Arthropod Symbiosis and Symbiont-based Control Strategies in European Union

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Synopsis of the Presentation

Part A: EU Symbiosis Actions

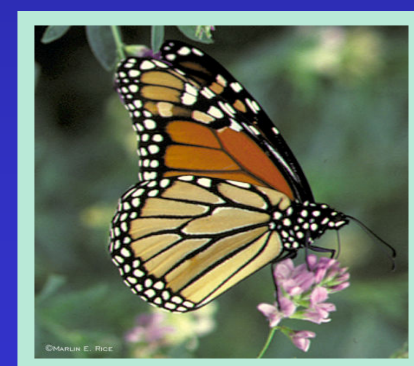
Part B1: Introduction to Wolbachia Biology

Part B2: Wolbachia-induced Cytoplasmic Incompatibility

Part B3: Ongoing studies and Conclusions

Part A: EU Symbiosis Actions

EU COST Action FA0701: Arthropod Symbioses: from fundamental studies to pest and disease management



Symbiosis

Anton de Bary, 1879:

“The living together of dissimilarly organisms”

Symbiotic bacteria in arthropods

- ❑ Supplement nutrition deficiencies
- ❑ Influence sex ratio
- ❑ Help cope with environmental factors

Genomics and Metagenomics

Symbionts:

- Buchnera, Blochmannia, Blattabacterium, Serratia symbiotica, Rickettsia species, Wolbachia, Wigglesworthia glossinidia, Sodalis glossinidius, …..

Arthropods:

- Many species including mosquito species, aphid, tsetse fly, Rhodnius bug, ….

Metagenomes:

- Bemisia tabaci, Bactrocera oleae, ….

Symbiont-based Control Strategies (SCS)

- Use symbionts for pest and disease management

- Ongoing efforts:
  - Pierce disease
  - Fiji disease virus
  - Chagas disease
  - Trypanosomiasis
  - Phytoplasmas
  - Malaria, Dengue virus, Lymphatic filariasis
  - Aphids, Whiteflies, Leafhoppers, Tephritids
  - …….

Active Projects

- ❑ Many international collaborations
- ❑ >150 funded projects at national level

Study both basic and applied aspects of Arthropod Symbioses

Reasons for the action - COST is required to coordinate research of EU scientists

Crossing research boundaries: to date, research on Arthropod Symbioses and SCSs has been fragmented and there is a need to bring together people working on various systems

Combining forces: to develop the tools required for implementing SCS and/or to characterize and exploit arthropod symbiosis and metagenomes demands experts from many fields. COST will improve the likelihood of these advances being made and carried into biotechnology

Providing cross-border training: Short term visits, personnel exchange, training and dissemination activities will improve scientific contact and collaboration between laboratories and countries

Main objectives

Overall: Develop arthropod-symbiosis into commercially available products to benefit society

- ✓ Promote data collection on arthropod symbiosis
- ✓ Support arthropod pest control programs on major pests and disease vectors
- ✓ Develop alternative control methods against pests and disease vectors
- ✓ Explore metagenomes of arthropods to identify novel products
- ✓ Promote public discussion on the legal and regulatory issues associated with SCS implementation

Secondary objectives

- Provide information on the diversity of symbiotic bacteria
- Provide information on host-symbiont interactions
- Develop and deliver research tools
- Provide resources for the scientific community
- Enhance public communication

Scientific program

Five specific working groups (WGs):

WG1 - Arthropod symbiont diversity

WG2 – Arthropod-symbiont metagenomes

WG3 - Host-symbiont interactions

WG4 - Symbiont-based control strategies

WG5 - Ethical, regulatory & commercial aspects of SCS

Each WG has a well-defined focus and 2-5 tasks

Organization and Dissemination Plan

- MC (chair, vice chair, WG co-ordinators)
- Regular meetings
- Workshops & Conferences
- STSMs
- Joint activities (IOBC, IBMA, COST862)
- Publications (scientific, technical guidelines, leaflets, book)
- Teaching activities
- Reports
- Website

Workshops

- “Assessment of arthropod symbiont diversity”
- “Symbiont dynamics: understanding symbiont population biology and its applications”
- “Joint meeting with COST 862”
- “Symbiont-based control strategies and commercialization”
- “Arthropod metagenomics and applications”
- “Pathogen-symbiont interactions in arthropod vectors”
- “Safety, registration and public perception”

Benefits

- ❖ Contribute to the discovery of novel gene products with potential for commercial exploitation
- ❖ Facilitate technology transfer from academia to the private sector
- ❖ Improve the competitiveness of European biological control SMEs
- ❖ Facilitate international contact between laboratories
- ❖ Provide a framework for young scientists to get trained in specialized area and connect with the private sector and academia
- ❖ Support and encourage scientific collaboration between laboratories, countries and companies

Target groups

- Academia - results on the arthropod symbiont diversity, microbial genomes, arthropod metagenomes, host-symbiont interactions and novel technologies for biological control will advance the research activities in diverse fields of life sciences

- Industry - Biological control (SCS for controlling arthropod pests and disease vectors); Pharmaceutical (novel products identified by [meta]genomics)

- The public - increased awareness about risks and benefits of SCS as well as environmentally-friendly control strategies of arthropod pests and diseases; reduced use of pesticides

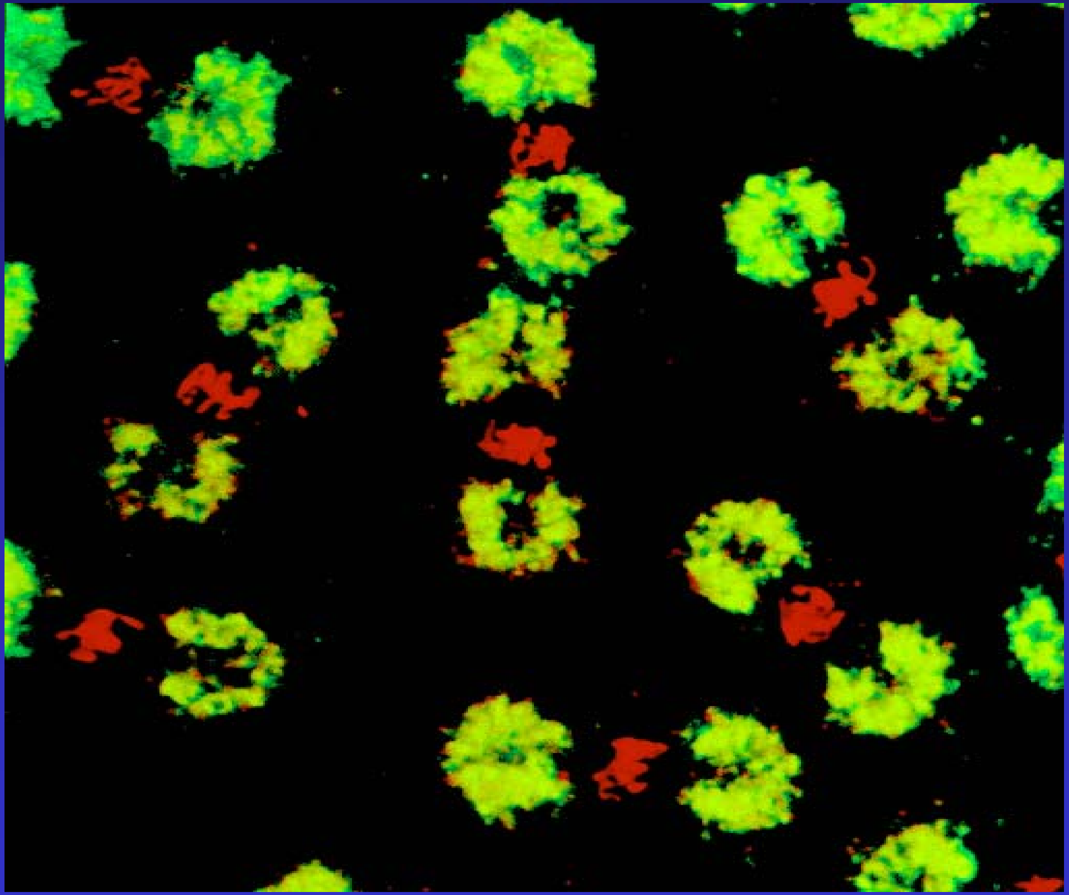
Participants

- Austria
- Czech Republic
- Denmark
- France
- Germany
- Greece
- Hungary
- Ireland
- Italy
- Israel
- Norway
- Portugal
- Slovenia
- Spain
- Sweden
- Switzerland
- The Netherlands
- United Kingdom

- **100 experts and 4 SMEs**
- **18 EU and 5 non-EU countries**

Part B1: Introduction to Wolbachia Biology

Wolbachia interacts with host microtubules



[Bourtzis' Lab]

Host Distribution of Wolbachia Strains

Arthropods

- Insects
- Mites
- Isopods
- Spiders
- Springtails

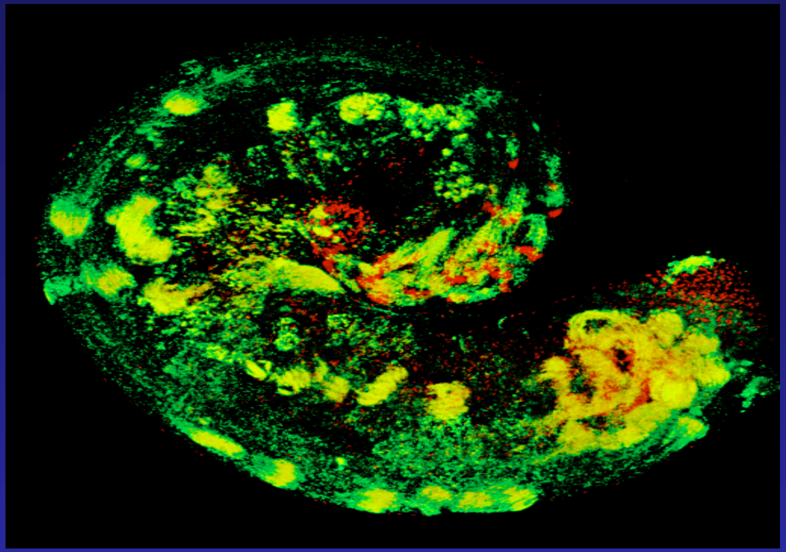
Nematodes

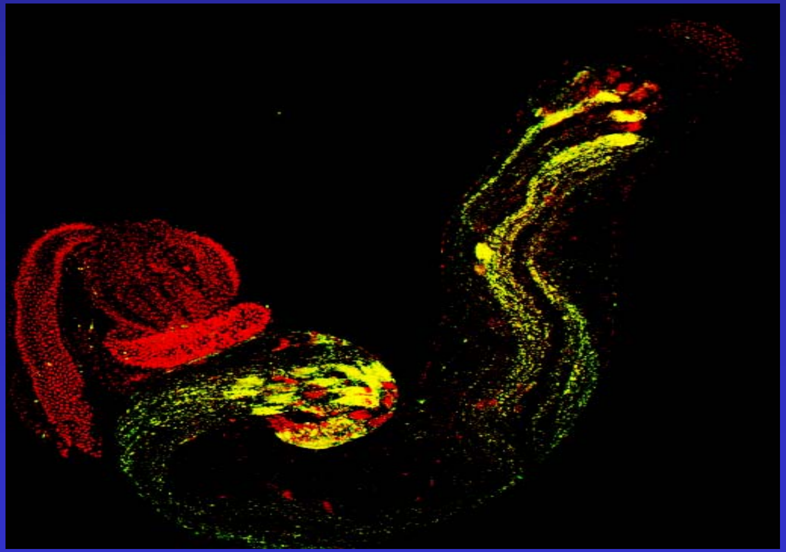
- Filarial worms

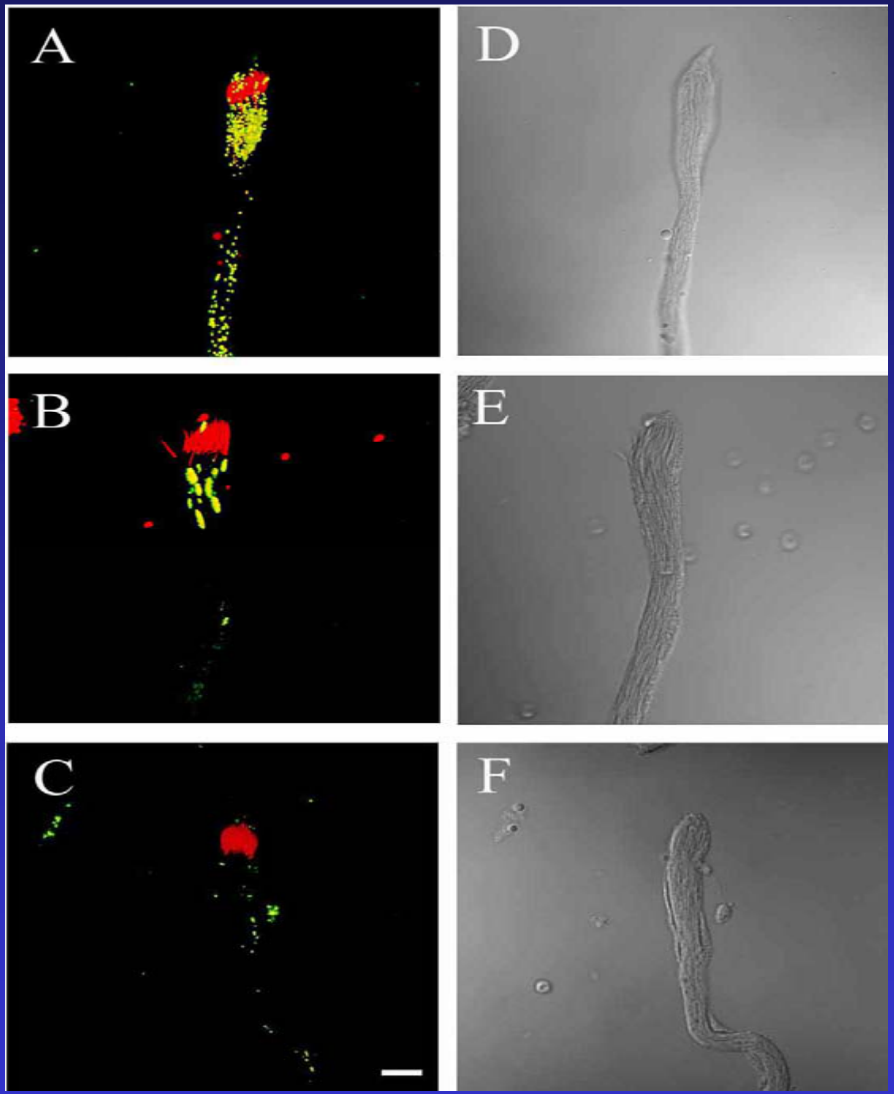
Not infected:

- many species of agricultural importance (e.g. *Bactrocera oleae*)
- many species of medical importance (e.g. *Anopheles gambiae*)
- many species of environmental importance (e.g. *Dendroctonus* spp.)

Wolbachia during *Drosophila* spermatogenesis







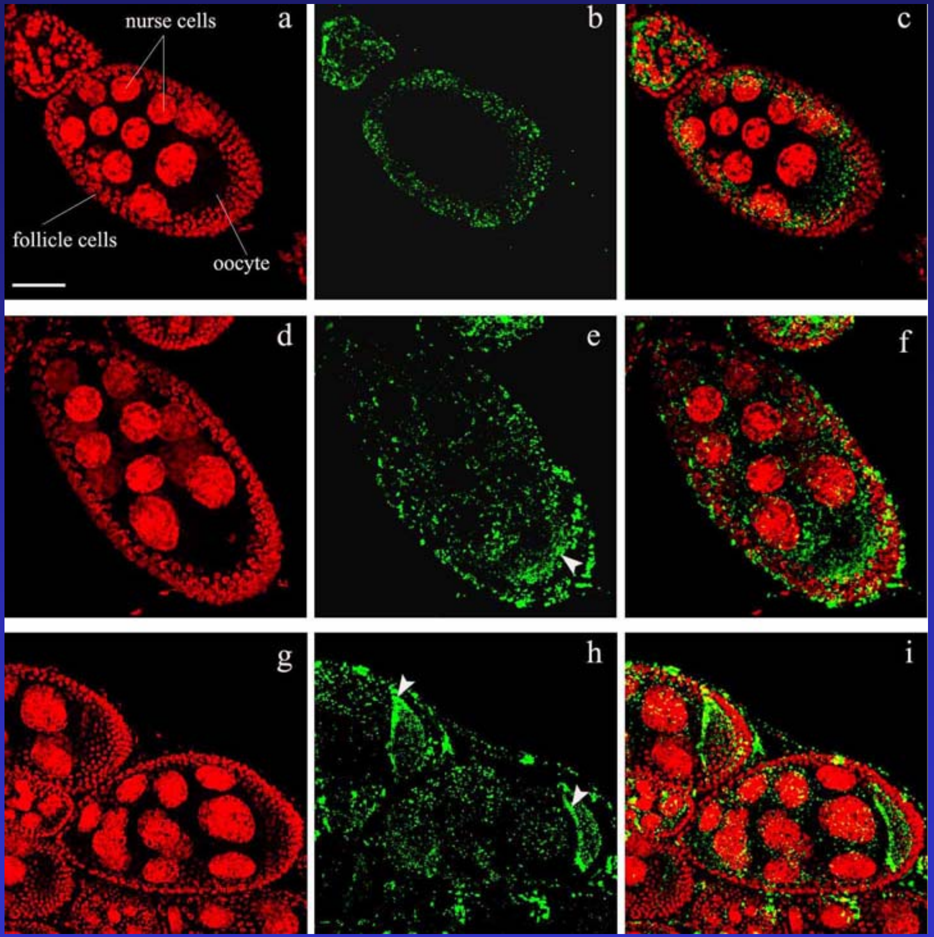
[Veneti et al. (2003), Genetics 164: 545-552; Clark et al. (2002), Mech. Devel. 111: 3-15; Clark et al. (2003), Mech. Devel. 120: 85-98]

Wolbachia during *Drosophila* oogenesis

D. simulans (*w*Ri)

D. melanogaster (*w*Mel)

D. simulans (*w*No)



oskar-like mRNA pattern

bicoid-like mRNA pattern

[Veneti et al. (2004) Appl. Env. Microbiol. 70: 5366-5372]

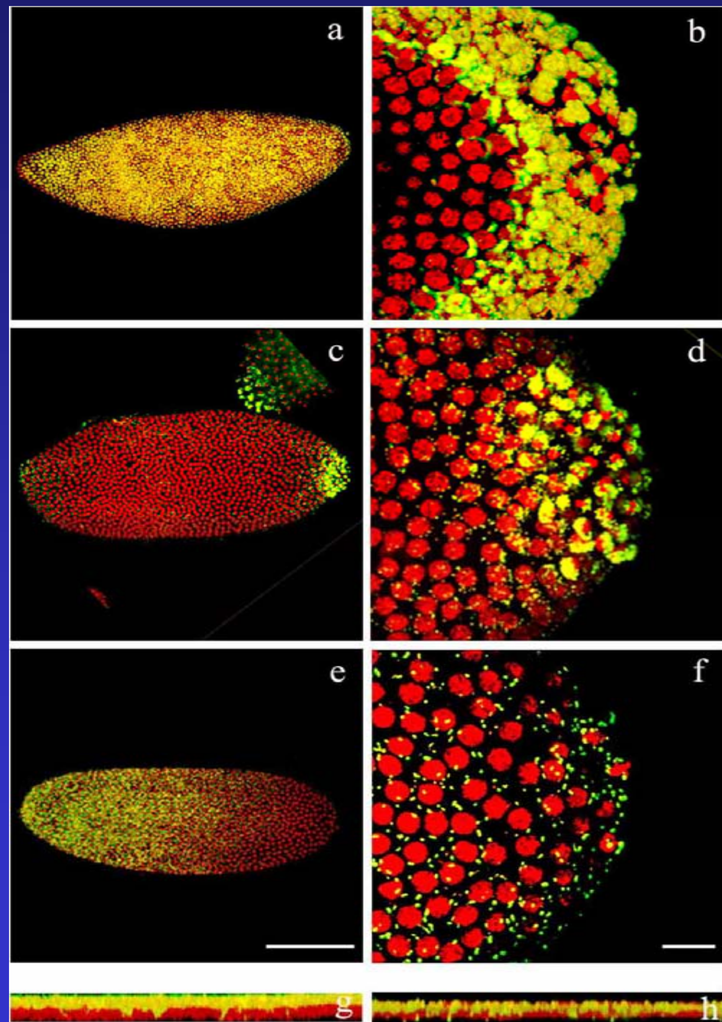
Wolbachia during *Drosophila* embryogenesis

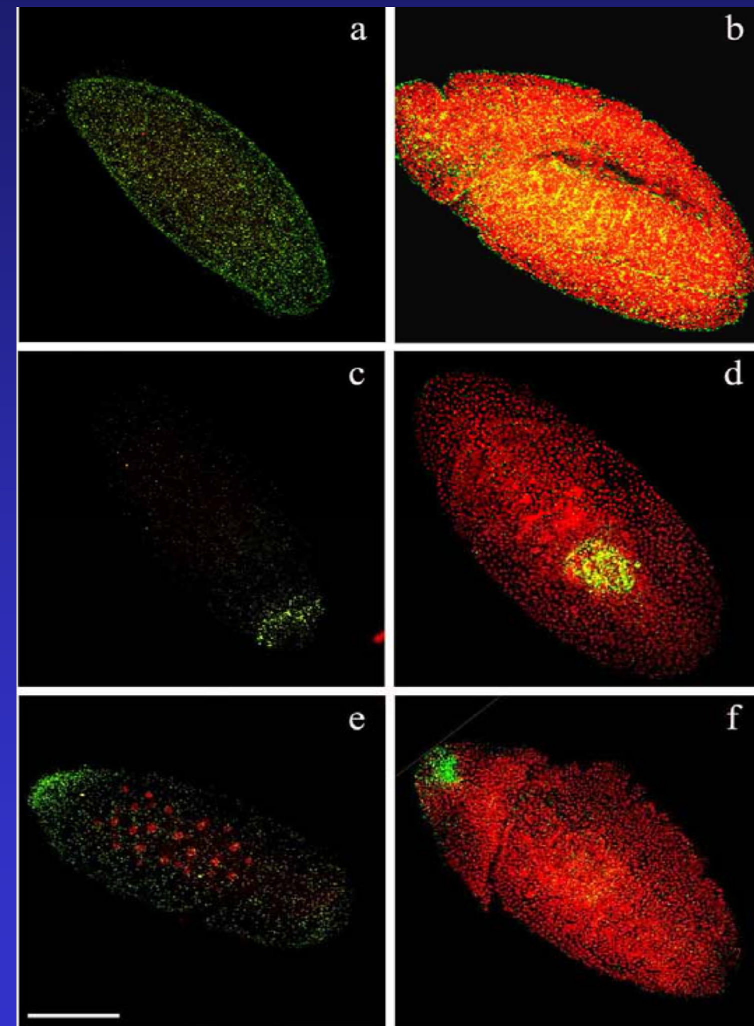
Embryo Pole cells

D. simulans (*w*Ri)

D. teissieri (*w*Tei)

D. simulans (*w*Ma)





[Veneti et al. (2004) Appl. Env. Microbiol. 70: 5366-5372]

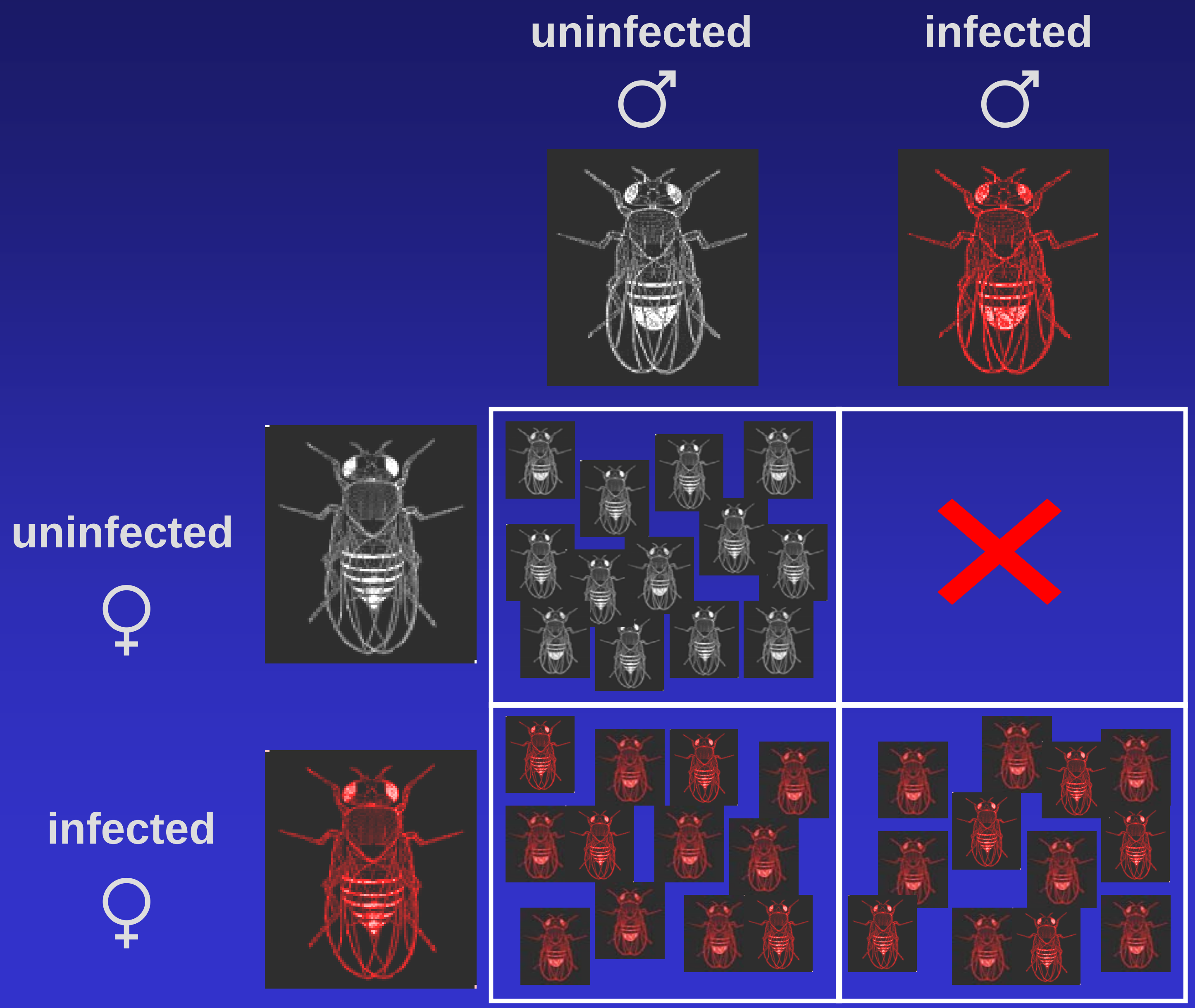
Wolbachia-Induced Reproductive Abnormalities

Wolbachia induce a number of reproductive alterations, such as:

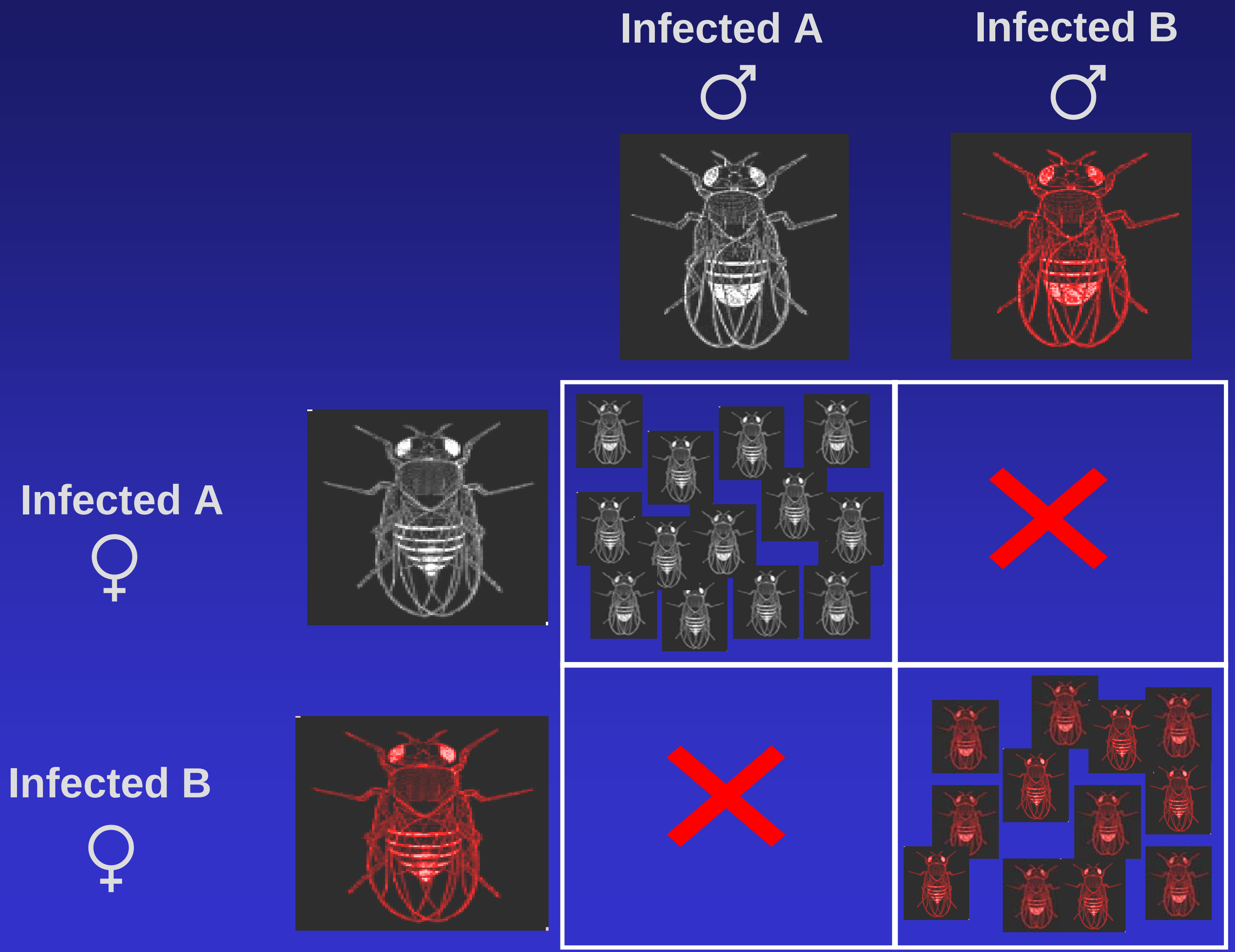
- Feminization
- Parthenogenesis
- Male-killing
- Cytoplasmic Incompatibility

- Spreading
- Curing - Antibiotics

Uni-Directional CI



Bi-Directional CI



The mechanism of cytoplasmic incompatibility

- Unknown molecular mechanism
- Modification – Rescue model
- Modification (imprinting) during spermatogenesis
- Rescue during fertilization/early embryogenesis

[Werren (1997) Annu. Rev. Entomol. 42: 587-609; Bourtzis et al. (1998), Nature 391: 852-853; Tram and Sullivan (2002), Science 296: 1124-1126]

Major Goals of Bourtzis' Lab

- To dissect host-*Wolbachia* symbiosis towards the elucidation of the mechanism of Cytoplasmic Incompatibility (CI)

- To use the mechanism of CI for applied purposes

Part B2: Wolbachia-induced CI

Wolbachia and Applied Biology

For example:

1. Asexuality

2. As an expression vector

3. As a tool for the modification of population age structure

4. As a spreading mechanism

5. As a tool for population suppression of insect pests

Wolbachia-induced Cytoplasmic Incompatibility as a tool to suppress med fly populations

Wolbachia transfer to the med fly *Ceratitis capitata*

- The entire work was done at the IMBB, Crete.

- Recipient: Benakeion strain

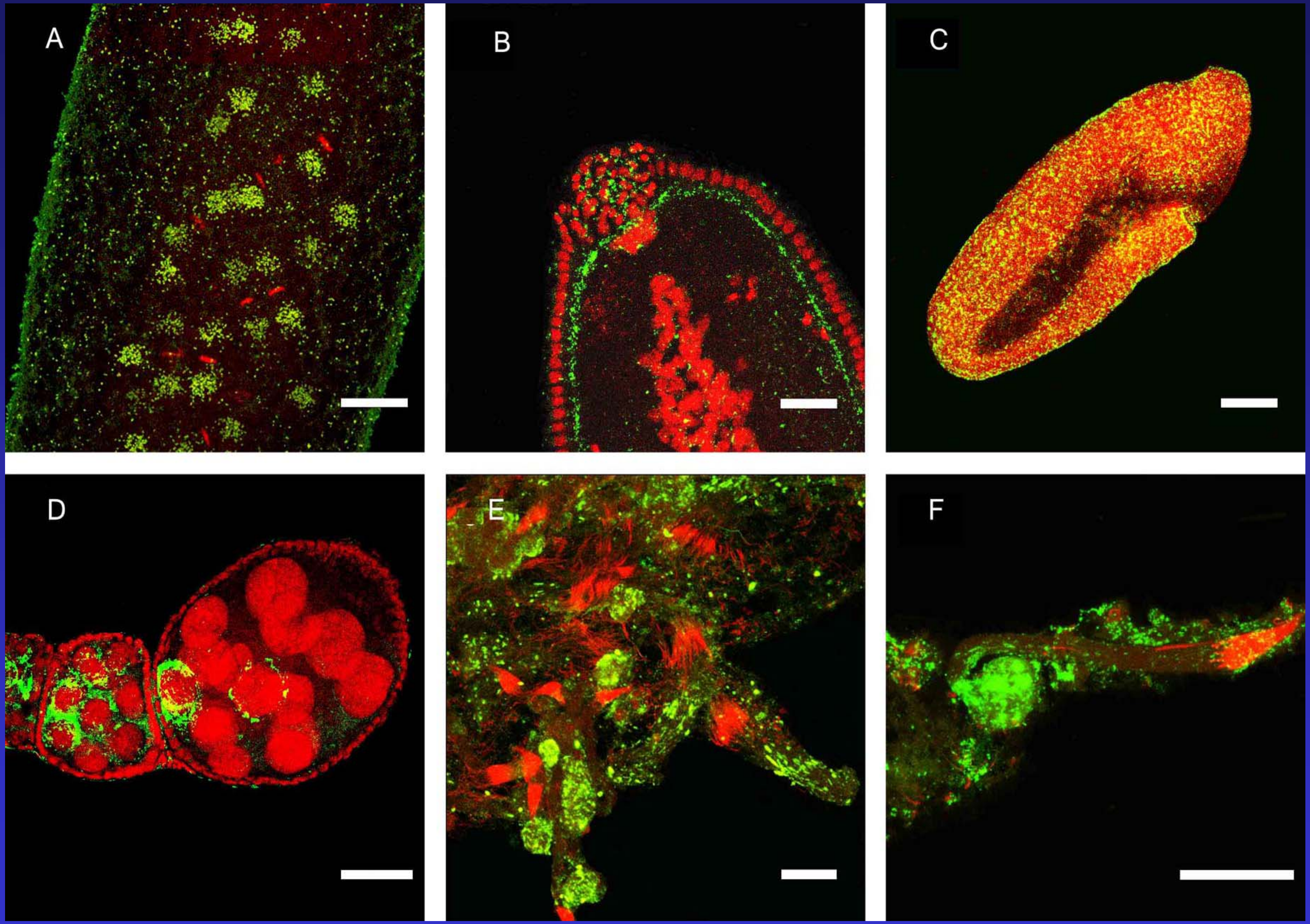
- Donor: *Rhagoletis cerasi*
  - Austria (*w*Cer1 + *w*Cer2)
  - Sicily (*w*Cer1 + *w*Cer3 + *w*Cer4)
  - collaborators: M. Riegler and C. Stauffer

- Two stably transinfected lines: 88.6 (*w*Cer2) and S10.3 (*w*Cer4).

- 100 % infection rates (for over 6 years now, 76 generations).

[Zabalou et al. (2004), PNAS, 101: 15042-15045]

Wolbachia Distribution in Transinfected Medfly



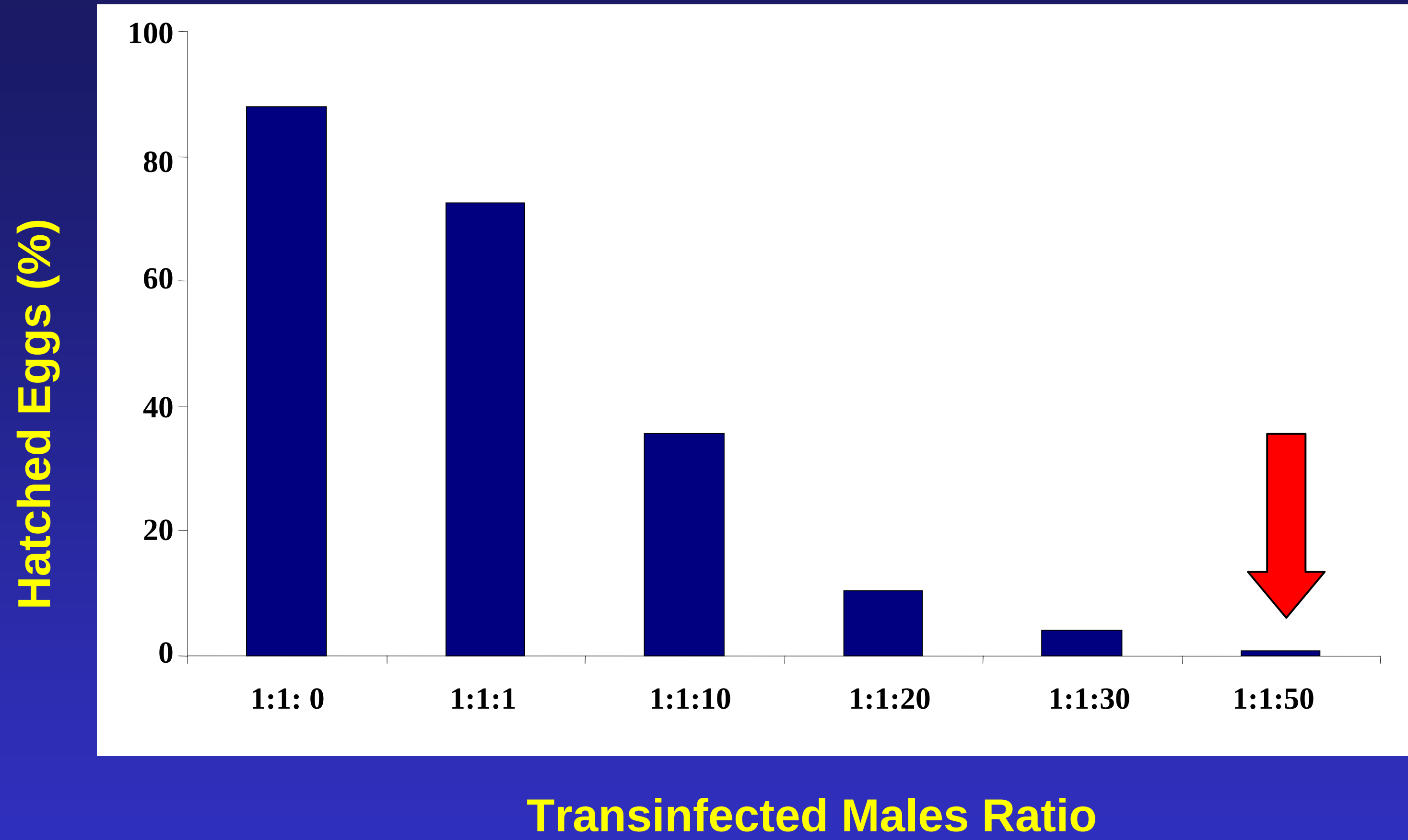
[Zabalou et al. (2004), PNAS, 101: 15042-15045]

CI assays with transinfected *Ceratitis capitata*

Cross (females x males)	Embryos scored	Embryonic mortality (%)
A)		
Uninfected Benakeion x WolMed 88.6 (*w*Cer2)	3000	100 ± 0
Uninfected Benakeion x WolMed S10.3 (*w*Cer4)	3000	100 ± 0
WolMed 88.6 (*w*Cer2) x Uninfected Benakeion	3000	16.73 ± 0.68
WolMed S10.3 (*w*Cer4) x Uninfected Benakeion	3000	32.03 ± 0.85
WolMed S10.3 (*w*Cer4) x WolMed 88.6 (*w*Cer2)	3000	100 ± 0
WolMed 88.6 (*w*Cer2) x WolMed S10.3 (*w*Cer4)	3000	100 ± 0
WolMed 88.6 (*w*Cer2) x WolMed 88.6 (*w*Cer2)	3000	64.77 ± 0.87
WolMed S10.3 (*w*Cer4) x WolMed S10.3 (*w*Cer4)	3000	67.25 ± 0.87
Uninfected Benakeion x Uninfected Benakeion	3000	12.17 ± 0.60
B)		
* WolMed S10.3 tet x WolMed S10.3 tet	1890	23.44 ± 0.97
** WolMed S10.3 tet x WolMed S10.3 tet	3000	11.80 ± 0.59
* WolMed 88.6 tet x WolMed 88.6 tet	2283	25.10 ± 0.91

[Zabalou et al. (2004), PNAS, 101: 15042-15045]

Suppression of medfly populations using *Wolbachia*-induced CI



Number of adults	300	300	300	306	290	520
Number of eggs scored	3000	3000	2097	1688	858	700

[Zabalou et al. (2004), PNAS, 101: 15042-15045]

Part B3: Conclusions

Incompatible Insect Technique (I.I.T.)

- Based on the mechanism of Wolbachia-induced CI
- Analogous to S.I.T.
- Effective sexing system is necessary
- Environmentally friendly technology
- Low technological input
- Low cost technology
- Higher competitiveness of released males
- Successful applications in the past (*C. pipiens* in India, a WHO project)

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