Un po' di new breeding technique per una difesa sostenibile e sicura

PROF, PhD BRUNO MEZZETTI

DIPARTIMENTO DI SCIENZE AGRARIE, ALIMENTARI ED AMBIENTALI

UNIVERSITA' POLITECNICA DELLE MARCHE, ANCONA

Email:b.mezzetti@univpm.it

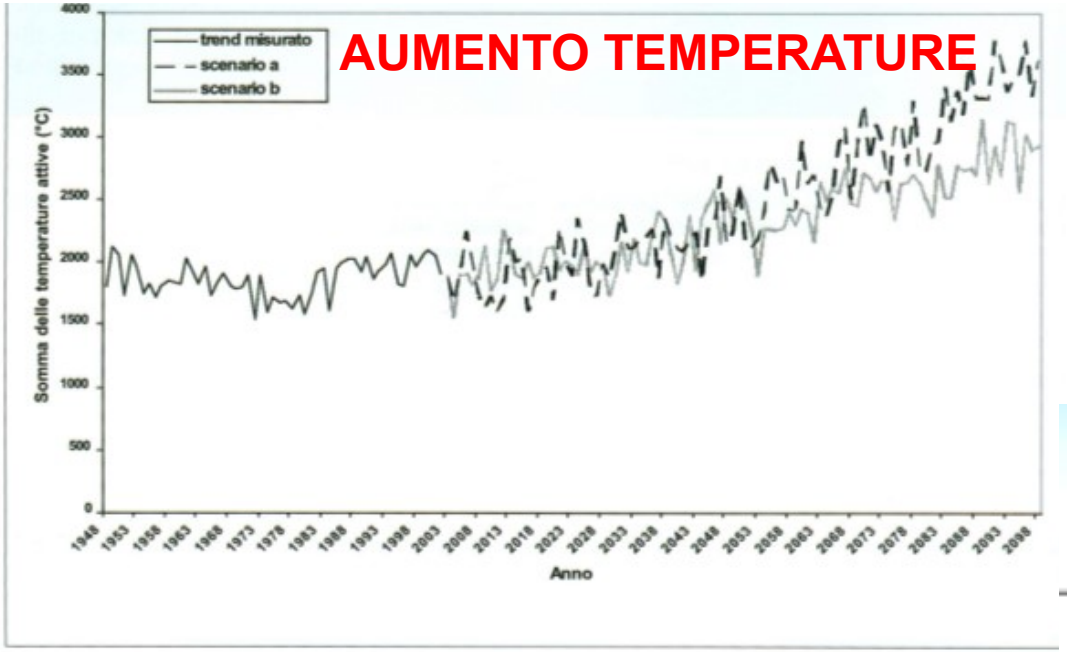
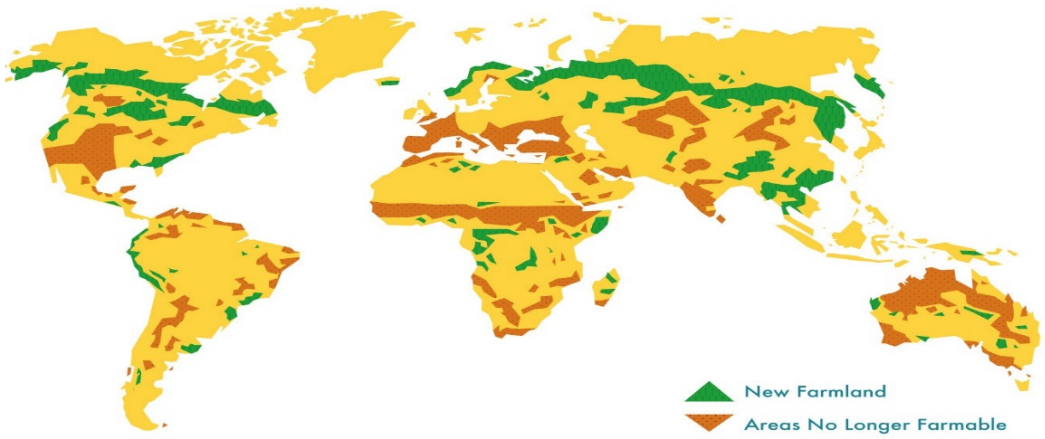


Fig. 1. Andamento della somma delle temperature attive osservato nel periodo 1948-2002 (linea continua) previsto secondo lo scenario a (linea tratteggiata) e lo scenario b (linea grigia) nel periodo 2003-2098.

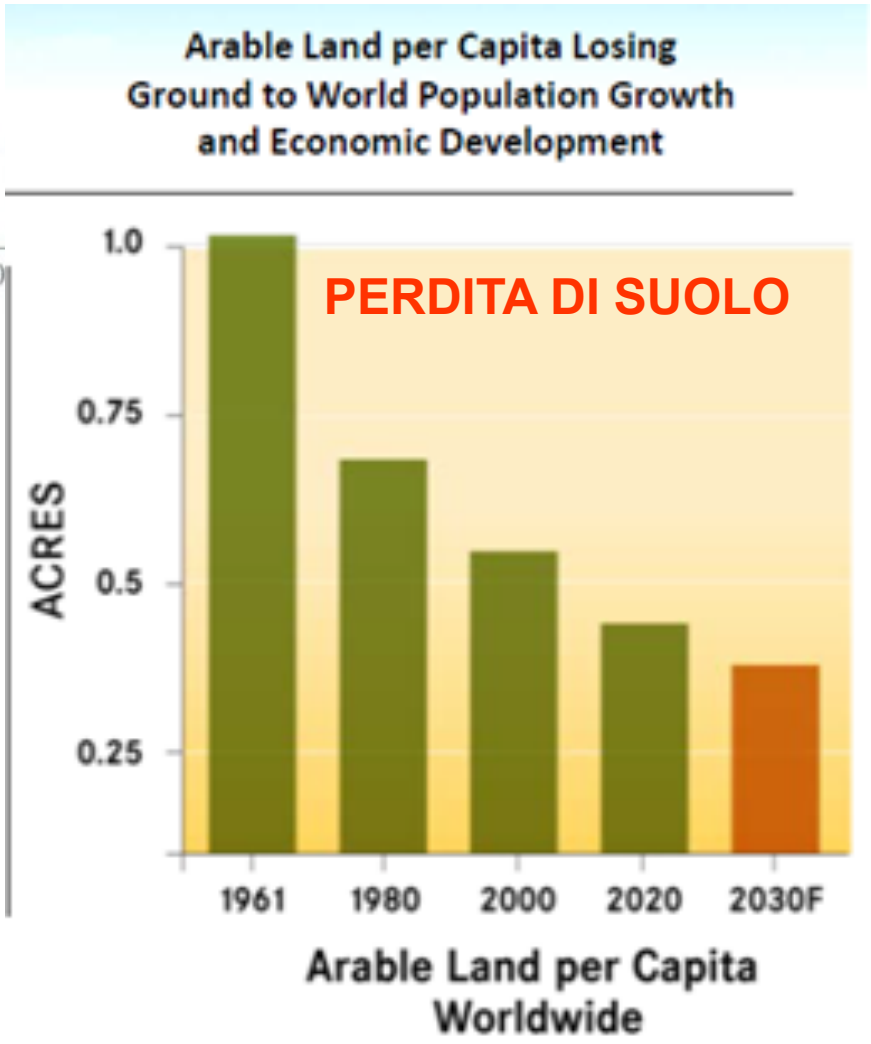
EMERGENZE:
CAMBIAMENTI CLIMATICI
RIDUZIONE SUOLO COLTIVATO

NUOVI AREALI DI COLTIVAZIONE



: dx.plos.org/10.1371/journal.pone.0107522

WWW.HOWWEGETTONEXT



Per l'Agricoltura del XXI secolo, il modello meccanica, chimica e genetica è da rivedere e integrare con tutte le conoscenze e verifiche scientifiche (agronomia, biologia, ecologia, pedologia, difesa, tecnologie..).

VALORIZZARE LA MULTIFUNZIONALITA' DELL'AGRICOLTURA: ALIMENTI, MATERIALI, ENERGIA,ORNAMENTO, PROTEZIONE AMBIENTALE, INCLUSIONE SOCIALE

Individuare nuove strategie per l'AGRICOLTURA SOSTENIBILE del futuro:

- a basso impatto ambientale
- elevata efficienza produttiva
- garanzia salute e reddito agricoltore
- qualità e salute consumatori

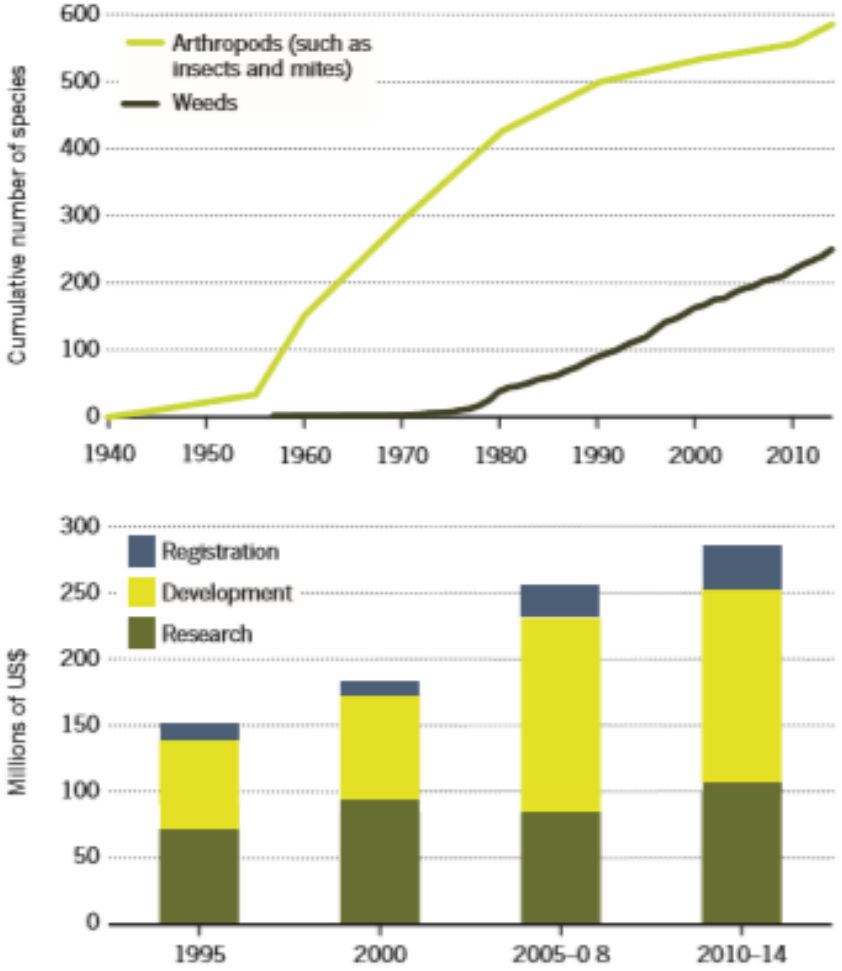
WHEN THE PESTICIDES RUN OUT

Resistance is exhausting the agricultural arsenal against insects, weeds and disease. New biological approaches could help.

BY BROOKE BOREL

THE RISE OF RESISTANCE

The number of pests (including insect and plant species) resistant to at least one form of synthetic pesticide has been steadily on the rise for decades, as has the cost of developing such chemicals.



SOSTENIBILITA': PESTICIDI, UN PROBLEMA MERAMENTE PERCETTIVO

5 principi attivi rameici: 5 Sintesi

Ossido rameoso:
$2\ Cu + ½\ O_2$ (P = 6 bar, T = 120 °C, con alcuni catalizzatori) → **Cu_2O**

Idrossido di rame: (in presenza di fosfati)
$CuSO_4 \cdot 5H_2O + 2\ NaOH + 5\ H_2O \rightarrow$ **$Cu(OH)_2$** + $Na_2SO_4 \cdot 10H_2O$

Solfato di rame tribasico:
$4\ CuSO_4 \cdot 5H_2O + 6\ NH_3 + 6\ H_2O \rightarrow$ **$CuSO_4 \cdot 3Cu(OH)_2$** $+ 20\ H_2O + 3\ (NH_4)_2SO_4$

Ossicloruro di rame:
$4\ Cu + 2\ HCl + 2\ O_2 + 2\ H_2O \rightarrow$ **$CuCl_2 \cdot 3Cu(OH)_2$**

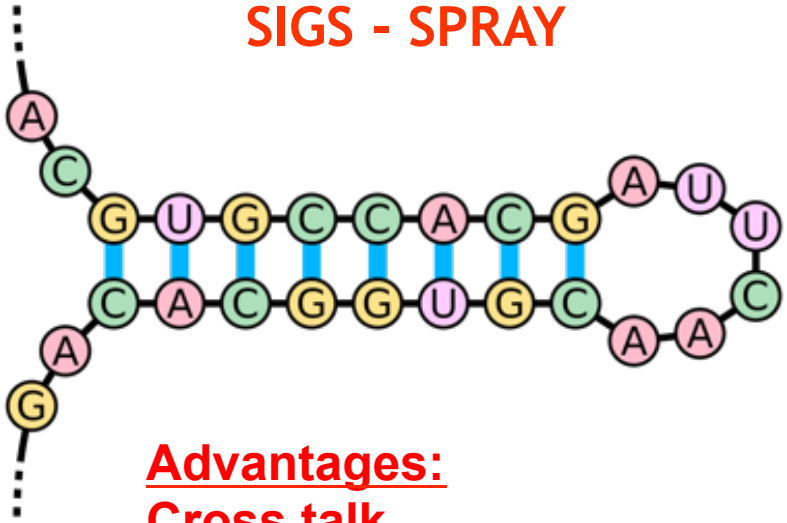
Poltiglia bordolese:
$4\ CuSO_4 \cdot 5H_2O + 3\ Ca(OH)_2 \rightarrow$ **$CuSO_4 \cdot 3Cu(OH)_2 + 3\ CaSO_4 \cdot 2\ H_2O$** $+ 14\ H_2O$

IT'S THE MOST WIDELY USED PESTICIDE IN ORGANIC FARMING

i saranno nuovi erbicidi o chi vuole può tornare a zappare.

RNAi versus CRISP/Cas9

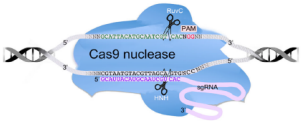
RNAi inhibits gene expression
in a sequence-specific manner
induced by double strand RNA
(dsRNA)
HIGS -TRANSGENICO
SIGS - SPRAY

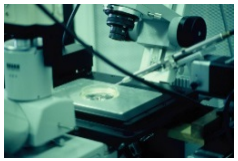


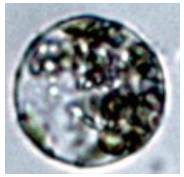
Advantages:
Cross talk
Mobility
Topical application

Gene Editing:

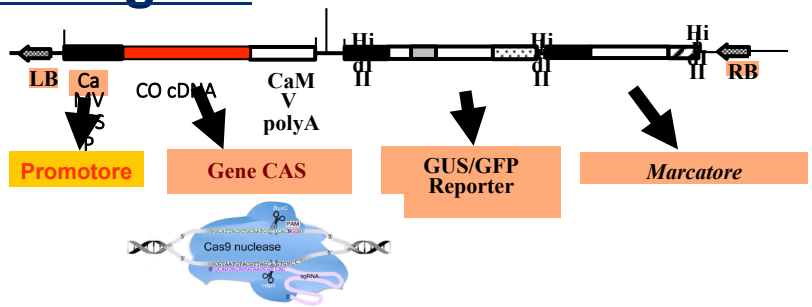
Transiente







Transgene



In planta gene silencing can be repalced by Gene Editing.

Conoscere I geni di resistenza da modificare

frontiers in Plant Science

REVIEW
published: 15 August 2017
doi: 10.3389/fpls.2017.01418

New Biotechnological Tools for the Genetic Improvement of Major Woody Fruit Species

*Cecilia Limera[1], Silvia Sabbadini[1], Jeremy B. Sweet[2] and Bruno Mezzetti[1]**

[1] Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy, [2] J. T. Environmental Consultants Ltd, Cambridge, United Kingdom

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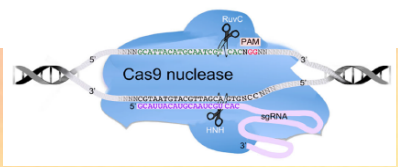
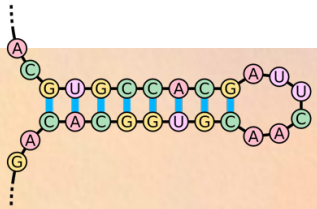
STUDIARE LA COMUNICAZIONE TRA ORGANISMI PER ISOLARE GENI DI RESISTENZA E/O METABOLITI/RNAi INDUTTORI DI RESISTENZA

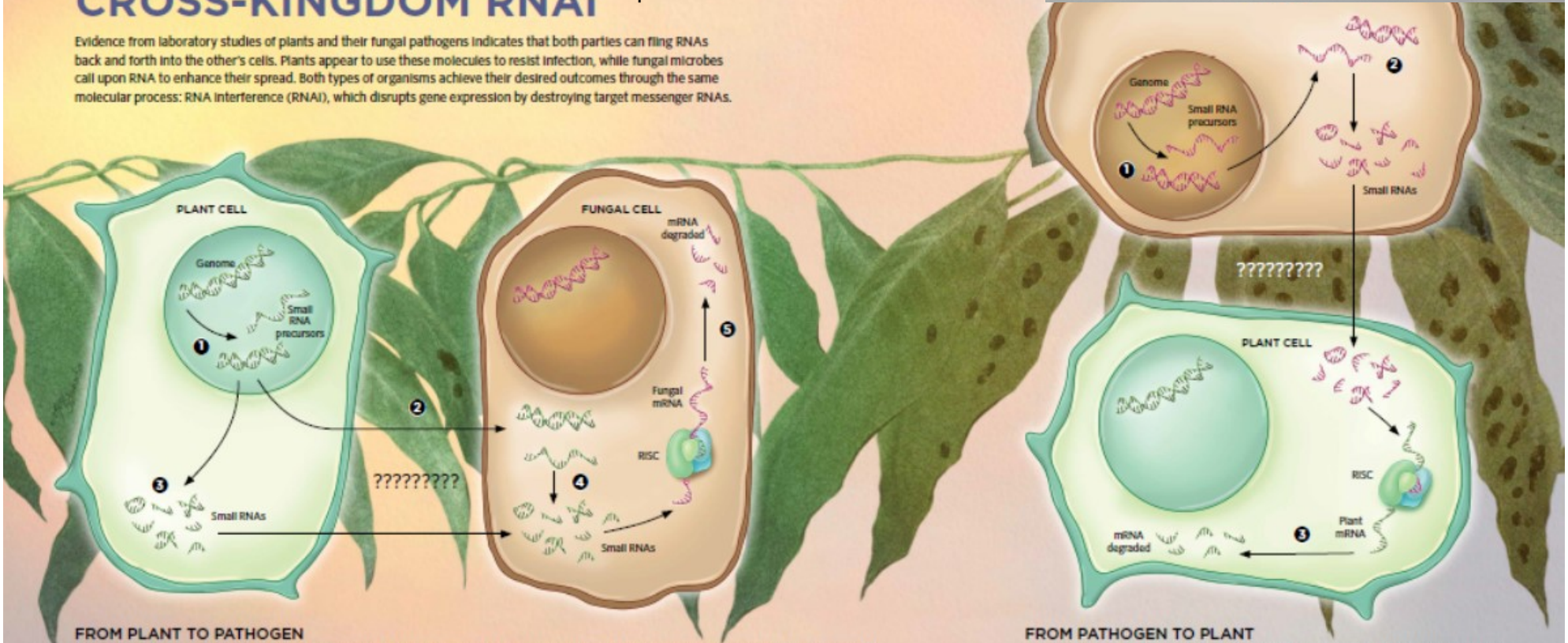




PESTICIDI MOLECOLARI

CROSS-KINGDOM RNAi

Evidence from laboratory studies of plants and their fungal pathogens indicates that both parties can fling RNAs back and forth into the other's cells. Plants appear to use these molecules to resist infection, while fungal microbes call upon RNA to enhance their spread. Both types of organisms achieve their desired outcomes through the same molecular process: RNA interference (RNAi), which disrupts gene expression by destroying target messenger RNAs.



FROM PLANT TO PATHOGEN

The plant produces a small RNA precursor, either a long double-stranded RNA or a pre-microRNA, with sequence similarity to a fungal gene ❶. Researchers have engineered the sequence into the genomes of crop plants or model organisms and demonstrated superior fungal resistance, although one recent study showed plants may naturally encode sequences to protect themselves against pathogens.

Evidence points to the idea that the small RNA precursors can pass directly to the fungal cell ❷ or undergo processing into small RNAs prior to transfer ❸. If the precursor leaves the plant intact, the fungus's processing machinery chops it up ❹. In either case, the result is a plant small RNA inside the fungal cell, though the mechanism of transfer remains unknown.

Upon additional processing in the fungal cell, a single strand of the small RNA becomes part of the RNA-induced silencing complex (RISC), which then destroys an mRNA with a matching sequence ❺. If the transcript is essential to fungus growth, the pathogen dies and the plant staves off disease.

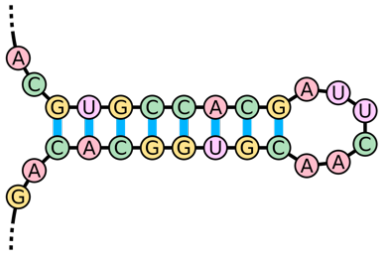
FROM PATHOGEN TO PLANT

Scientists have also discovered that fungal pathogens can send RNAs into plant cells to aid their invasion. Similar to the reverse process, the fungus generates small RNA precursors whose sequences complement those of plant mRNAs ❶. A fungal protein slices up the small RNA precursors to produce small RNAs ❷, which are then passed over to the plant cell via unknown means.

Inside the plant cell, the small RNAs are incorporated into the plant's RISC and direct the complex to degrade the target transcript ❸. If the genes affected are involved in plant immunity, the fungal infection expands.

HIGS

Chewing insects



RNAi activity in tabacco targeting the cytochrome p450 monoxygenase gene (CYPAE14) of the cotton bollworm, Helicoverpa armigera inhibited expression of the CYPAE14 in this lepidopteron pest. The inhibition of CYPAE14 expression in these insects led to their increased sensitivity to the natural defence compound, gossypol, produced by the plant. (Mao et al., 2011)

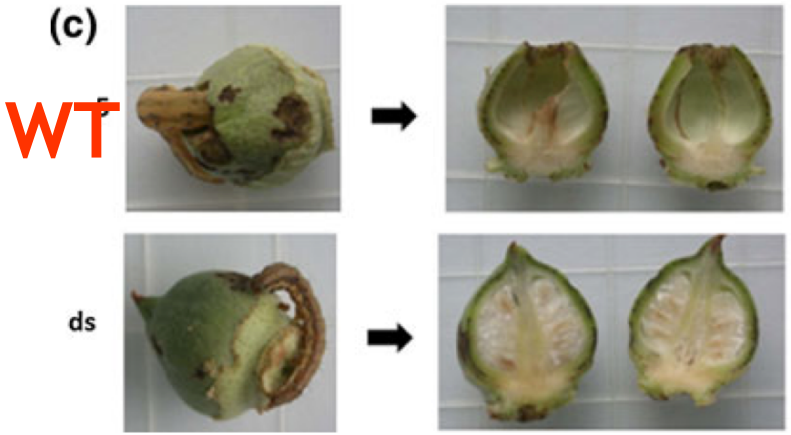
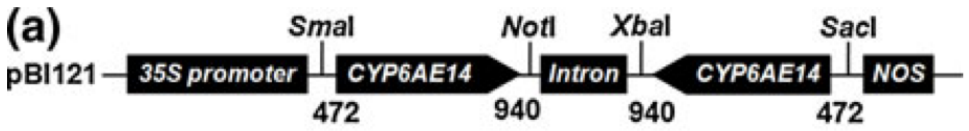


Image of larvae on cotton boll. Larvae previously reared on leaves of control R15 or ds6-3 T2 plants for 10 days were transferred to cotton boll for another day



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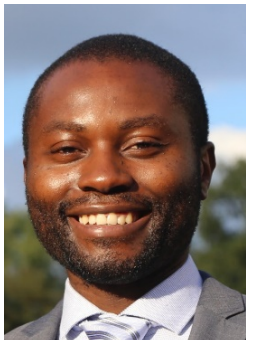
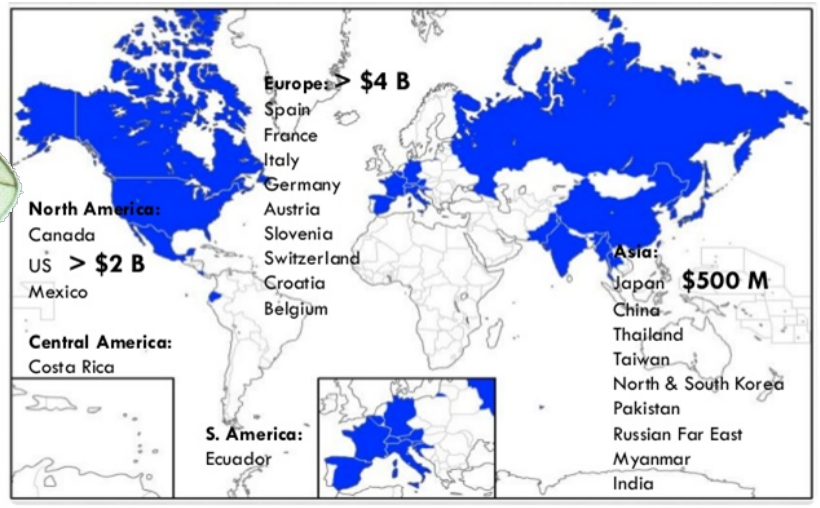
ANOTHER IMPORTANT EMERGENCY IN EU:

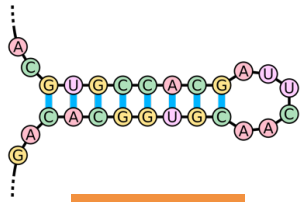
RNAi: virus-induced gene-silencing against invasive wing-spotted fruit fly *Drosophila suzukii*



Dr. Clauvis Taning

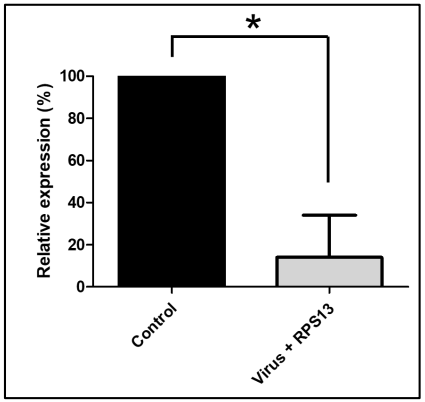
UNIVERSITEIT GENT

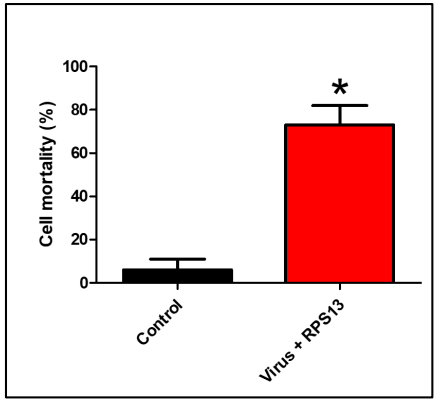




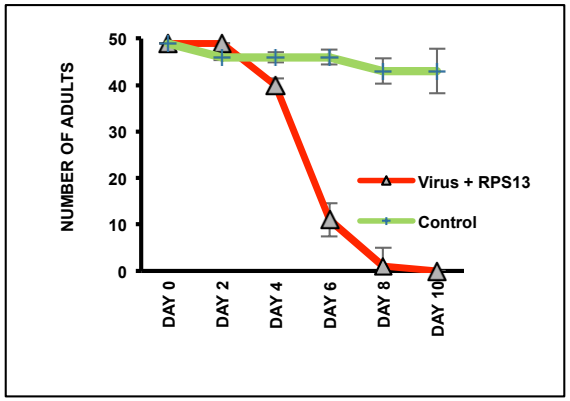
VIGS

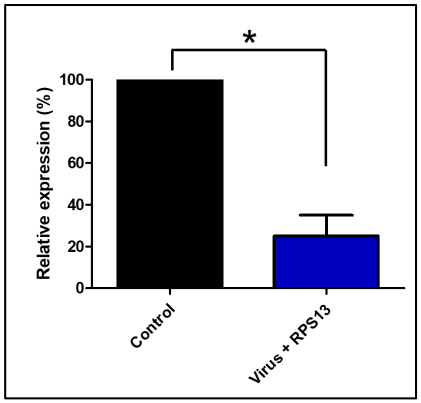
VIGS in cells with engineered Flock House Virus





VIGS effects in adult flies

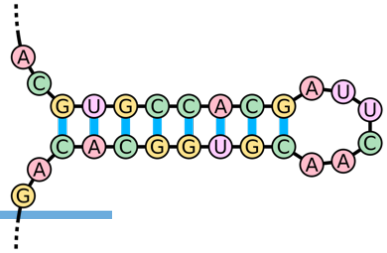




Frontiers in Physiology, 3 July 2018; https://doi.org/10.3389/fphys.2018.00805

HIGS

I case study:
PRSV-RESISTANT TRANSGENIC PAPAYA



In 1992 a papaya expressing the coat protein of *papaya ringspot virus* (PRSV), shown fully resistance to the virus under field condition (Fitch *et al*. 1992).

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nature plants

ARTICLES

PUBLISHED: 19 SEPTEMBER 2016 | ARTICLE NUMBER: 16151 | DOI: 10.1038/NPLANTS.2016.151

Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection

Ming Wang[1], Arne Weiberg[1†], Feng-Mao Lin[2], Bart P. H. J. Thomma[3], Hsien-Da Huang[2] and Hailing Jin[1*]

Very high potentials for controlling diseases in horticultural plants

HIGS

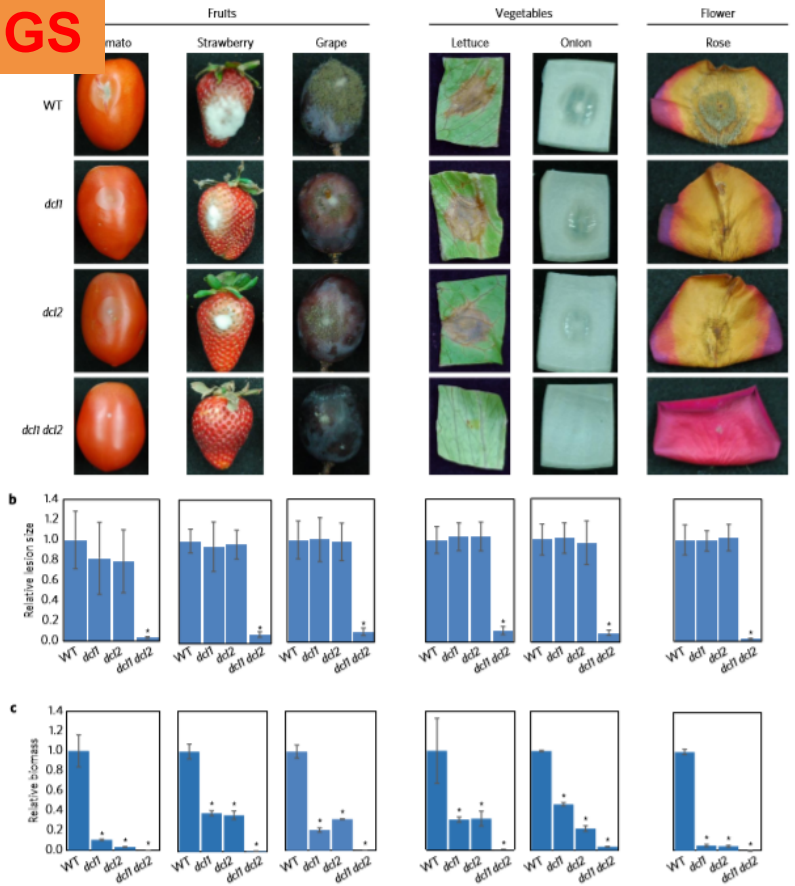

Figure 1 | *B. cinerea dcl1 dcl2* double mutant, but not the *dcl1* or *dcl2* single mutants, displays reduced virulence on fruits, vegetables, and flower petals. **a**, *B. cinerea dcl1 dcl2* double mutant shows compromised virulence on fruits (tomato, strawberry and grape), vegetables (lettuce and onion) and flower petals (rose), and *B. cinerea dcl1* and *dcl2* single mutants showed similar virulence as the WT strain. **b**, Relative lesion sizes of the infected plant samples were measured 3 days post inoculation (dpi) for lettuce, onion and strawberry and 5 dpi for tomato, grape and rose petal using ImageJ. Error bars indicate the standard deviations (s.d.) of ten samples. **c**, *B. cinerea* relative DNA content (relative biomass) was measured by quantitative PCR. Error bars indicate the s.d. of three technical replicates. Asterisks indicate statistically significant differences ($P < 0.01$). Similar results were obtained from at least three biological replicates.

SIGS

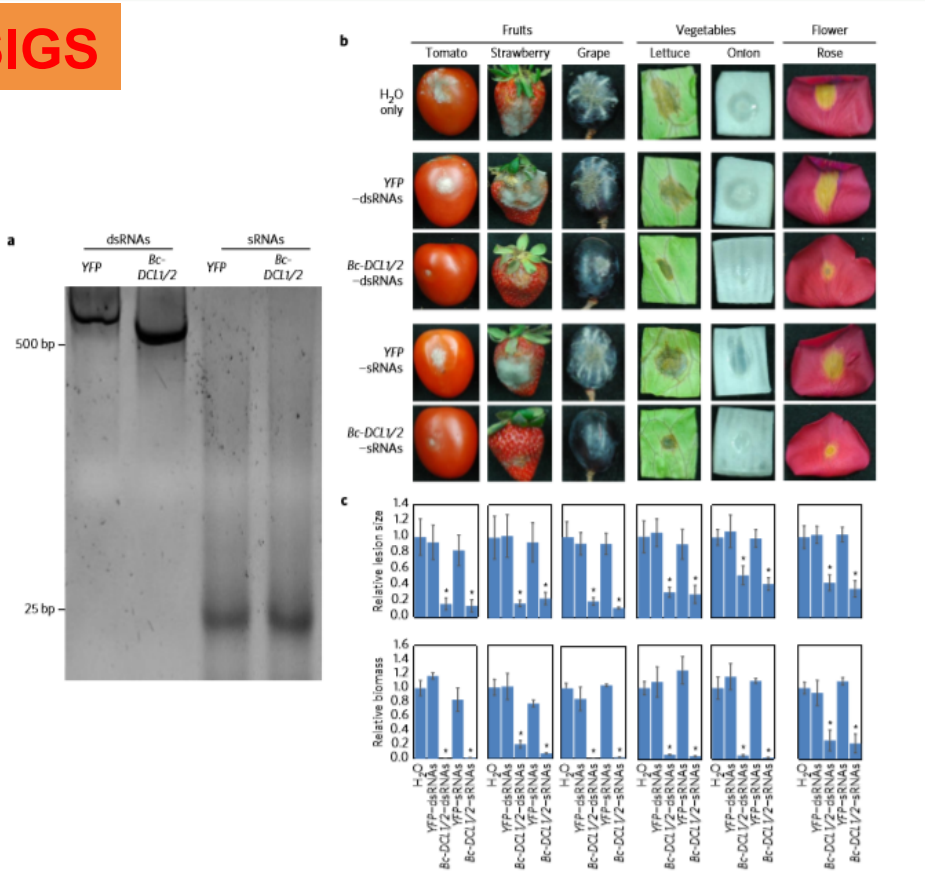

Figure 4 | Externally applied *Bc-DCL1/2*–sRNAs and –dsRNAs inhibited pathogen virulence on fruits, vegetables, and flower petals. **a**, *Bc-DCL1/2*–dsRNAs and –sRNAs, as well as *YFP*-dsRNAs and –sRNAs, were synthesized and processed, and 100 ng of RNAs was analysed on a native PAGE gel to check the quality. **b**, External application of *Bc-DCL1/2*–dsRNAs and –sRNAs (20 µl of 20 ng µl^{-1} synthetic RNAs) inhibits the virulence of *B. cinerea* on fruits (tomato, strawberry and grape), vegetables (lettuce and onion) and flower petals (rose) compared with the treatments using water, *YFP*-dsRNAs and –sRNAs. **c**, The relative lesion sizes and fungal biomass were measured at 3 dpi for lettuce, onion, rose and strawberry and at 5 dpi for tomato and grape fruits using ImageJ software and quantitative PCR, respectively. Error bars indicate the s.d. of te[illegible] biomass, respectively. Asterisks indicate statistically significant differences (

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HIGS

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 12 September 2011
doi: 10.3389/fmicb.2011.00185

Synthetic RNA silencing in bacteria – antimicrobial discovery and resistance breaking

Liam Good[1] *and James E. M. Stach*[2]*

[1] Department of Pathology and Infectious Diseases, Royal Veterinary College, University of London, London, UK
[2] School of Biology, Newcastle University, Newcastle upon Tyne, UK

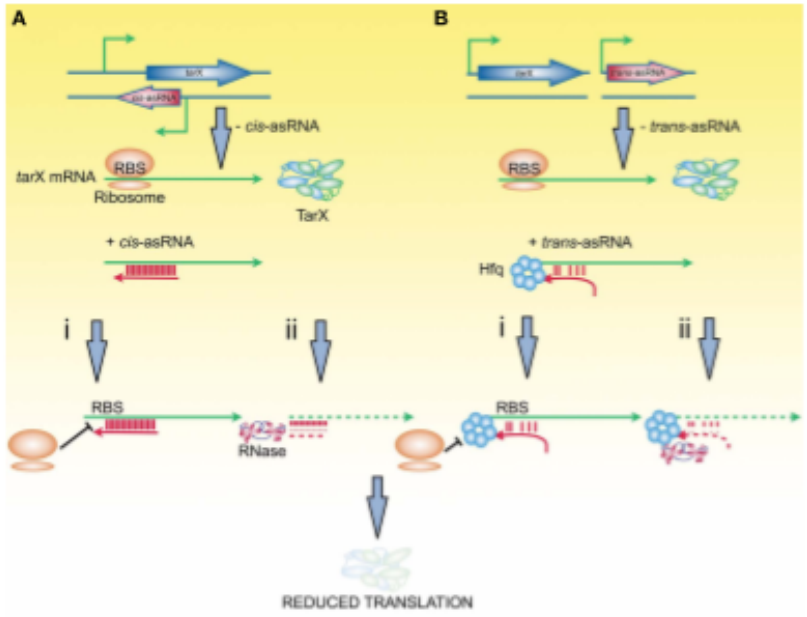
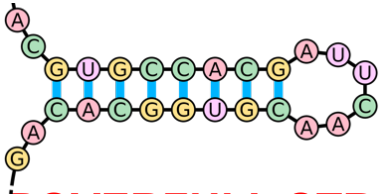


FIGURE 1 | Natural RNA silencing in bacteria. (A) Antisense RNAs (asRNAs) that are *cis*-encoded share high degrees of complementarity with the target mRNA. **(B)** asRNAs that are *trans*-encoded have limited complementarity with the target mRNA and, in some species, require the an RNA chaperone (Hfq) to facilitate binding. In either case, once the asRNA is bound to the target mRNA, translation of the target gene (*tarX*) is silenced by inhibition of ribosome binding to the target mRNA (i); induced RNase degradation of the asRNA:mRNA hybrid (ii) or a combination of both processes.



A POVERFULL STRATEGY TO SOLVE IMPORTAN EMERGENCY IN EU, eg. *Xilella fastidiosa* AFFECTING OLIVE PRODUCTION

Acting on bacteria

Or acting on the vector *Philaenus spumarius*

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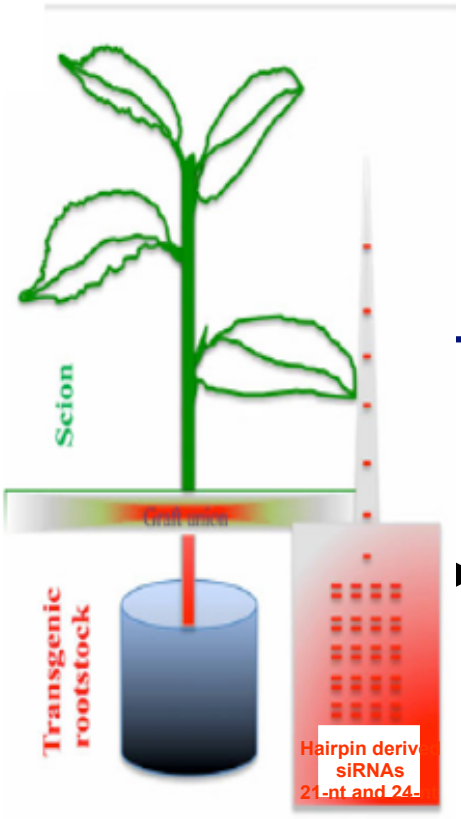
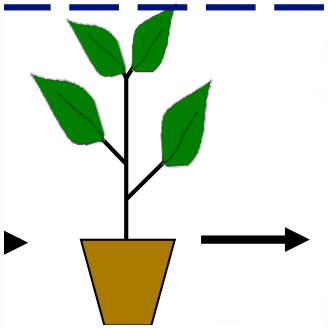
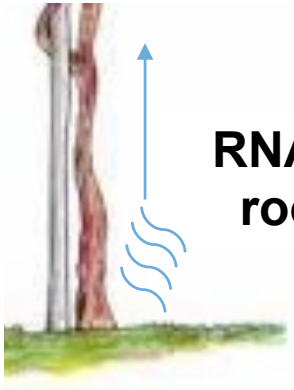
RNAi-based Rootstock grafted on a wild type scion



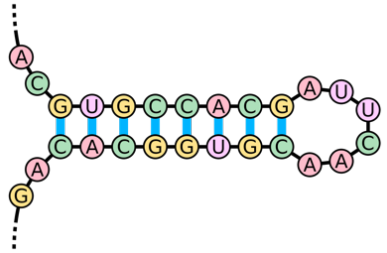


RNAi-based plant



RNAi-based rootstock

Non-Transgenic scion



Non-Transgenic resistant fruits

Zhao & Song (2014). Plant biotechnology journal

Advantages in the use of a silenced rootstock:

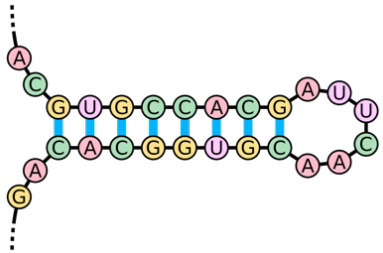
- The scion maintains its genetic inheritance.
- There is not gene flow because pollen and seed are not genetically modified.

PUBBLIC ACCEPTANCE

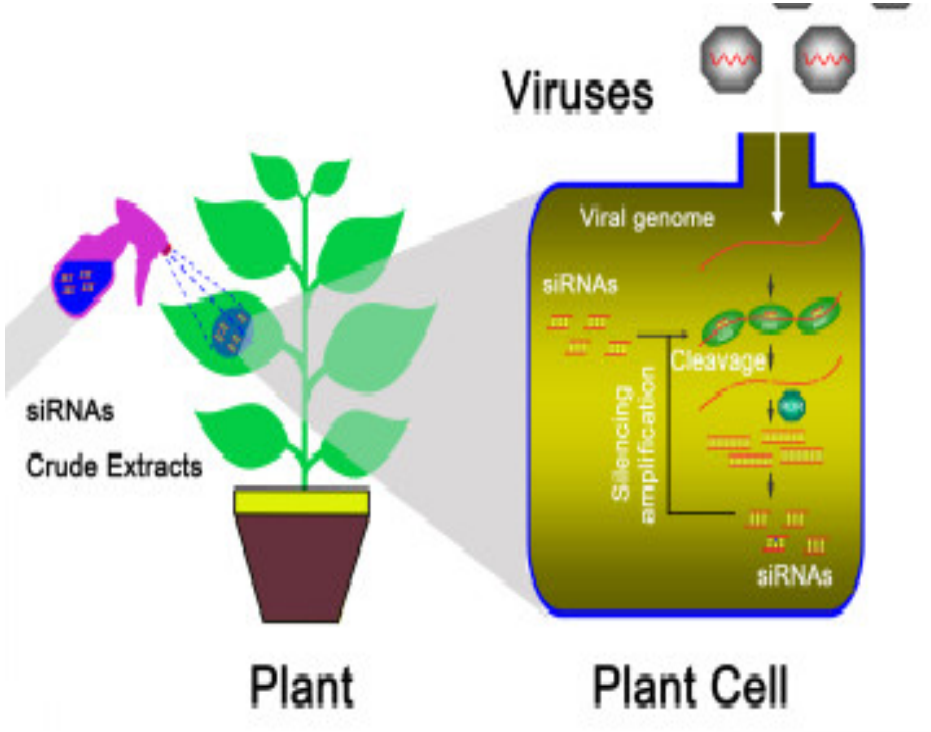
LOW ENVIRONMENTAL RISK

SIGS

Exogenous induction



3) Application of dsRNA on plant surface by spray.



Insect
Fungi
Bacteria
…
Contol fruit ripening

C. Duan, C. Wang and H. Guo (2012) Application of RNA silencing to plant disease resistance Silence 2012, 3:5.

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Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

To be or not to be….. GMO

RNAi applications in question

- Stable expression of dsRNA in plants — GMO **HIGS**
- Cis/Intragenic plants — **no / GMO**
- Intermediate GMOs — **no GMO**
- Grafting on GM-rootstocks — **no GMO**
- Genome editing — **Gene editing is GM, says European Court (25/07/2018)** — GMO
- **RNAi Spray – SIGS No GMO – sostituiranno gli attuali pesticidi**

TO SOLVE OPPOSITION AGAINST G

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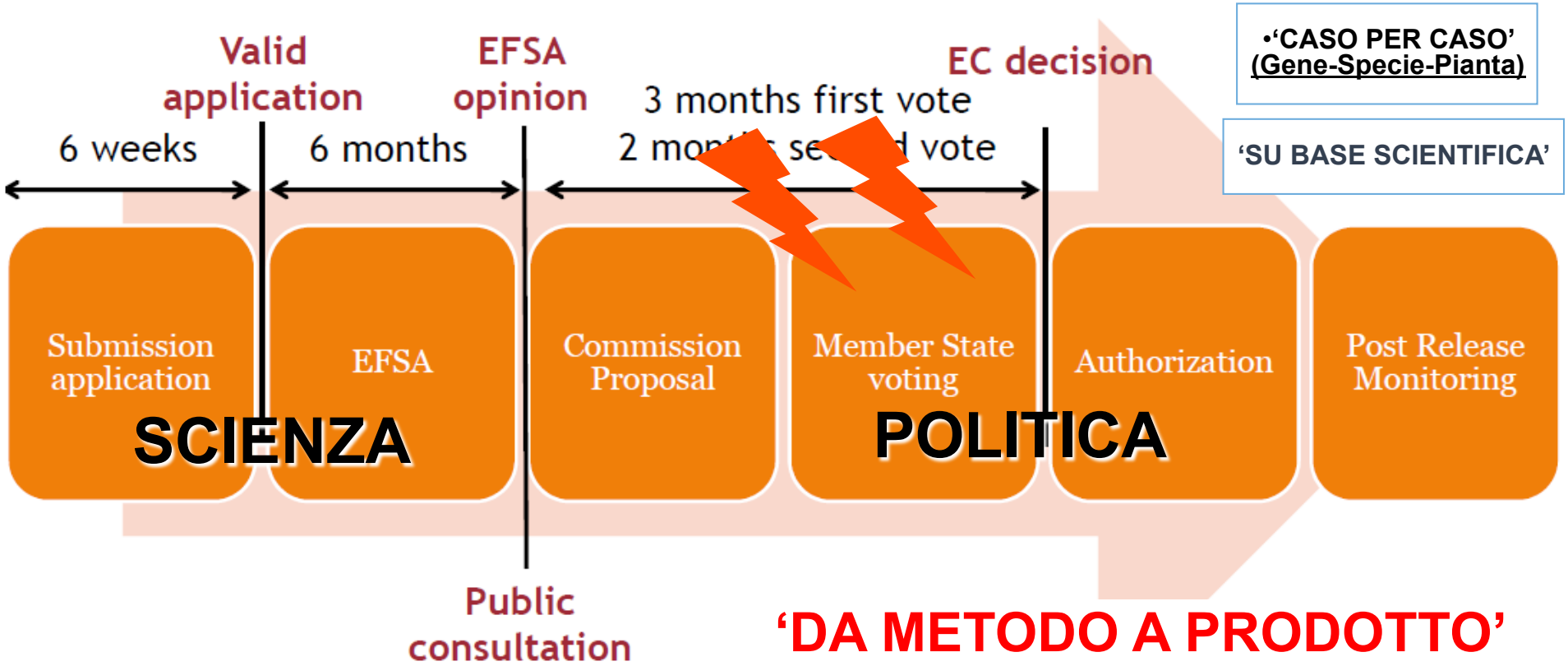
1) Come gestire l'incubo delle Regole EU sugli OGM.

Direttiva EU 1829/2003 Food and Feed



- •Rischio Salute Uomo
- •Rischio Ambiente
- •Rischio sistemi agricoli

- •'CASO PER CASO' (Gene-Specie-Pianta)

'SU BASE SCIENTIFICA'

'DA METODO A PRODOTTO'

'INCERTEZZA E RISCHIO D'IMPRESA' - 'GARANZIA PER IL CONSUMATORE '

QUALE STRATEGIA D'USO DELL'INNOVAZIONE PER LO SVILUPPO SOSTENIBILE?

FACTS:
Socio-economical impact of RNAi technology measured in number of pantent released in different areas and by companies.

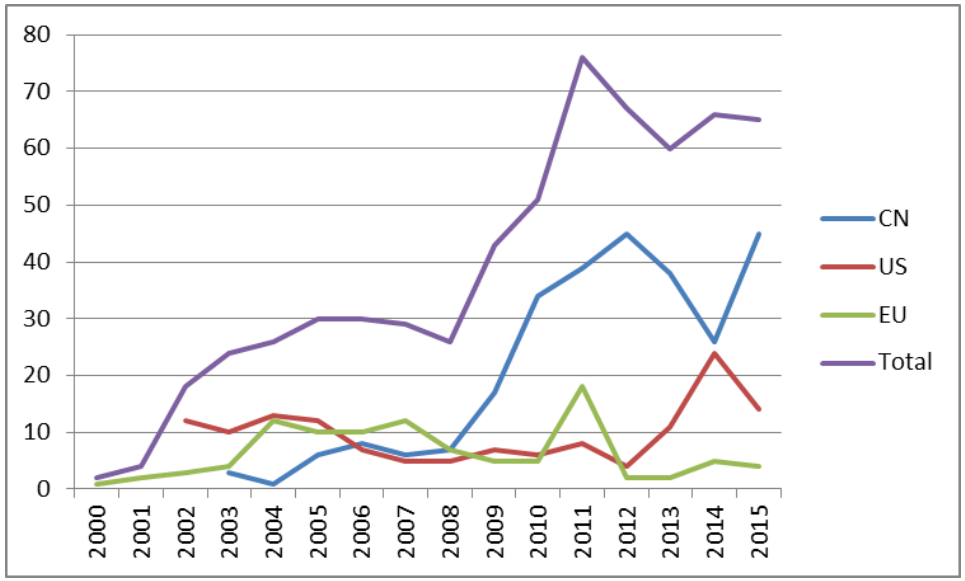


Figure 1. Time trend for plant-RNAi inventions
Source: own elaboration on Espacenet data

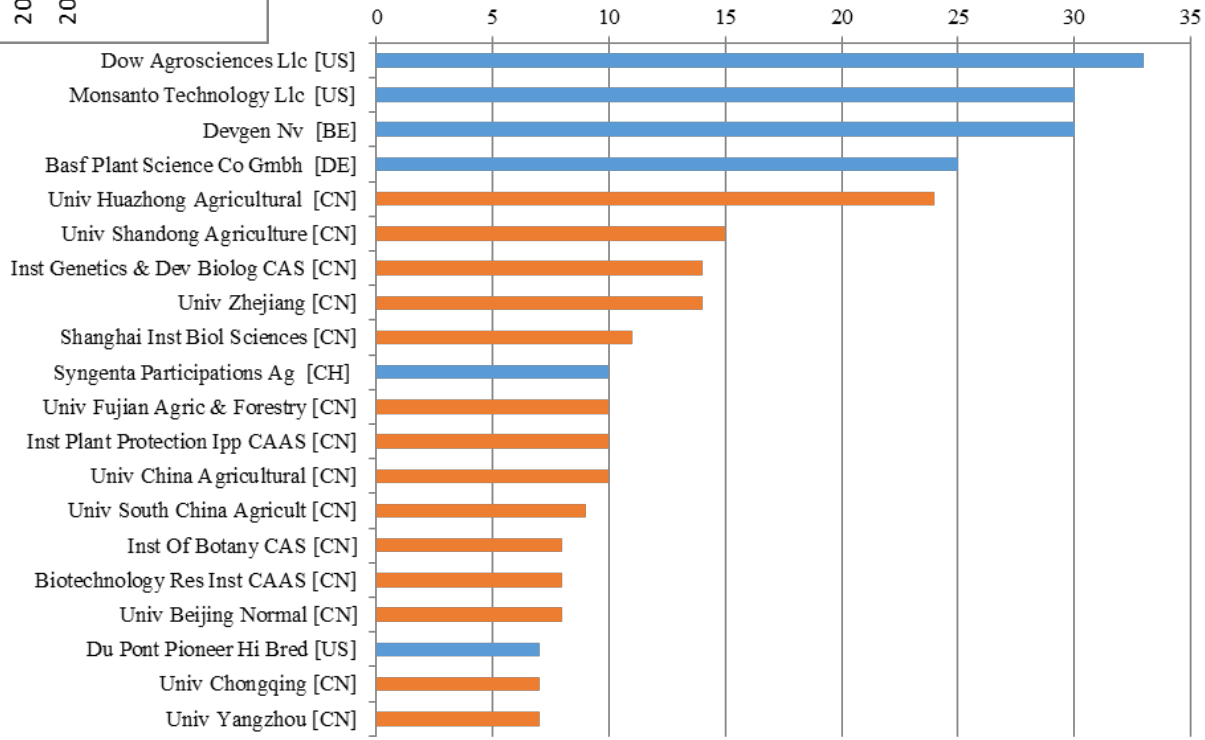


Figure 2. Plant RNAi patent_ Top Assignee
Note: In red colour Chinese assignee. **Source:** Elaborations on Espacenet data

D. Frisio and V. Ventura, 2018. RNAi in agriculture: a Chinese interference? In press

iPlanta

Quale strategia: affrontare i punti critici che hanno fermato le biotecnologie vegetali

4) Mostrare i prodotti. Negli ultimi 15 anni, a causa delle difficoltà create nei diversi paesi (blocco politico con sanzioni, assenza di finanziamenti e paura della distruzione da parte di anti-OGM) e dell'idea che tutto avrebbe potuto essere risolto dalle nuove regole sulla cisgenesi e sul genome editing, la ricerca sulle biotecnologie vegetali è stata quasi completamente bloccata e, in particolare, sono state realizzate pochissime sperimentazioni in serra e in campo.

5) Importanza della scienza interdisciplinare. La maggior parte dell'industria privata considera la scienza accademica un 'caos', non solo per la ricerca ma anche per la scarsa fiducia/conoscenza del processo di regolamentazione.

6) Replicare alle ONG. Il movimento anti-OGM è portato avanti dall'attività di molte ONG ed organizzazioni che operano a livello globale e che trovano sostegno a livello locale.

Senato della Repubblica
Sen. Prof. Elena Cattaneo

Alla c.a. del sig. Michele Serra
La Repubblica
Roma, 7 dicembre 2018

Gentile Sig. Serra,

la sua risposta su La Repubblica del 24 novembre mi fornisce l'occasione per approfondire alcuni aspetti che, per il contesto della sua iniziale segnalazione ("L'Amaca", La Repubblica, 21 novembre 2018) e comprensibili questioni di spazi del giornale, mi era stato impossibile affrontare nella mia lettera del 23 novembre.

Lo farò ora, dopo molti colloqui, letture e ore di studio che da tempo dedico a capire il "**prodotto biologico**". In particolare, desidero capire perché i consumatori lo trovino nei supermercati a costi elevati, cioè quali sarebbero le migliori qualità del prodotto che ne giustificano il maggior prezzo (fino al 110% in più e in alcuni casi anche oltre). Ma anche quali siano le garanzie di sicurezza sui prodotti biologici e le verifiche a monte e a valle della filiera, di quante risorse necessiti "il biologico" per essere realizzato (anche in termini di terra) e quanto se ne produca per unità di suolo. C'è anche da comprendere in modo più accurato "quanti gas serra emette", quali pesticidi usa e se e quanto questi sono dannosi per l'ecosistema. In sostanza, mi chiedo quali siano le proiezioni temporali e le conseguenze di "un mondo a biologico", con quale utilità e per chi. Per comprendere, mi informo. Lo faccio anche per confrontare questo processo agricolo con l'agricoltura