THIRD FAO/IAEA INTERNATIONAL CONFERENCE ON AREA-WIDE MANAGEMENT OF INSECT PESTS

Efficient Sex Separation in Aedes Mosquitoes Using Image Analysis and *Elimination of Females by Laser Beams*

Carlos Tur Lahiguera

GENERALITAT VALENCIANA
CONSELLERIA DE AGRICULTURA, MEDIO AMBIENTE, CAMBIO CLIMÁTICO Y DESARROLLO RURAL

Tragsa Grupo

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INTRODUCTION

Background

AW- IPM PROGRAM AGAINST MEDFLY IN VALENCIA REGION

Sterile Insect Technique



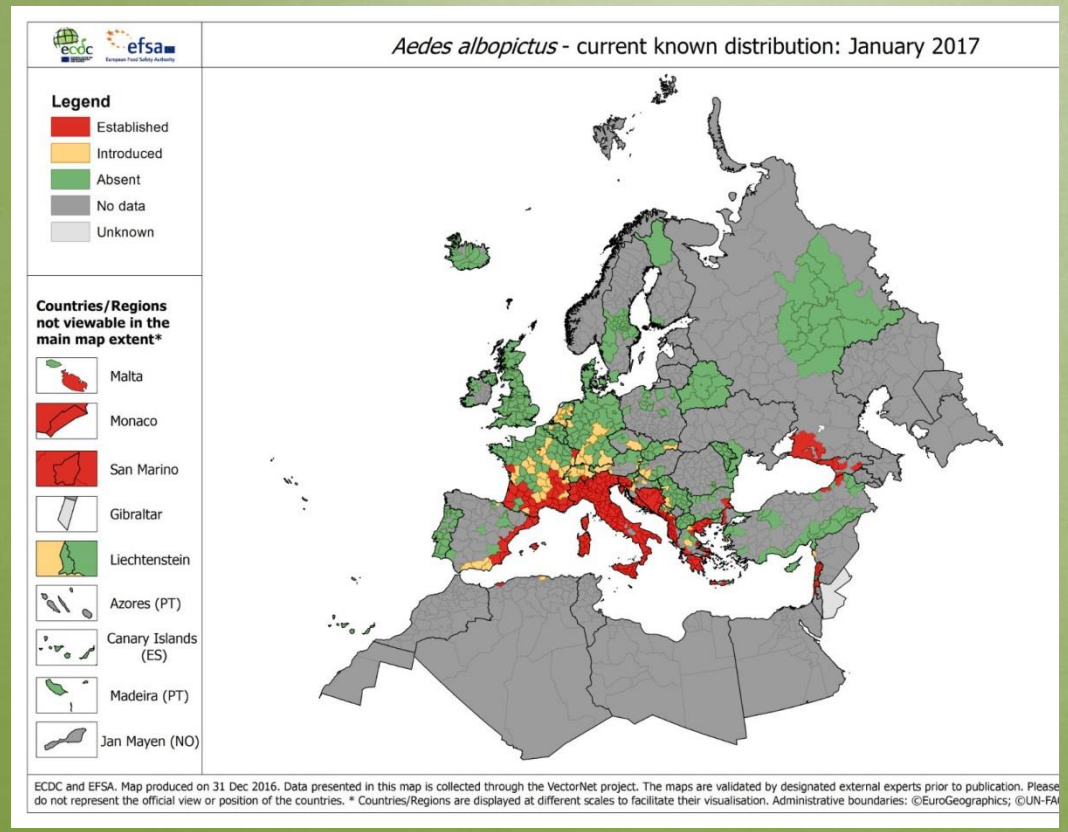







Vectors involved in transmission in Spain: *Aedes albopictus*

Current Distribution in Europe: January 2.017
(European Centre for Disease Prevention and control)



MAIN OBJECTIVE

Aedes albopictus SIT for suppression

ONGOING ACTIVITIES

- **Preparatory activities for an SIT pilot project in Valencian region**
- Development of equipment and procedures for *Aedes albopictus* mass rearing

SIT PILOT PROJECT IN VALENCIAN REGION 2.018

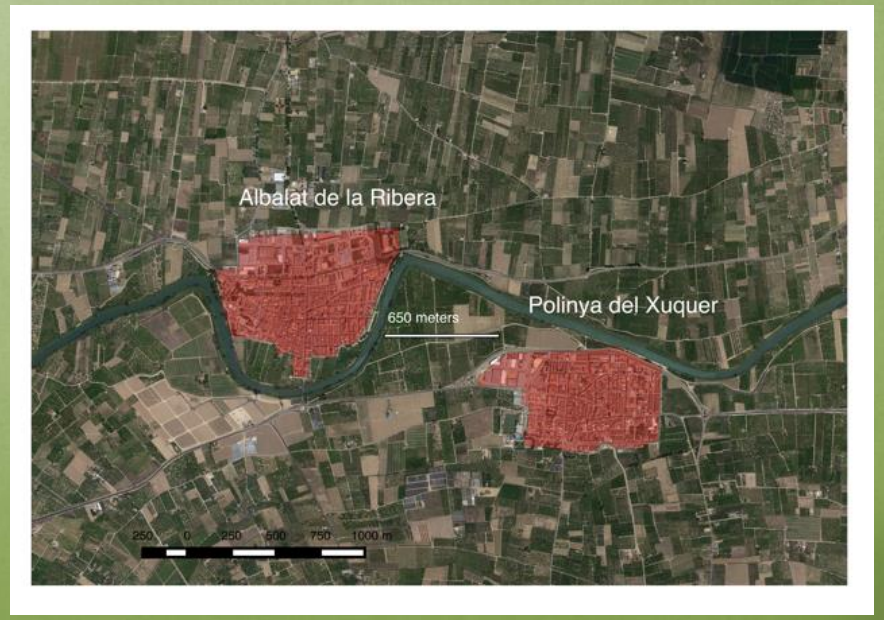
Municipalities:

Polinya del Xuquer

Surface: 44 Ha
Population: 2.546 Inhabitants

Albalat de la Ribera

Surface: 51 Ha
Population: 3.485 Inhabitants



DEVELOPMENT OF EQUIPMENT FOR *AEDES ALBOPICTUS* MASS REARING

Eggs

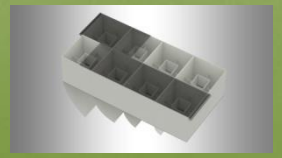
Adapted mass rearing cages based on the IAEA previous design

Pupae

Sex sorter prototype

Coordinated Research Project (CRP) "Exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes."

Adults



Only male adult cages & Release containers

Coordinated Research Project (CRP) "Mosquito Handling, Transport, Release and Male Trapping Methods"

AEDES SEX SEPARATION

Main bottleneck for *Aedes* SIT

- There is lack of a system that could efficiently separate mosquitoes by sex since the females must be eliminated given that they are the vehicles for transmitting diseases to human through their bites.

SEX SORTER PROTOTYPE

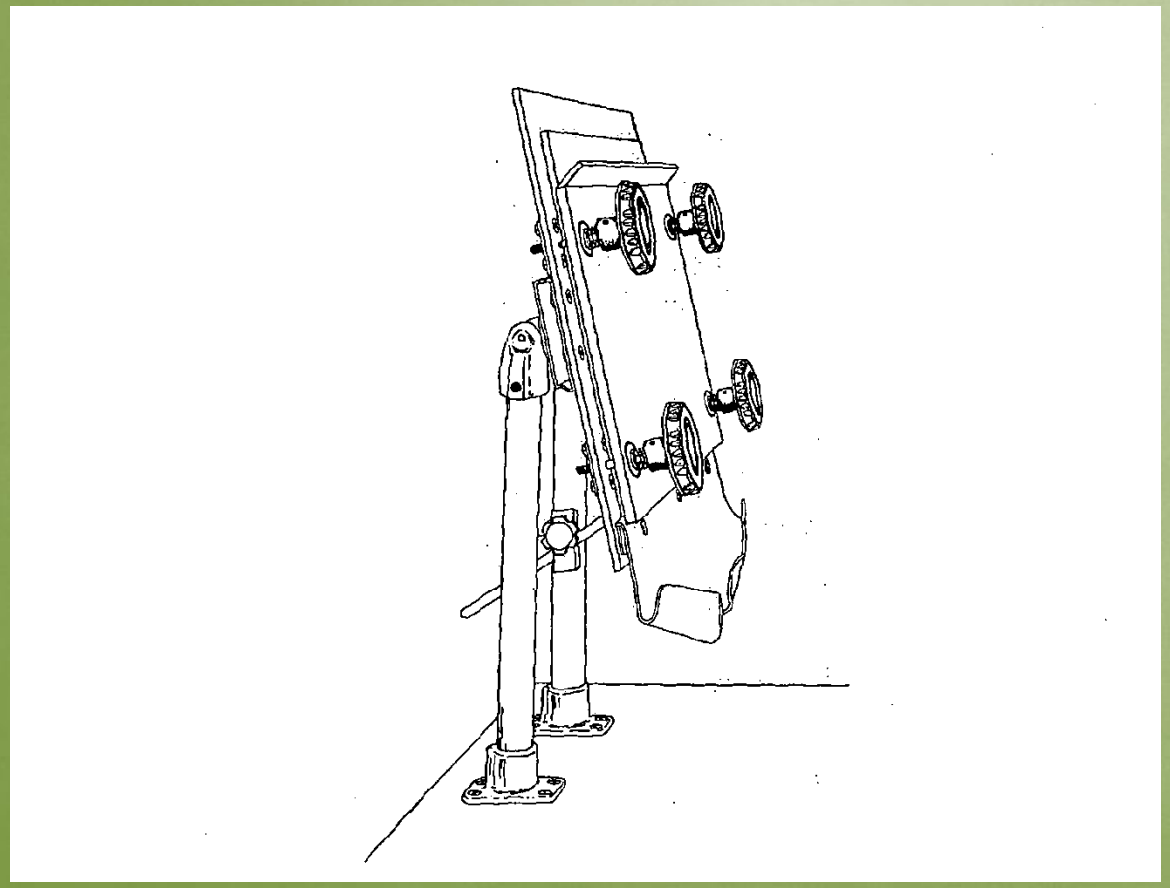
Coordinated Research Project (CRP)

- "Exploring genetic, molecular, mechanical and behavioral methods of sex separation in mosquitoes."

MAIN OBJECTIVE

- "DEVELOPMENT OF A SYSTEM FOR OBTAINING ONLY MALES WITH A SEX PURITY SCALABLE FOR AN SIT OPERATIONAL PROGRAM BASED ON SEXUAL DIMORPHISM"

CURRENT SEX SEPARATION METHODS BASED ON SIZE

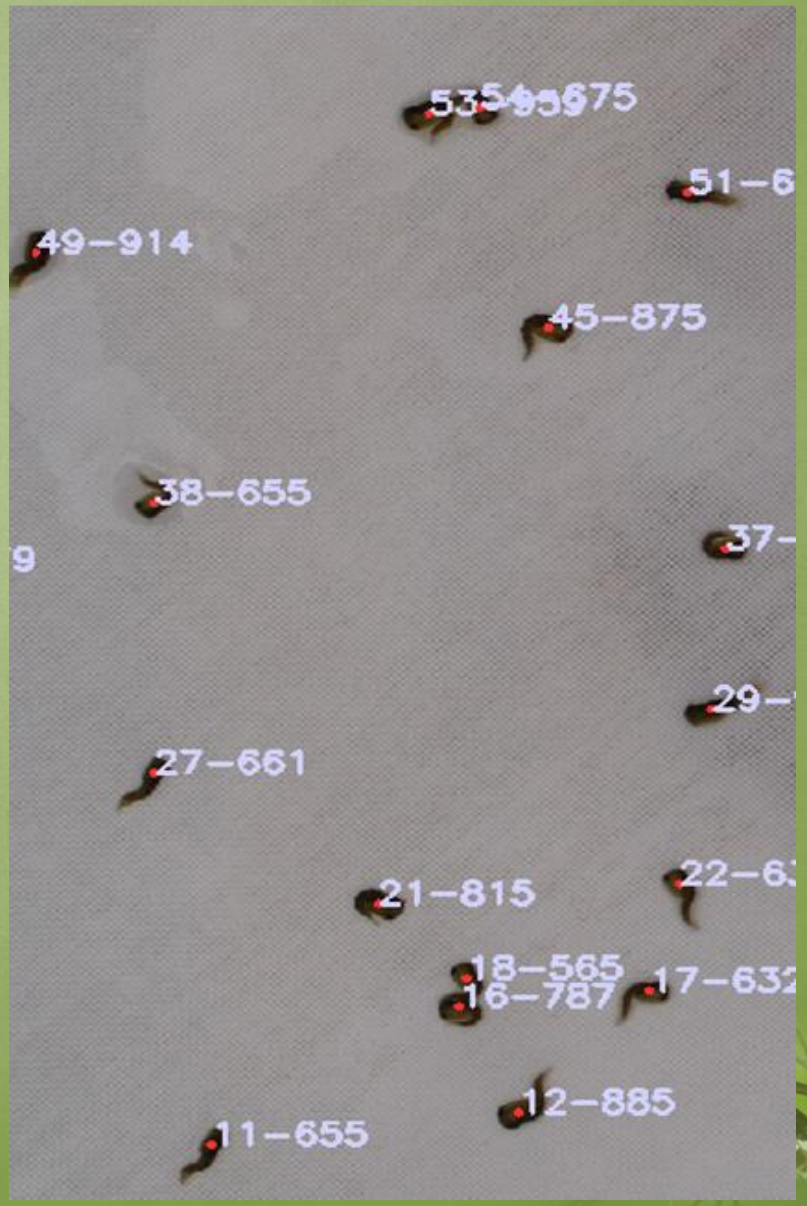


Adjustable glass plates

Sieving

PRINCIPLE

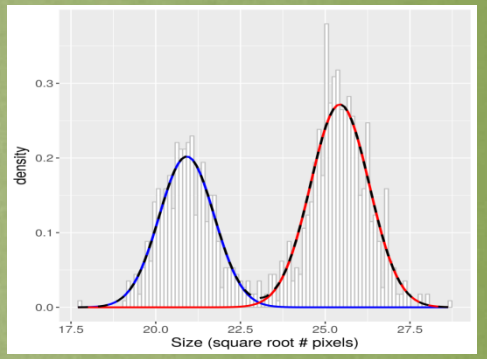
- Image analysis becomes a powerful tool to differentiate males from females based on size.



DEVELOPMENT PROCEDURES

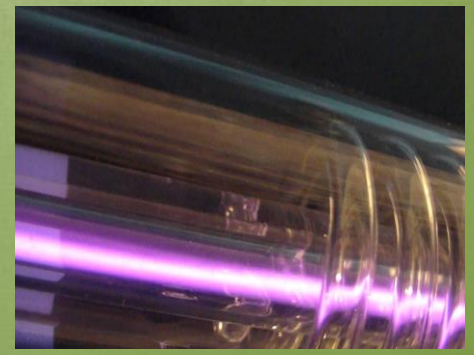
FIRST OBJECTIVE:

CALCULATE THE **THRESHOLD SIZE** TO OBTAIN THE MAXIMUM NUMBER OF MALES WITH A SEX PURITY SCALABLE FOR AN SIT OPERATIONAL PROGRAM"



SECOND OBJECTIVE:

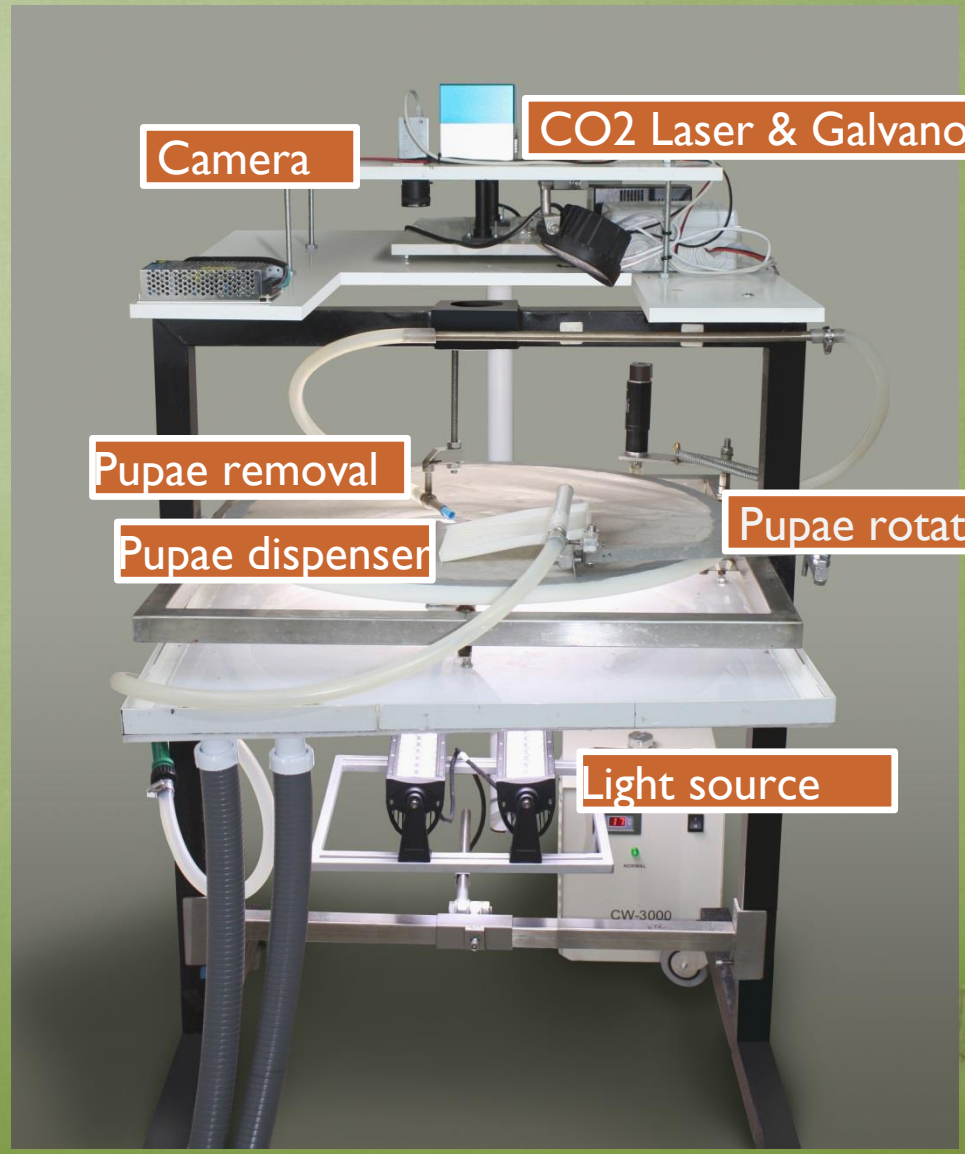
Aedes albopictus **FEMALE REMOVAL** USING A COMPUTER CONTROLLED LASER BEAM



SEX SORTER PROTOTYPE

MAIN COMPONENTS

1. Pupae dispenser
2. Pupae rotating disc
3. Image acquisition (camera and light source)
4. Image analysis
5. Galvanometer and CO2 laser beam
6. Pupae removal



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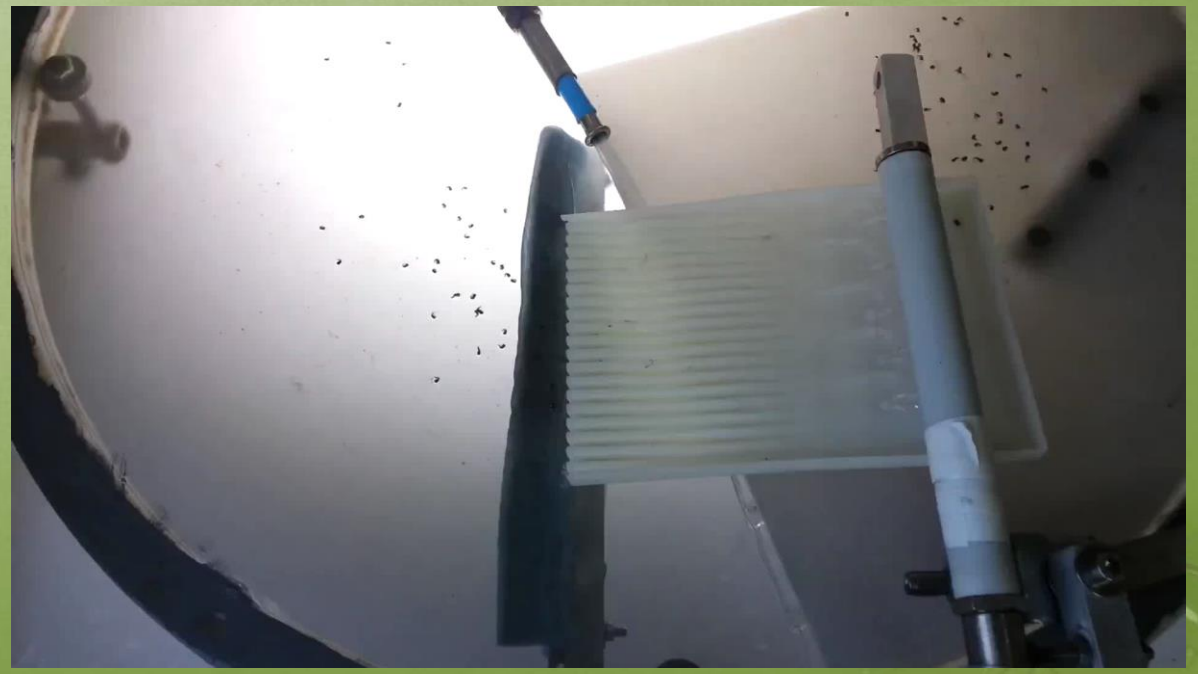
Pupae placement in a plate in dry conditions



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The plate turns and pupae pass through the image analysis area

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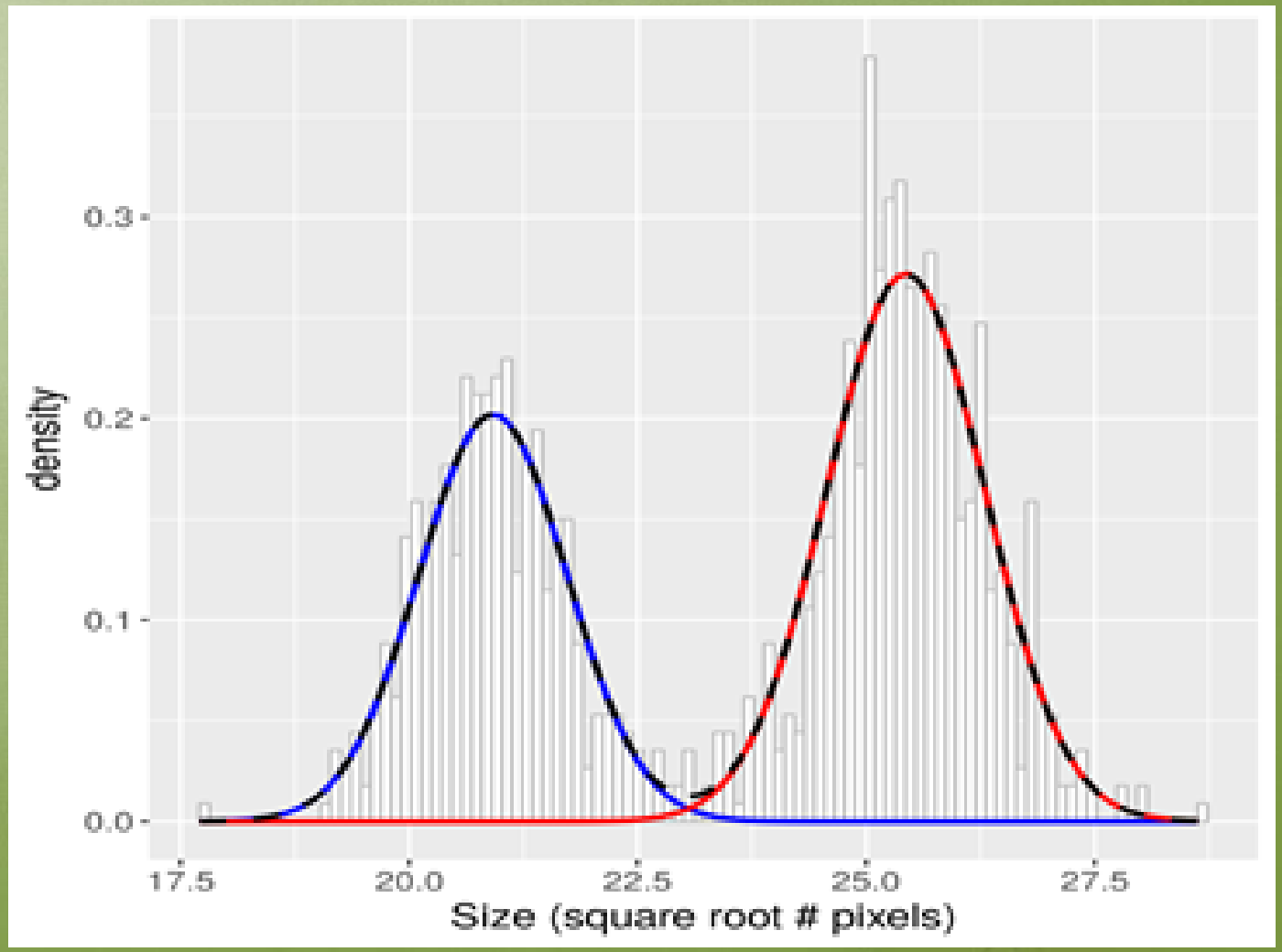
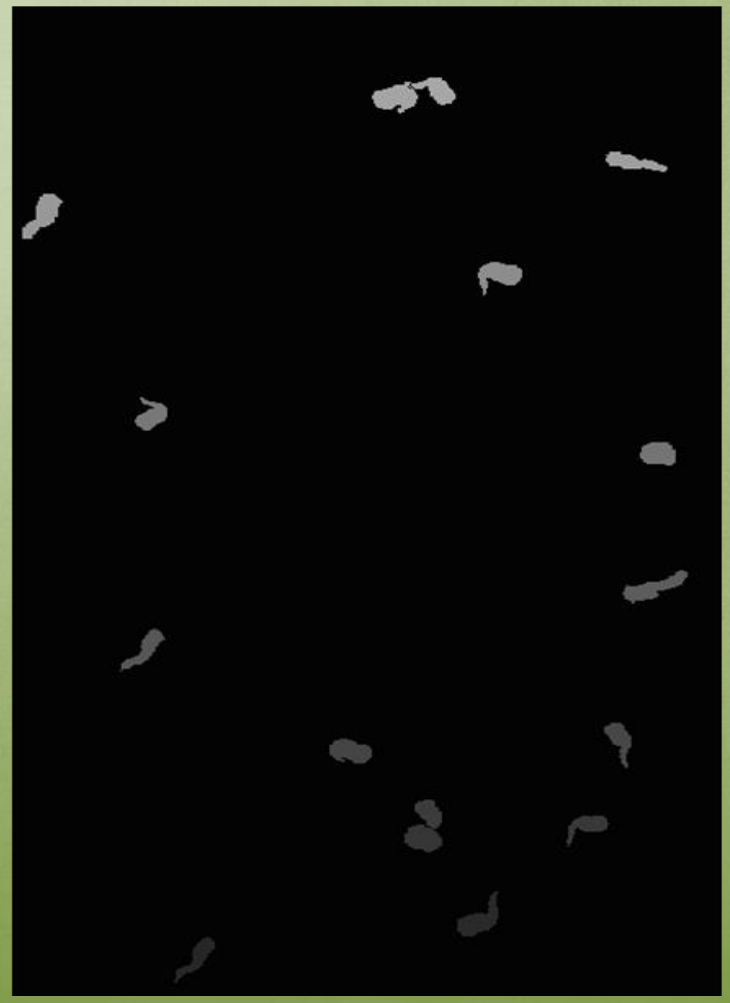
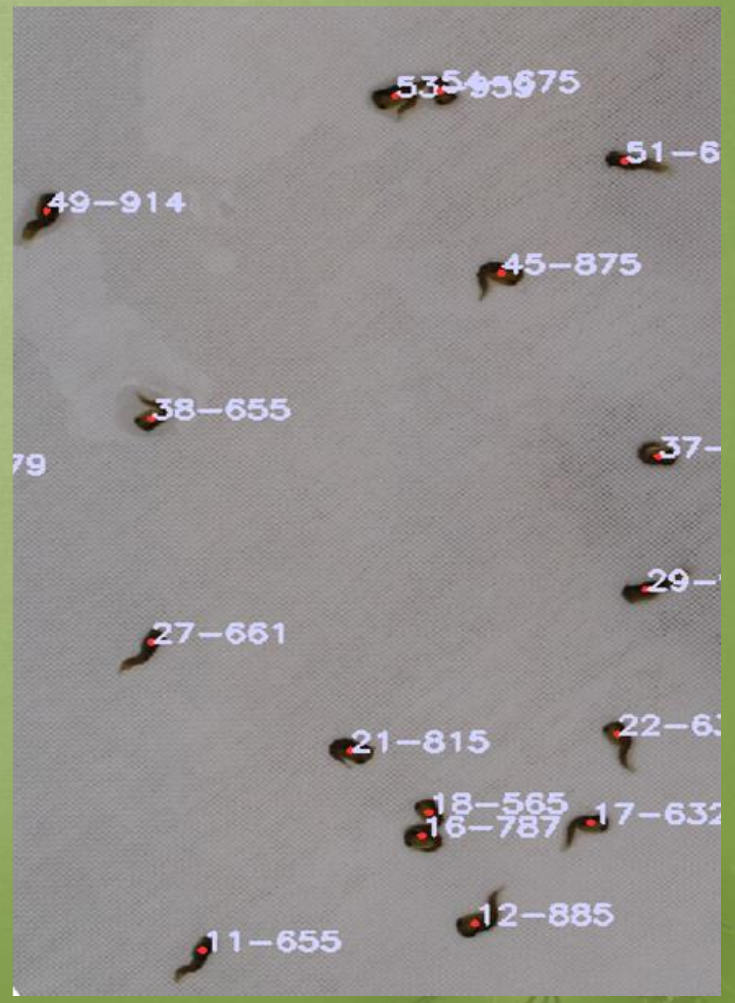


FREQUENCY DISTRIBUTION CURVES OF SIZE FOR MALES AND FEMALES CALCULATION

Image acquisition in dry conditions



Watershed algorithm



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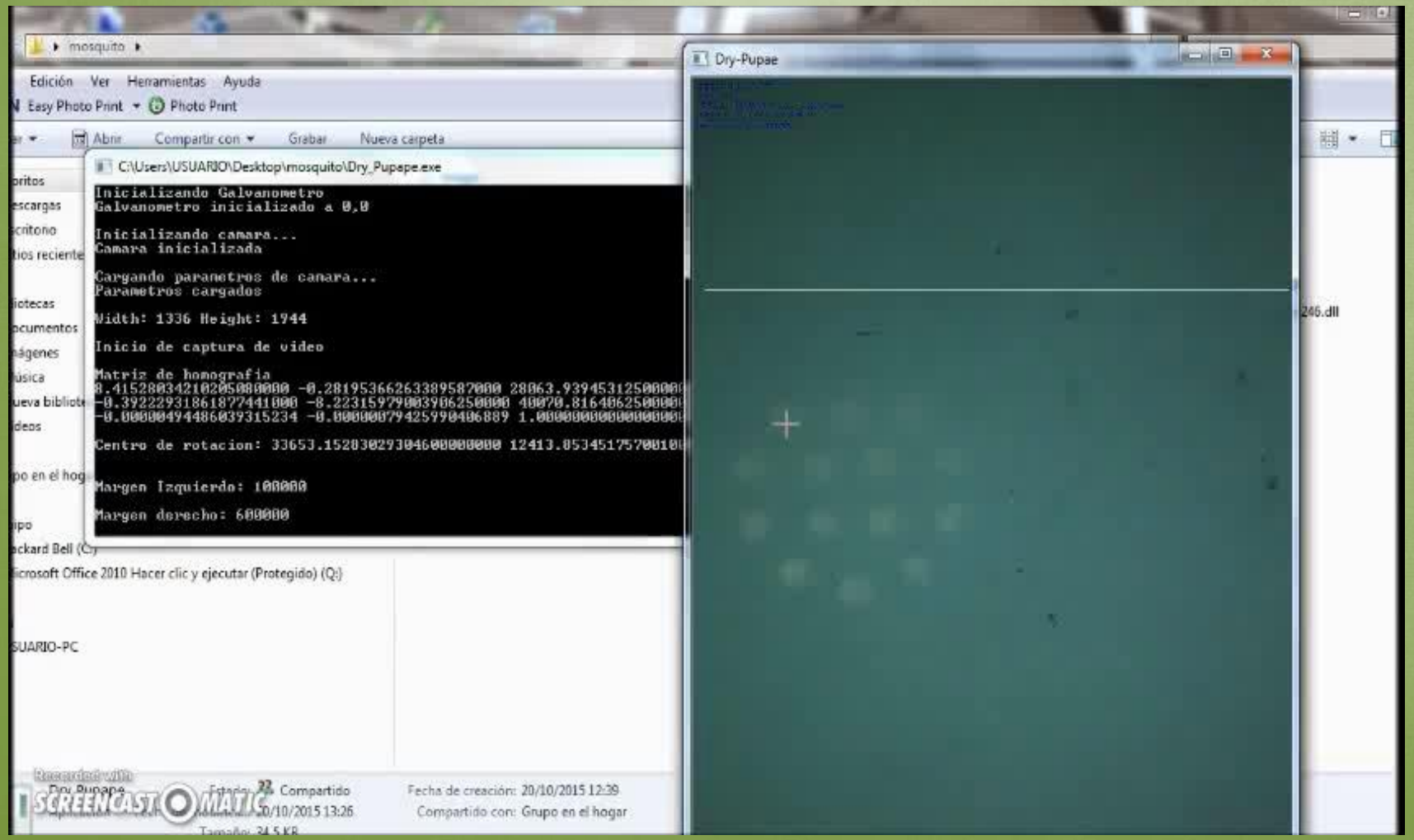
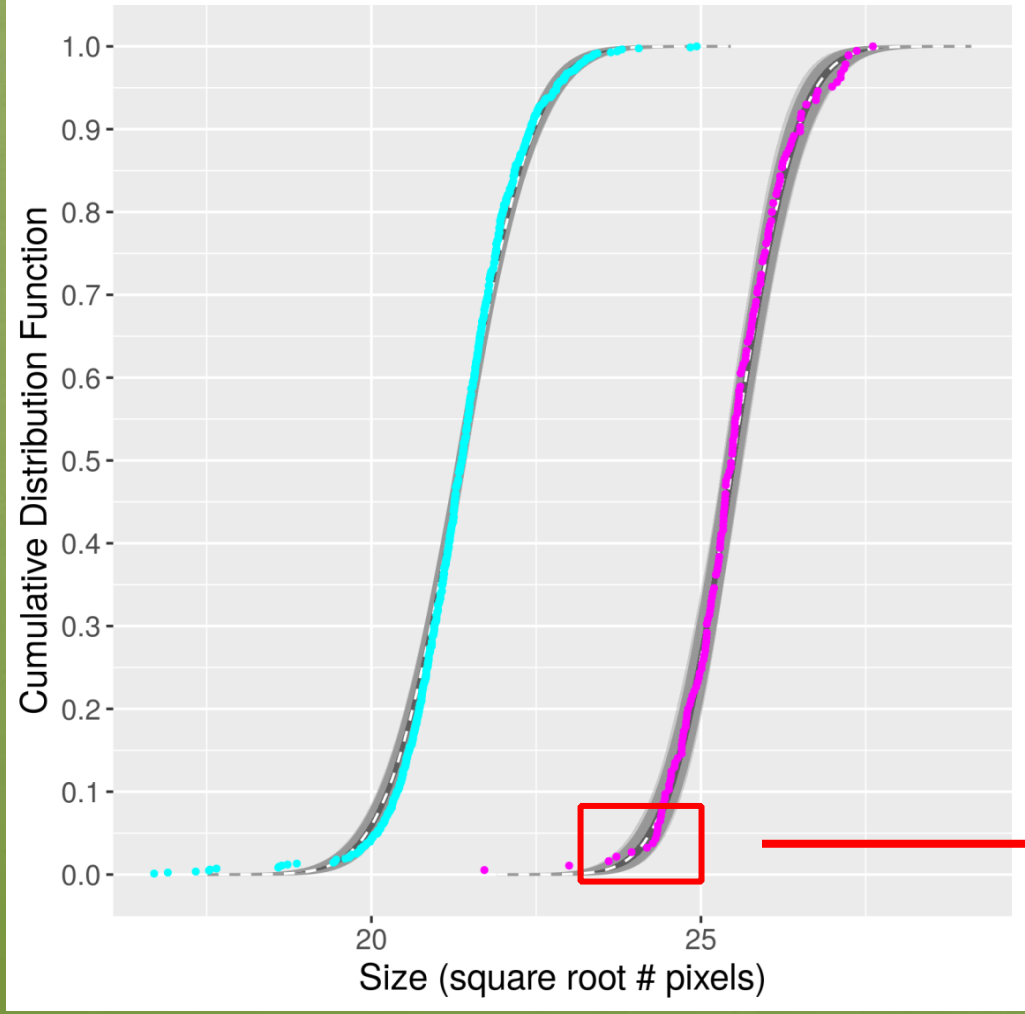


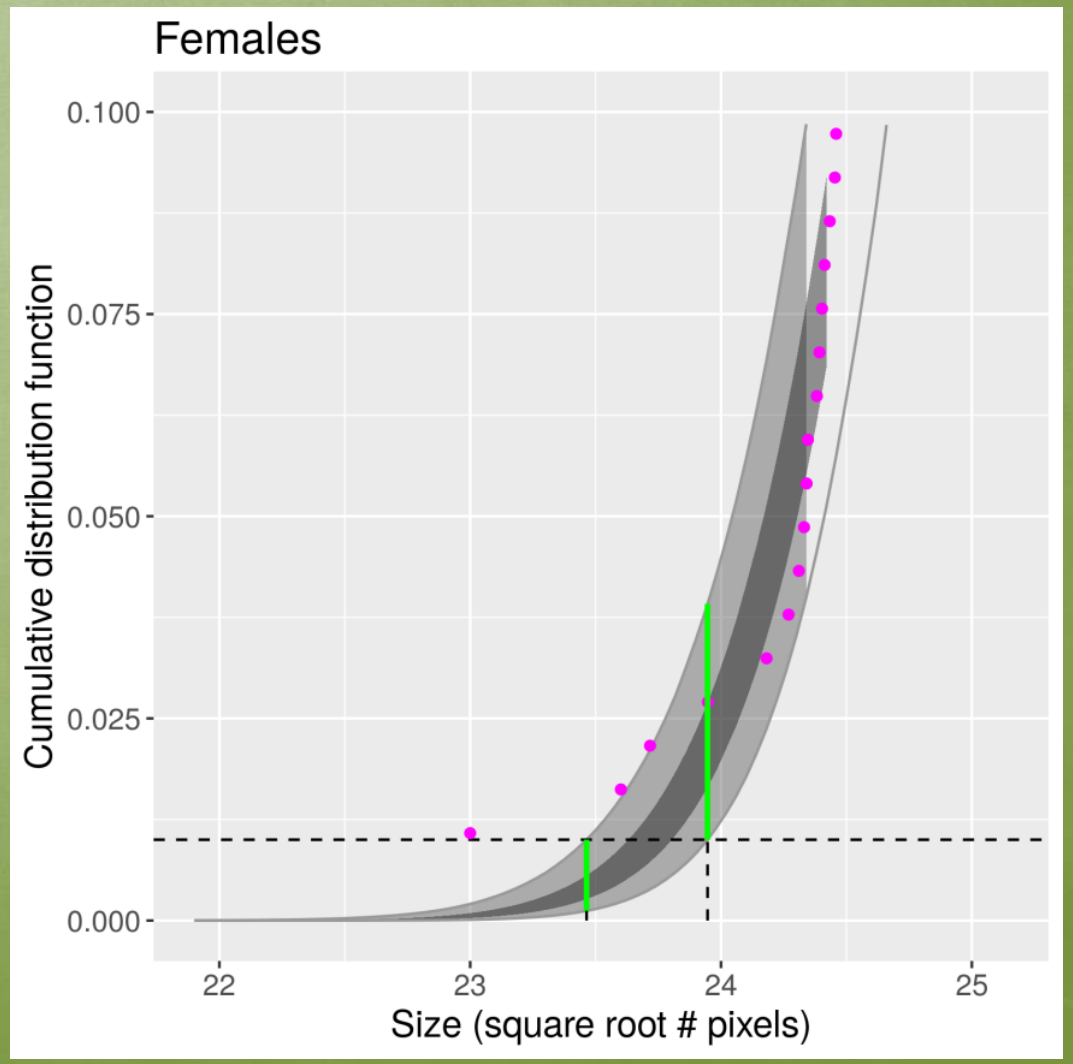
1. Pupa Id
2. Size calculation
3. X, Y Coordinates
4. Time

FREQUENCY DISTRIBUTION CURVES OF SIZE FOR MALES AND FEMALES

Cumulative distribution function F(x): Percentage of males/females below an area X.

Expectation Magnification algorithm





SEX SORTER PROTOTYPE

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6. **Pupae removal**

SECOND OBJECTIVE:

Aedes albopictus **female removal** using a computer controlled laser beam

ADVANTAGES AND DISADVANTAGES

- Target productivity of one million male pupae per day (laser Ton: few ms to few ns, galvanometer 40.000 points/sec, pupae administration, cameras/software: 5-7 frames/sec)
- Unattended technology
- Accurate count of the number and size of pupae (Quality control parameters)
- Can also be used for female sexing (lab tests)
- Possibility of working with local strains
- No need of filter colony (amplification system for GSS)
- Applicable in different species with sexual dimorphism (*albopictus, aegypti, polynesiensis*…)
- Sexing efficiency depends on standard rearing conditions

FUTURE PLANS

- Productivity can be increased by reducing pupae manipulation and improving pupae dispenser
- Similar tests will be carried out with other species next months in Seibersdorf laboratories
- An industrial version is planned

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Thank you very much