Research on Screwworms: Male-only Strains, Cryopreservation and Reducing Ammonia in Mass Rearing

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Screwworm Damage

Livestock

Wildlife

Pets

Humans
Efficacy of the SIT against screwworms

Before Eradication Program

Current Distribution
Estimated **annual benefits** to producers (in US dollars) from eradication of screwworms:
- USA – $896.1 million
- Mexico – $328.6 million
- Central America - $87.8 million

$1,312.5 million

Estimated **total cost** of eradication program in North America, through 2005, is $1,290 million

Estimated **annual losses** to producers (in US dollars) in South America from screwworms:
- Brazil - $1770 million
- Argentina - $618 million
- Colombia - $264 million
- Uruguay - $210 million
- Venezuela - $199 million
- All others Countries - $445 million

$3,506 million

From Vargas-Terán et al. 2005
SIT Program:
Bisexual release...both sterile males and females
Advantages of a genetically modified, transgenic male-only strain with a fluorescent protein marker gene

• More efficient population suppression in the field with male-only release
• Considerable savings in diet costs if females die before pupal stage
• Essentially double the capacity of the plant *
• Identification of released sterilized flies using fluorescent marker
• If males are genetically sterilized, no need for radiation treatment. Males should be more competitive and costs reduced.

* Allowing for new programs OR to address outbreaks
Insertion of genetic material to screwworm eggs.

**Microinjection**

Position needle to eggs

Inject DNA

DNA ‘constructs’

Larvae under white light

Same larvae under fluorescent light

100’s of putative lines were screened – single component and double component.
Female-specific overproduction of tTA is lethal (single component)

Females die due to excessive production of tTA, which interferes with gene transcription. Death as late larva/pupa.

Luke Alphey, Oxitec
Components needed for an early-acting female killing system (double component)

- **Early Promoter**
  - tTA

- **Reaper**
  - tTA
  - tetO promoter
  - *STOP
  - **transformer intron**

- **Reaper**
  - RPR

- **No Tetracycline**
- **Transcription**

Isolated early-promoters from blow fly genes

Isolated blow fly cell death genes

Death as embryo or young larva

Rebecca Edman
MS (2013)
All in one
tTA driver +
Hid effector
Risk Assessment:
Transgenic male-only New World Screwworm

✓ Assess **fitness** characteristics of strain (fertility, fecundity, pupal weight)
✓ Assess male mating **competitiveness**
✓ Assess potential for **outcrossing** to closely related species (*C. macellaria*)
✓ Assess potential for **extended range** (e.g. survival at cooler temperatures)
✓ Assess efficacy of female-killing in **different genetic backgrounds**
✓ Assess **stability** of transgenic line (i.e. does transgene re-mobilize?). If necessary “stabilize” by recombination-excision of one *piggyBac* end.
Summary and perspectives

• Developed sexing strains that are 100% female lethal (single component) but female mortality is late in larval development.
• Best line now being tested (mass rearing characteristics) by Technical Direction of COPEG.
• Sexing strains that have early female mortality are being evaluated (double component and all-in-one).
• Applied to start limited field evaluations.
Cryopreservation of screwworm embryos

All operations by hand.
100’s per day.

Robotics applied to cryopreservation.
1000’s per day.
Cryopreservation of screwworm embryos

- The current production strain (J-06) and the backup (Valledupar) have been cryo-preserved.

- The ARS research strains, including all male only lines, are now being cryo-preserved.
Ammonia reduction and other rearing enhancements

Potassium permanganate (KMnO$_4$) reduces ammonia and replaces formaldehyde.

Soy powder replaces dry milk.

Tetracycline is required in larval diet for adult colony replacement of male only lines.
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STRI

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THANK YOU
For your attention
Steven R. Skoda

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