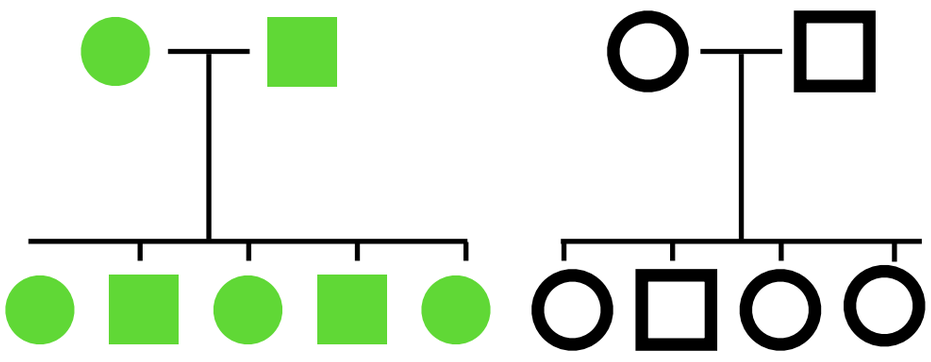
https://www.genecopoeia.com/wp-content/uploads/2013/11/Targeted-locus-cs9-small.jpg

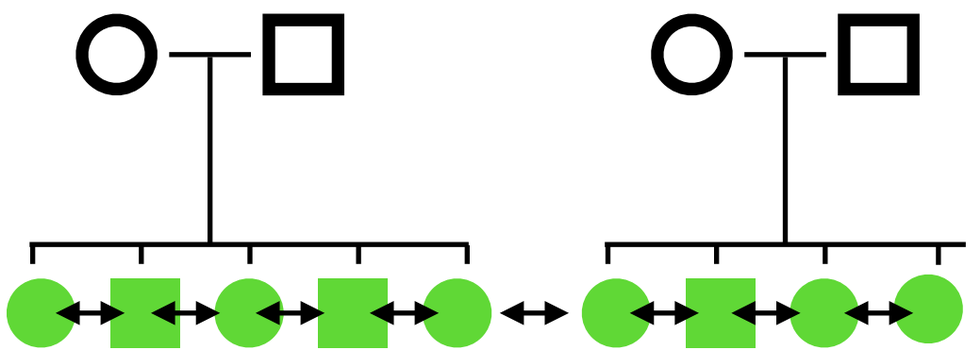
Responding to environmental challenges in crops

Vertical inheritance
(transgenic crops)

versus

Horizontal infection
(viral)





need to anticipate conditions prior to planting

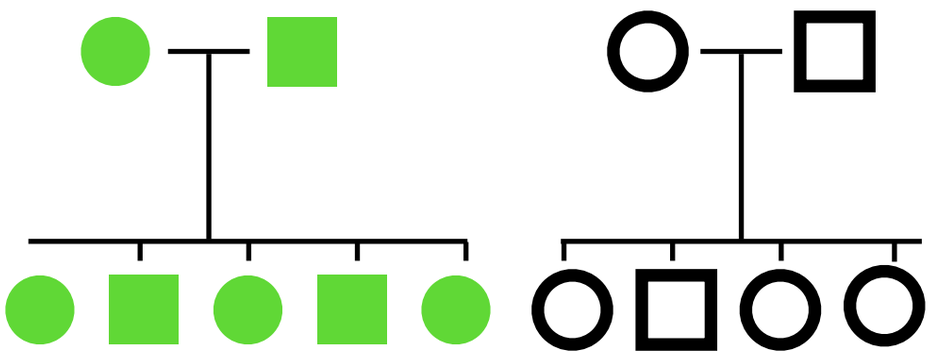
Speed and Flexibility

Responding to environmental challenges in crops

Vertical inheritance
(transgenic crops)

versus

Horizontal infection
(viral)



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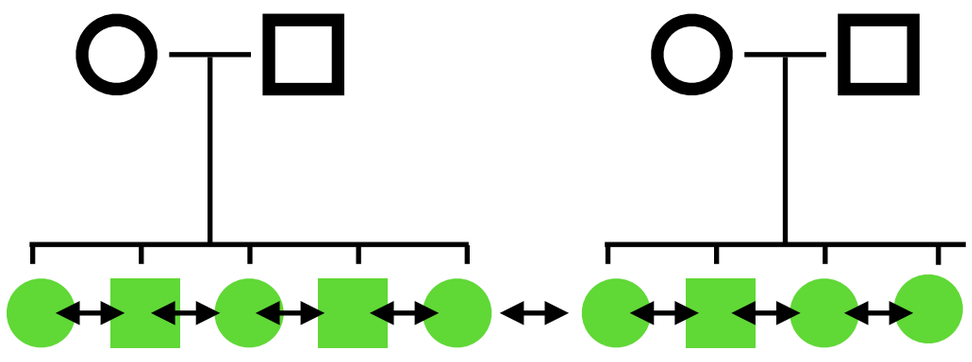


Speed and Flexibility

"Viruses, pests, fungi, herbicides, drought, pollution, salinity, flooding, and frost"

Virus (biocontrol)

human release of viruses as biocontrol agents

European rabbits – myxoma virus (MYXV) and rabbit hemorrhagic disease virus (RHDV)

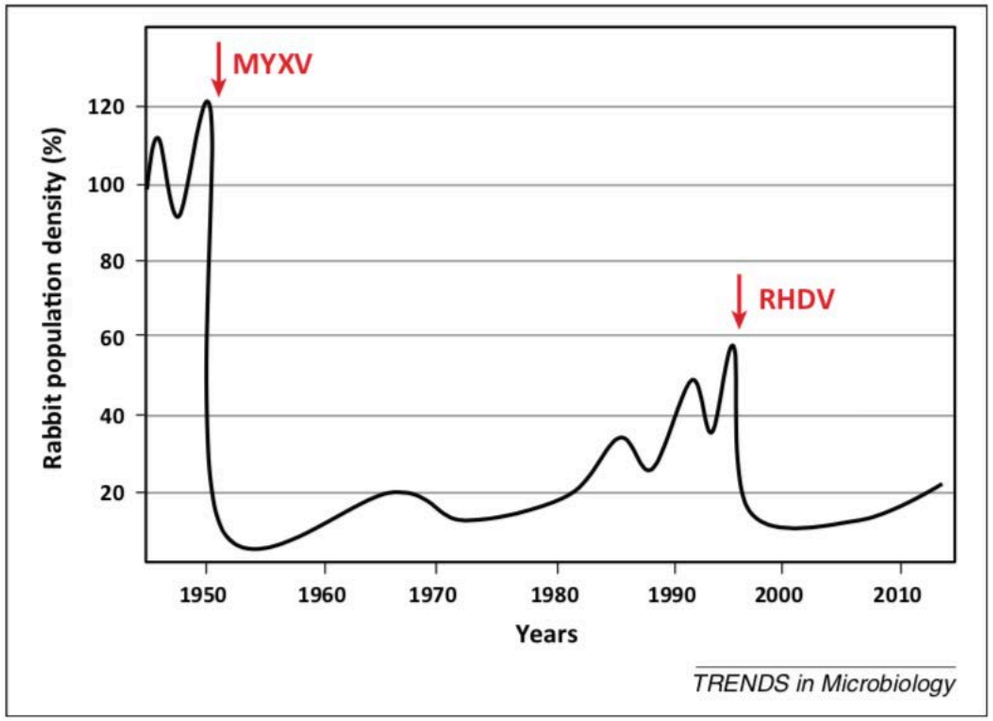


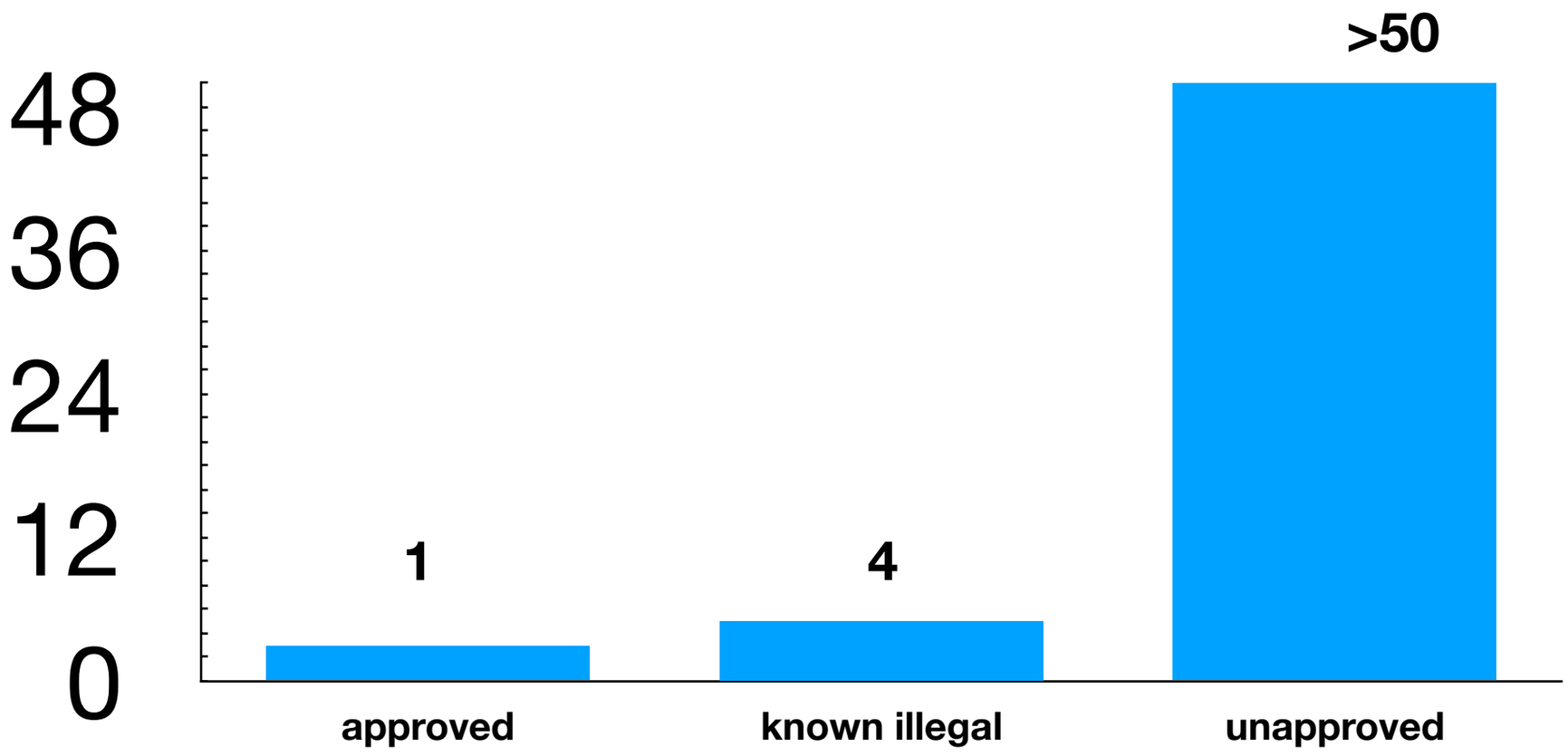
Figure 1. Temporal impact of MYXV and RHDV on rabbit populations in Australia. The relative rabbit abundance in Australia since 1945. Rabbit population density is nominally set to 100% in 1945. The arrows indicate the timing of the release of MYXV in 1950 and RHDV in 1995. Adapted from [4]. Abbreviations: MYXV, myxoma virus; RHDV, rabbit hemorrhagic disease virus.

Di Giallonardo, F.; Holmes, E. C. Viral biocontrol: grand experiments in disease emergence and evolution. *Trends in Microbiology* **2015**, *23*, 83–90,.

Transmissible viruses might not stay where you put them

- particularly if they are perceived as being effective

Trans-national movements of mixomatosis and rabbit haemorrhagic disease viruses since 1950s



Angulo, E.; Cooke, B. **First synthesize new viruses then regulate their release?** The case of the wild rabbit. *Molecular Ecology* **2002**, *11*, 2703–2709

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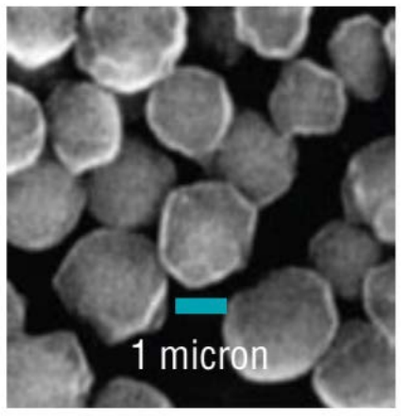
Virus (biocontrol)

human release of viruses as biocontrol agents

Established commercial use in agriculture



Virus-infected larva, showing abnormally pale color and sluggish behavior.

Target is insect larvae not plants

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GM virus

Field trial of a genetically improved baculovirus insecticide

Jennifer S. Cory, Mark L. Hirst, Trevor Williams, Rosemary S. Hails, David Goulson, Bernadette M. Green, Timothy M. Carty, Robert D. Possee, P. Jane Cayley* & David H. L. Bishop

NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR, UK
* Roussel Uclaf Environmental Health, McIntyre House, High Street, Berkamstead HP4 2DY, UK

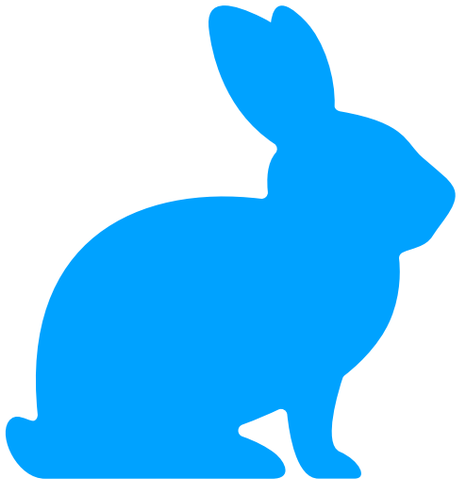
IMPROVEMENT of biological pesticides through genetic modification has enormous potential and the insect baculoviruses are particularly amenable to this approach[1,2]. A key aim of genetic engineering is to increase their speed of kill, primarily by the incorporation of genes which encode arthropod or bacterially derived insect-selective toxins[3–11], insect hormones[12,13] or enzymes[14,15]. We report here the first, to our knowledge, field trial of a genetically improved nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica* (AcNPV) that expresses an insect-selective toxin gene (AaHIT) derived from the venom of the scorpion *Androctonus australis*[16–18]. Previous laboratory assays with the cabbage looper, *Trichoplusia ni*, demonstrated a 25% reduction in time to death compared to the wild-type virus, but unaltered pathogenicity[6] and host range[19]. In the field, the modified baculovirus killed faster, resulting in reduced crop damage and it appeared to reduce the secondary cycle of infection compared to the wild-type virus.

- 1993 UK
- Virus expresses a scorpion toxin
- Improves insecticidal effect of spraying virus on plants- plant is not the target
- Caged plants

GM virus

2.2. Experimental area

The experiment took place in Isla del Aire, an island of 34 Has located 1 km to the east of Menorca



- **2000 Spanish Island**
- **To protect wild rabbit populations utilized for hunting from myxomatosis and rabbit haemorrhagic disease.**
- **Example of “transmissible vaccine”**

Torres, Juan M, Carmen Sánchez, Miguel A Ramírez, Mónica Morales, Juan Bárcena, Joan Ferrer, Enric Espuña, Albert Pagès-Manté, and José M Sánchez-Vizcaíno. “First Field Trial of a Transmissible Recombinant Vaccine against Myxomatosis and Rabbit Hemorrhagic Disease.” *Vaccine* 19, no. 31 (August 14, 2001): 4536–43.

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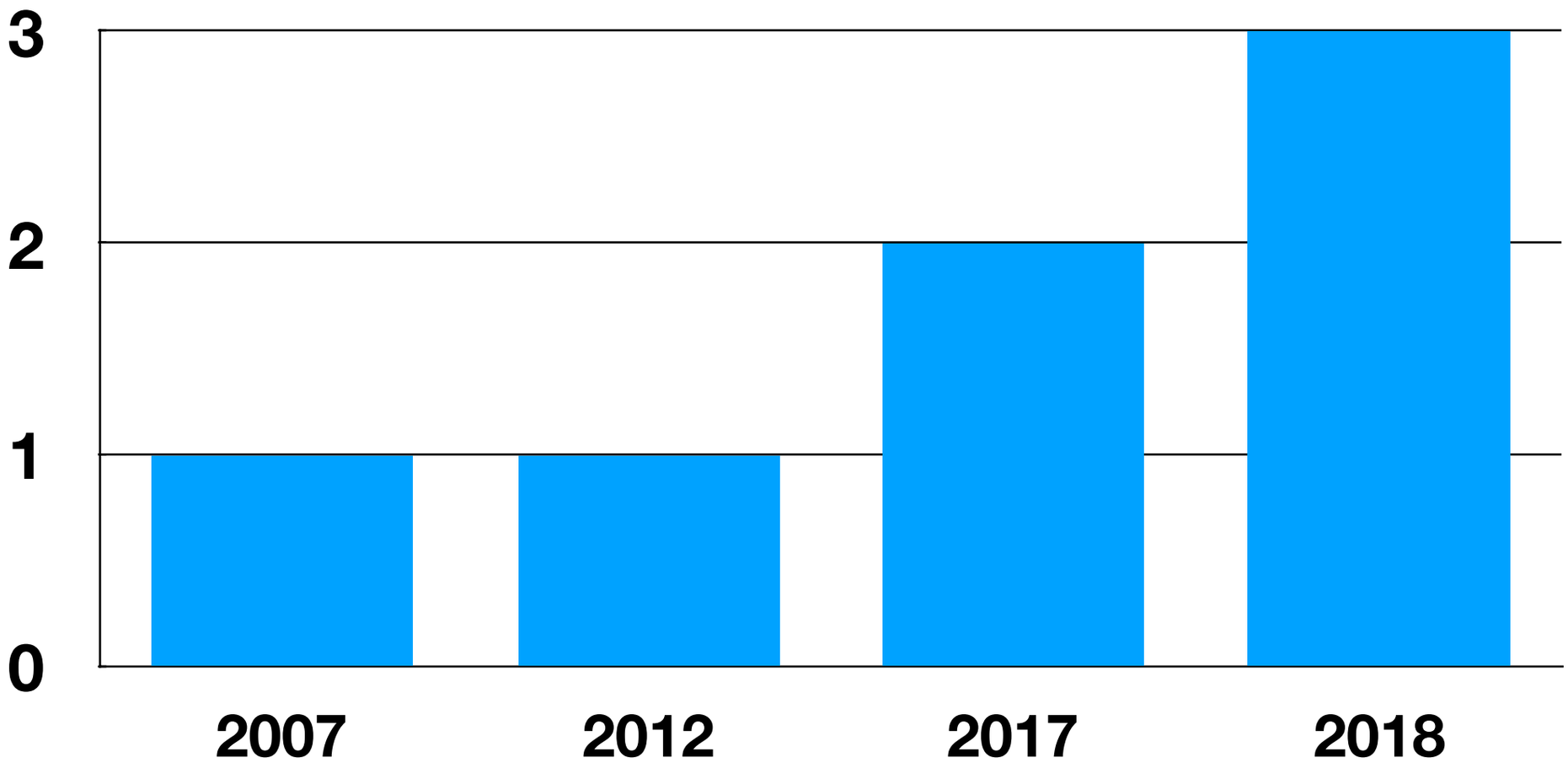
GM virus

Number of scientific publications with "*transmissible vaccine*" in their title



GM virus

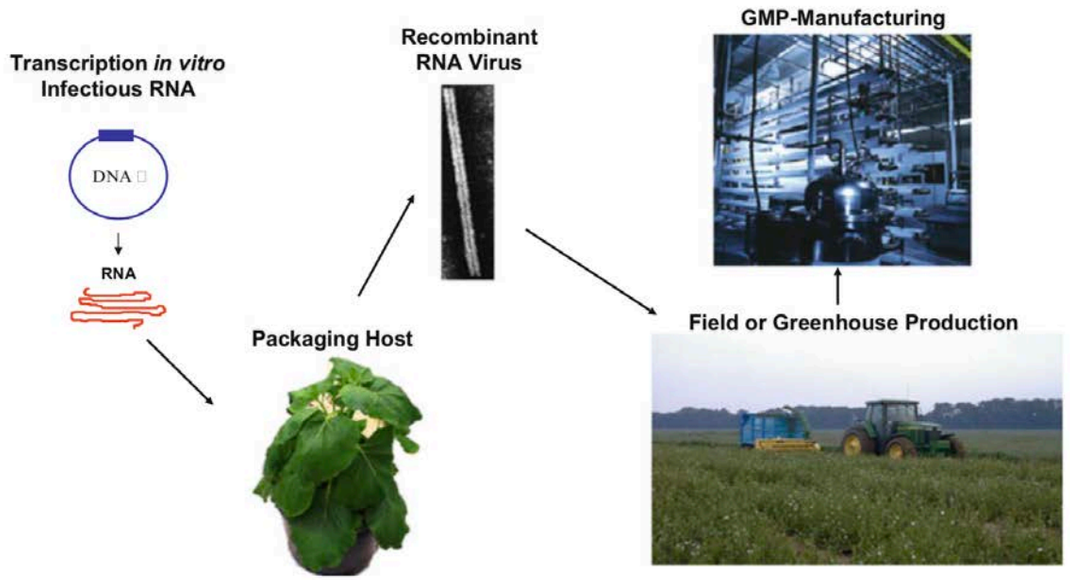


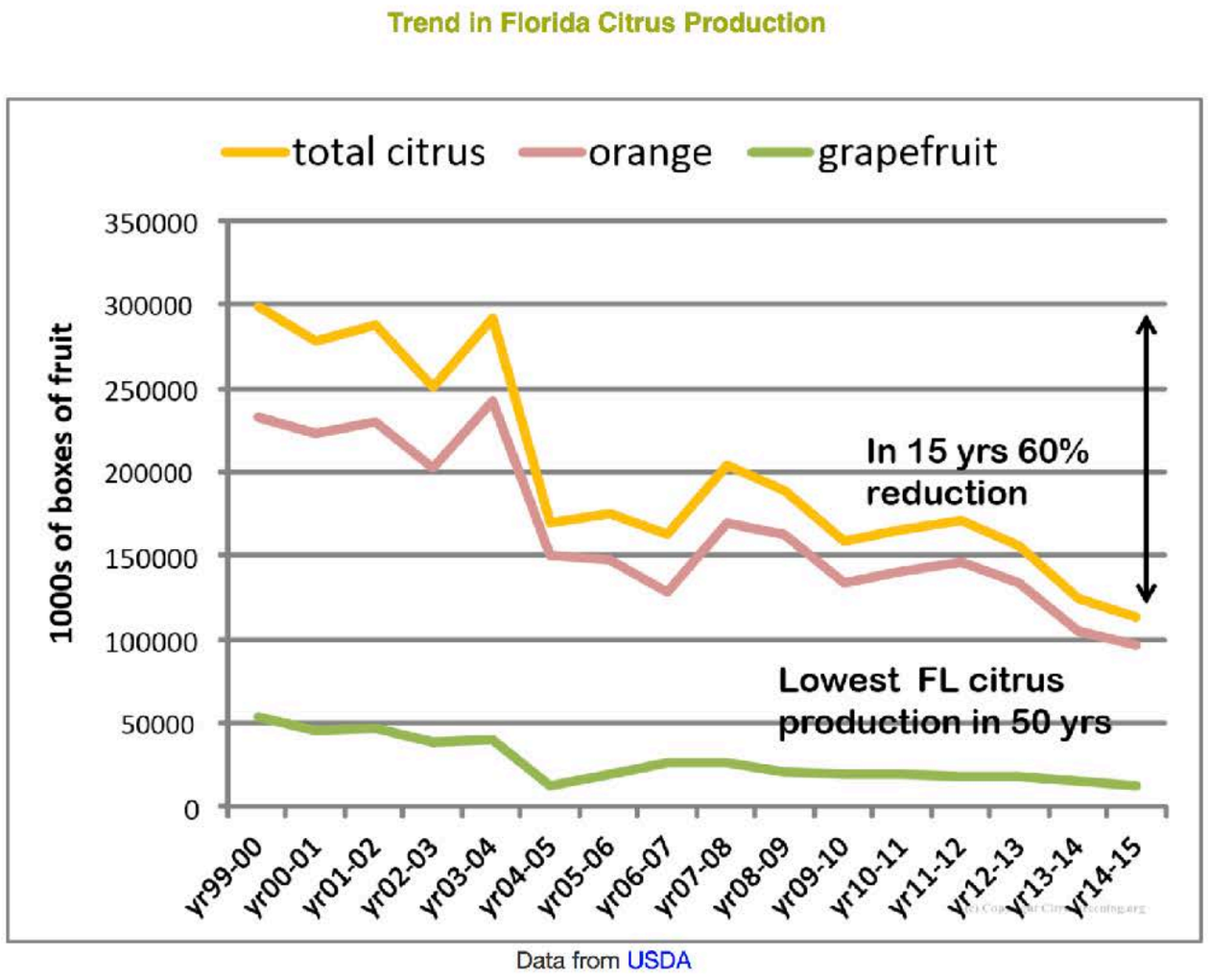
Fig. 5.1 The GENEWARE® expression system. Gene sequences are introduced into a plasmid containing the virus cDNA downstream of the native TMV U1 coat protein subgenomic promoter and upstream of the U5 subgenomic promoter, coat protein gene, and 3′ non-translated region. RNA transcripts are produced in vitro using T7 RNA polymerase and are used as inoculum on a packaging host (*Nicotiana benthamiana*—Nb). Recombinant virions are isolated from the packaging host and tested for intact gene encoding recombinant protein using genetic and functional tests. These are then mass inoculated on Nb grown in greenhouses or *Nicotiana excelsiana* grown in the field (shown in figure). Transfected plant tissue is harvested in mass, and proteins are extracted and purified in a facility capable of cGMP manufacturing

- **starting 2009 Kentucky (USA)**
- **Transient expression of transgenic protein for harvesting**
- **GM virus is sprayed to infect plant cells with virus**
- **Commercial company**

Pogue, Gregory P., Fakhrieh Vojdani, Kenneth E. Palmer, Earl White, Hugh Haydon, and Barry Bratcher. "Production of Pharmaceutical Grade Recombinant Native Aprotinin and Non-Oxidized Aprotinin Variants Under Greenhouse and Field Conditions." In *Commercial Plant-Produced Recombinant Protein Products: Case Studies*, edited by John A. Howard and Elizabeth E. Hood, 65–80. Biotechnology in Agriculture and Forestry. Berlin, Heidelberg: Springer Berlin Heidelberg, 2014.

GM virus

Protecting Orange trees from bacterial disease using GM viruses

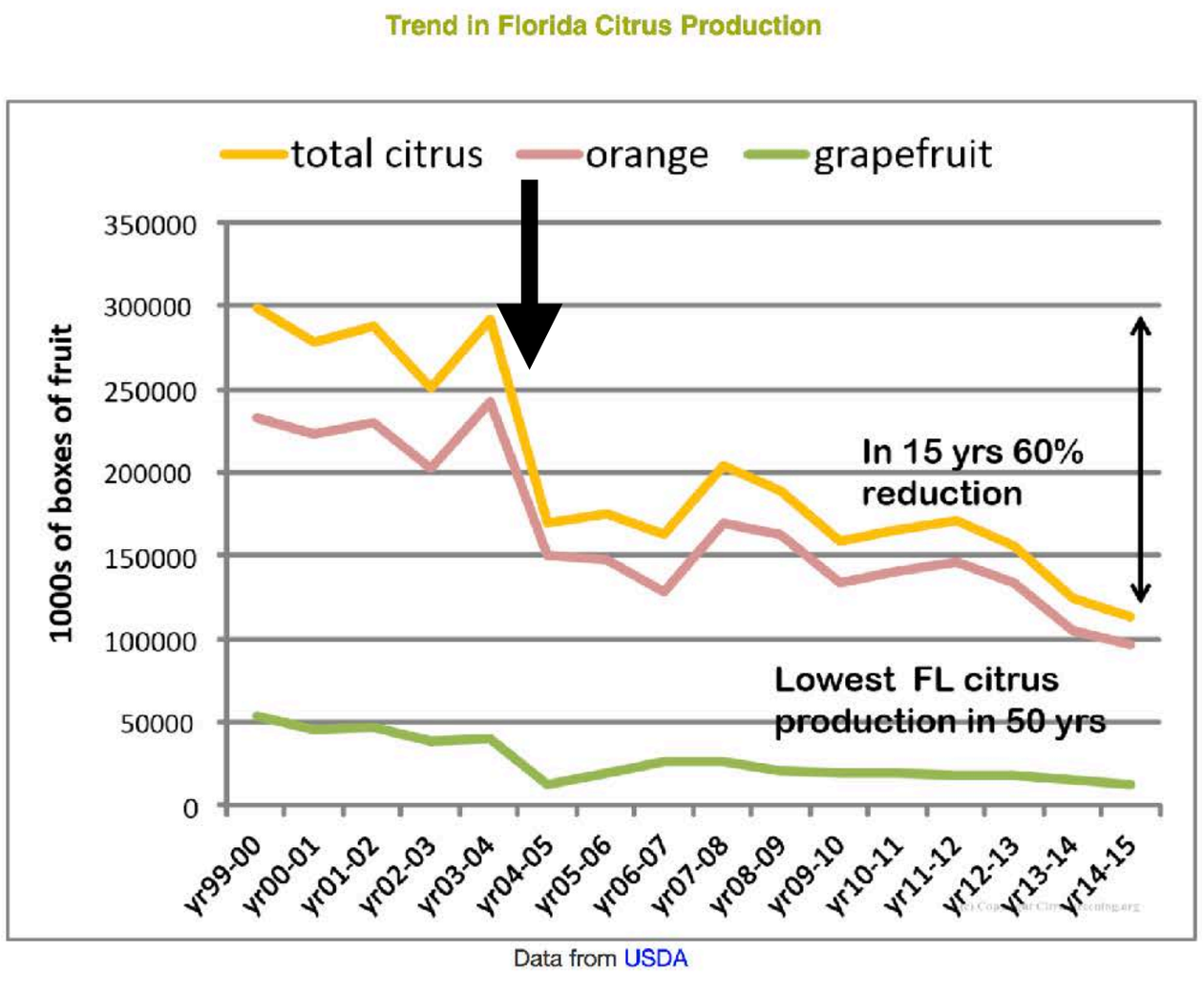


https://citrusgreening.org/disease/impact

GM virus

Protecting Orange trees from bacterial disease using GM viruses

In 2005 a bacterial disease of citrus trees enters the USA- citrus greening (Huanglongbing)



https://citrusgreening.org/disease/impact

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GM virus

Geneticists enlist engineered virus and CRISPR to battle citrus disease

Desperate farmers hope scientists can beat pathogen that is wrecking the US orange harvest.

Heidl Ledford

16 May 2017

Joe Raedle/Getty

Tangerine groves in the southern United States are vulnerable to a disease known as citrus greening.

No gene editing involved in citrus program

MAX-PLANCK-GESELLSCHAFT EWS

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SOUTHERN GARDENS CITRUS

GM virus

- **Field trials of GM virus started in 2010**
- **Citrus tristeza virus expressing spinach defensin proteins 2, 7 and 8 derived from spinach**
- **Virus strain not pathogenic to tree**
- **Only infects phloem**
- **Virus strain n*ot insect vectored***
- **2017 - current 400 acre experiment**
- **2018 USDA EIS and public comment period.**

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GM virus Transmissibility

SOUTHERN GARDENS CITRUS

Grafting of GM virus infected limbs reportedly required

HEGAA virus

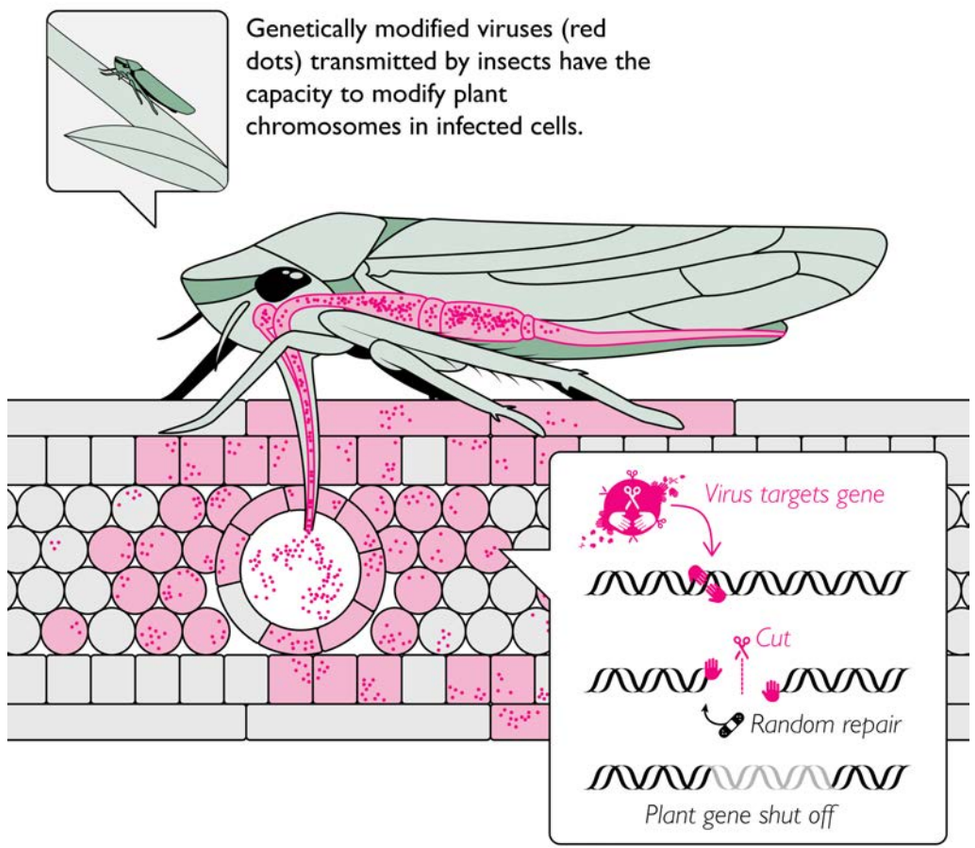
Horizontal Environmental Genetic Alteration Agents

HEGAAs

In the environment a GM virus targets the genome sequence of a second species -using CRISPR

DARPA

Broad Agency Announcement
Insect Allies
BIOLOGICAL TECHNOLOGIES OFFICE
HR001117S0002
November 1, 2016



HEGAA virus

Largely unpredicted regulatory issues with HEGAAs

HEGAAs with somatic gene editing capability: Crop genomes may be modified where the specified chromosomal target is present, i.e. the crop is targetable for chromosomal editing (Fig. 2). Even allowing for future technological developments in gene editing, it is unlikely that all plants in a field will receive the same modification at the intended chromosomal target site. This is quite different to the specific laboratory-generated genetic modifications and associated descriptions of their properties that bio-regulators currently consider.

HEGAAs with germline gene editing capacity: The introduction of imprecise edits into the germplasm of crops could considerably complicate efforts to protect this globally critical resource that, since ancient times, governments have played a fundamental role in securing for the future (see already developed laboratory-confined viral dependent editing systems (*11*, *12*)).

HEGAA virus + insect dispersal

Broad Agency Announcement

Insect Allies

BIOLOGICAL TECHNOLOGIES OFFICE

HR001117S0002

November 1, 2016

Table 1: Insect Allies Program Structure and Objectives – Overview

Tech Areas	Phase 1 *12 Months*	Phase 2 *18 Months*	Phase 3 *18 Months*
1-Virus	Select viruses specific to target crop and insect vector that can deliver a single transgene	Increase gene load and maintain stability of multiple transgenes	Deliver genes for enhanced plant trait(s) via one or more viral vectors
2-Insect	Identify and select insect vector appropriate for target crop and viruses	Reduce strain mortality, improve transmission qualities, and incorporate a single conditional lethal safeguard	Improve dispersal and host specificity for release in larger enclosure, and incorporate multi-factor conditional lethal safeguard(s)
3-Plant	Identify and select crop with established virus and insect vector interactions for transformation of mature plants	Stably transform multiple mature plants within a closed monoculture with multiple (≥3) genes	Stably transform multiple mature plants in a complex, multi-species contained community for enhanced trait(s)
End-of-Phase Demo	*Demonstration One:* Phase 1 Integrated Laboratory Demo	*Demonstration Two:* Phase 2 Integrated Small Greenhouse Demo	*Demonstration Three:* Phase 3 Integrated Large Greenhouse Demo

3 viral consortia announced staring July 2017

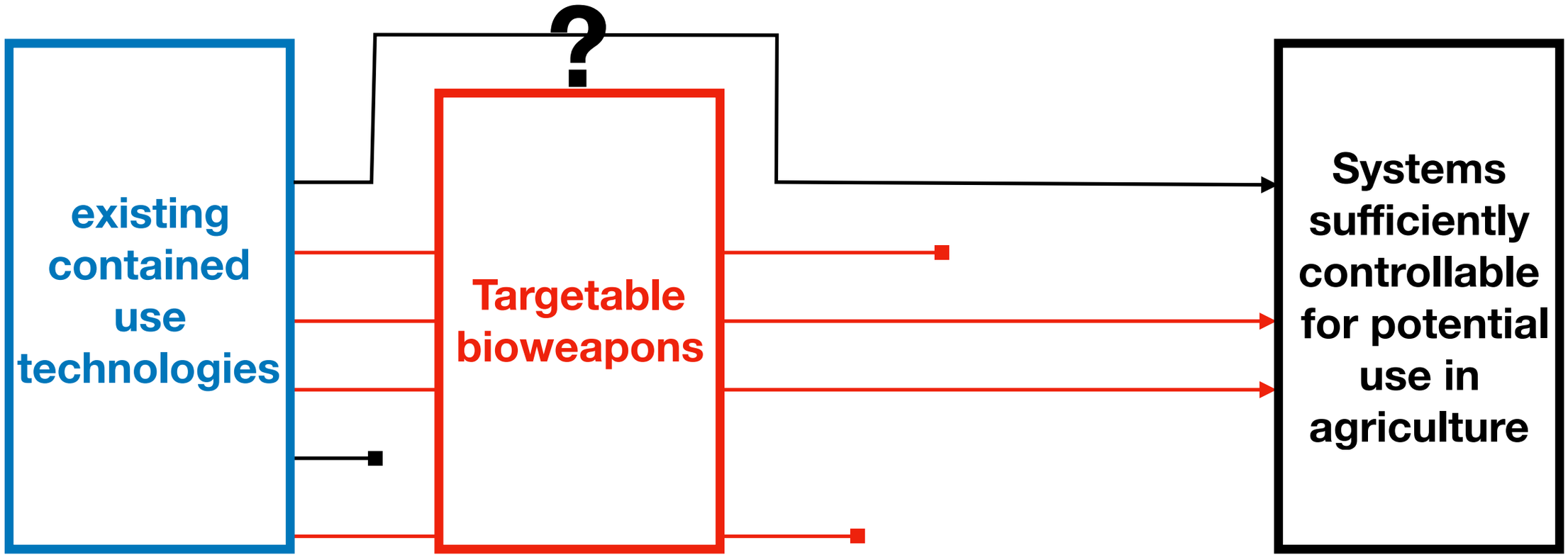
Broad Agency Announcement Insect Allies, Biological Technologies Office, HR001117S0002 November 1, 2016, (available at https://www.fbo.gov/utils/view?id=40638c9e7d45ed8310f9d4f4671b4a7b).

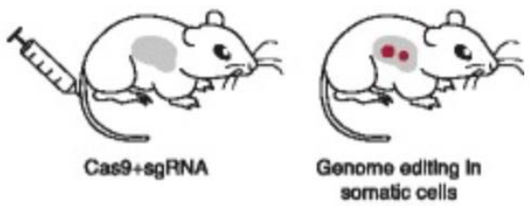
Proportionate public / global scrutiny

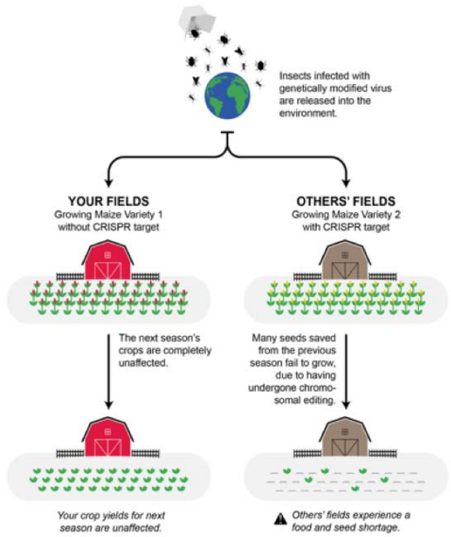
	Citrus trees	DARPA "Insect Allies"
Obvious farming demand for a solution	Yes	No
Transmissibility of virus	Reportedly low (grafting)	High (insect dispersal)
Genetic modification of germ line a concern	No	Yes
Regulatory uncertainty	Yes	Yes
Public scrutiny by competent	**USDA- EIS & Risk assessment & statutory public comment period.**	**Nothing public***

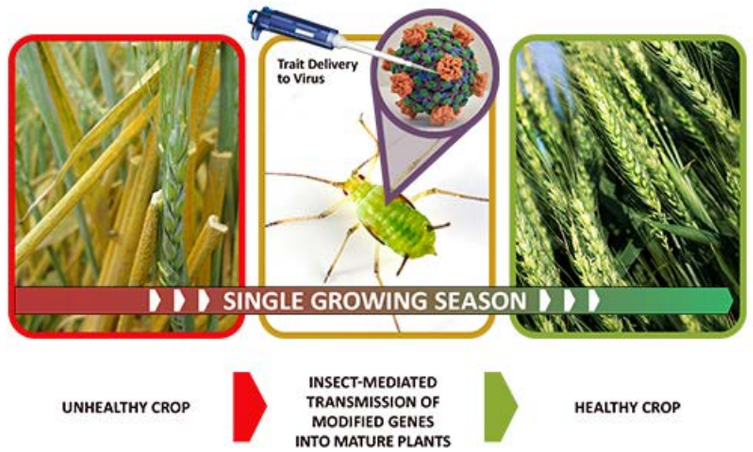
*1.Kuiken, T. DARPA's Synthetic Biology Initiatives Could Militarize the Environment. *Slate* **2017**.

Genetically Modified Viruses in the Environment









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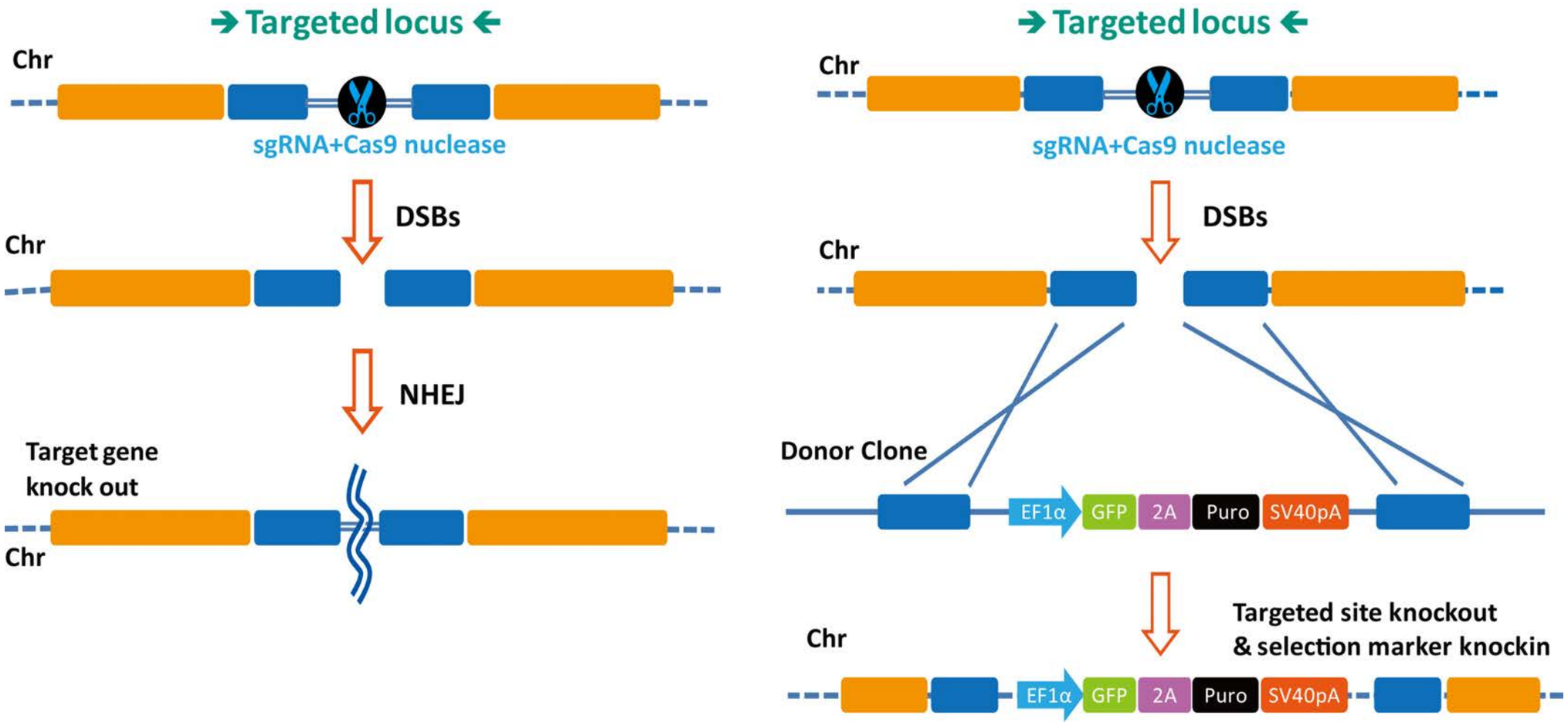

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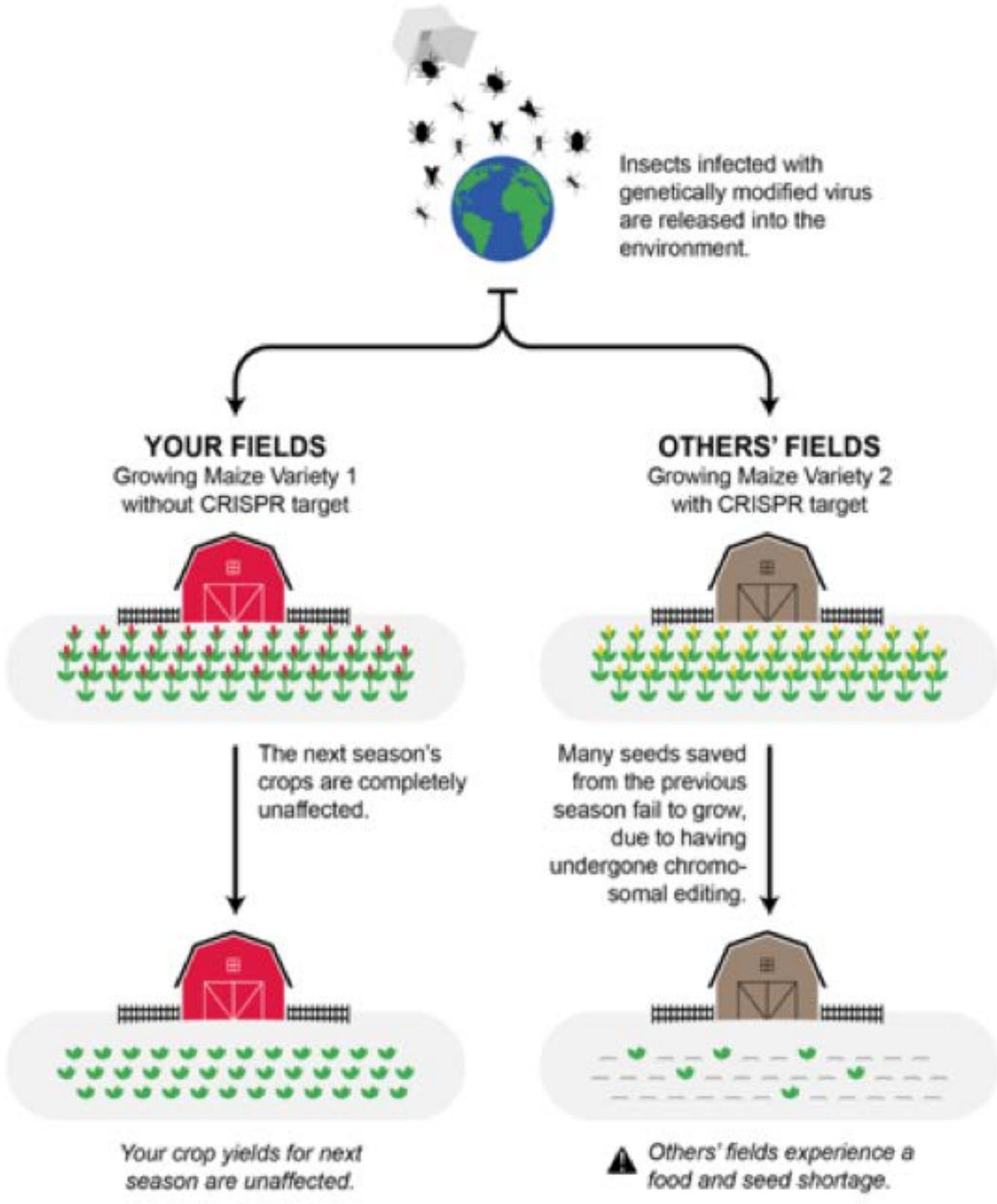
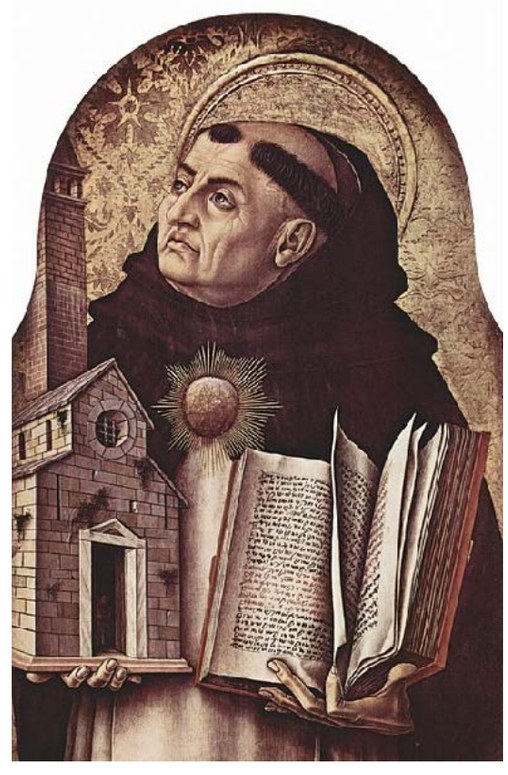

Insects infected with genetically modified virus are released into the environment.
YOUR FIELDS
Growing Maize Variety 1 without CRISPR target
OTHERS' FIELDS
Growing Maize Variety 2 with CRISPR target
The next season's crops are completely unaffected.
Many seeds saved from the previous season fail to grow, due to having undergone chromosomal editing.
Your crop yields for next season are unaffected.
Others' fields experience a food and seed shortage.

The doctrine of double effect



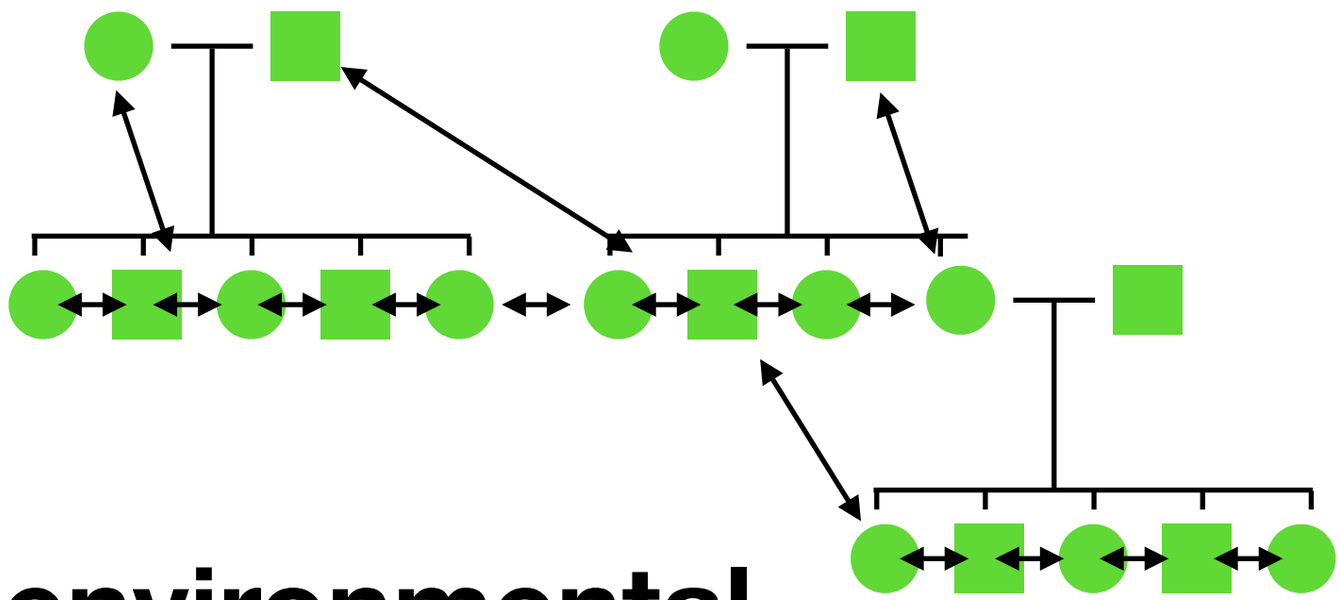
Thomas Aquinas Summa Theologiae, IIa-IIae Q. 64, art. 7; 1200s;

1. The *nature-of-the-act condition.* The action must be either morally good or indifferent.
2. The *means-end condition.* The bad effect must not be the means by which one achieves the good effect.
3. The *right-intention condition.* The intention must be the achieving of only the good effect, with the bad effect being only an unintended side effect.
4. The *proportionality condition.* The bad effect must not be disproportionate to the good effect.

Even legitimate biosecurity or biosafety concerns should not *'undermine the promotion and use of biotechnologies for human development'*
(article X of the Biological Weapons Convention)

Are programs which operate close to the blurred lines dividing peacetime and wartime applications obligated to project robust and plausible motivations for their work?

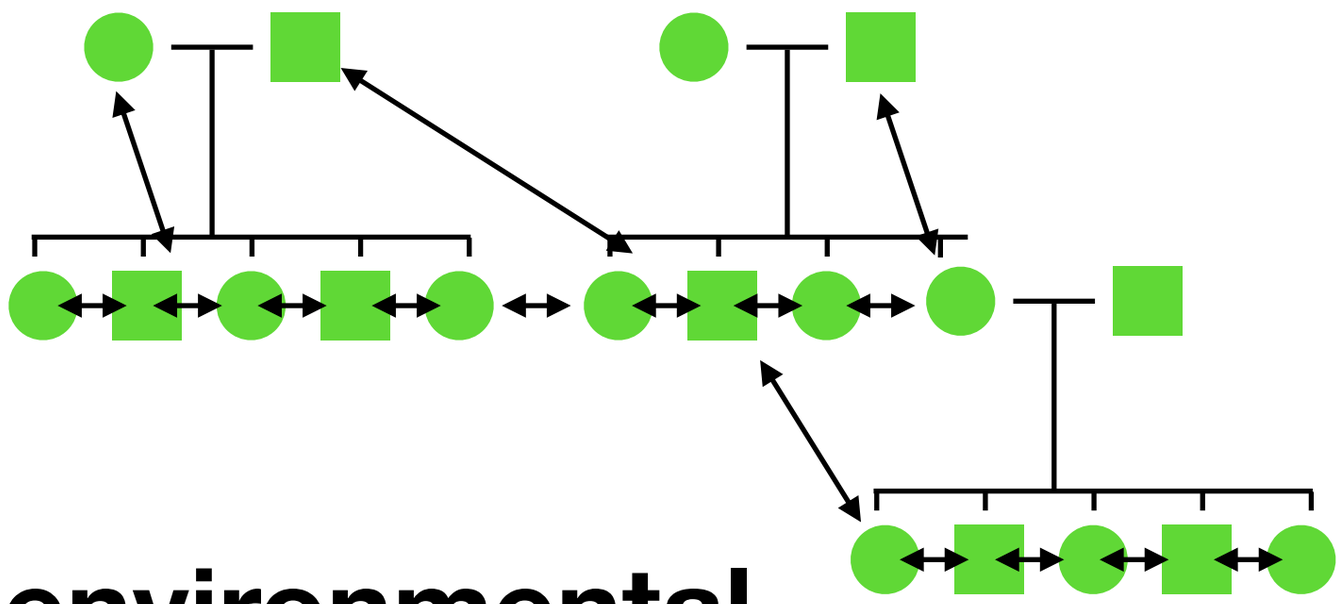
Horizontal infectious solutions offer Speed and Flexibility



Horizontal environmental approaches have outwardly always held some attractions.

They will continue to do so

Horizontal infectious solutions offer Speed and Flexibility



Horizontal environmental approaches have outwardly always held some attractions.

They will continue to do so

so far none are HEGAAs

Thanks

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POLICY FORUM

DUAL-USE RESEARCH

Agricultural research, or a new bioweapon system?

Insect-delivered horizontal genetic alteration is concerning

By R. G. Reeves[1], S. Voeneky[2], D. Caetano-Anollés[1], F. Beck[2], C. Boëte[3]

research contracts (*2*–*4*). In July 2017, th
first of three consortia announced that the
had been awarded a contract from DARP

http://web.evolbio.mpg.de/HEGAAs/