How can we tell insects were irradiated?

Catriona Condon, Giancarlo Lopez-Martinez, Woodward Bailey, Laura Jeffers, Robert Meagher and Dan Hahn.

Department of Entomology and Nematology, University of Florida.
and USDA-APHIS-PPQ

Irradiation as a phytosanitary treatment

Kill or Sterilize pests in commodities.

Facilitate trade – exports and imports, international and domestic.

Tolerated by fresh produce. No pesticides!

Insects can be alive post irradiation, but sterile.

A Need for Standards:
Development of generic doses of irradiation that can be used across commodities and pests.

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- Generic dose of 150 Gy is accepted for all species of fruit flies

- Generic dose of 400 Gy has been proposed for all insects except lepidoptera pupae and adults, but not accepted

- Industry demand, but a few barriers exist for national and international plant protection organizations that regulate trade in fresh commodities.
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Tolerated by fresh produce. No pesticides!
Insects can be alive post irradiation, but sterile.

E-beam irradiator at Fla Dept Ag Gainesville, FL

Commodities with live insects can be held or even rejected.

Irradiated or not?

Irradiated pupae, almost all emerged adults.

Irradiated or not?

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0 Gy 400 Gy 400 Gy
Irradiated insects can still look healthy

We need a test to verify irradiation!

What is needed in an irradiation biomarker?
1. Accurate indicator of sterility, for live insects.
2. Broadly effective across insect pests.
3. Fast and performed away from the lab.
4. Persist several weeks after irradiation.
5. Dose range of 50 to 400 Gy (maybe 1kGy)

Inspiration from Human Diagnostics

Many tests in development ...but low doses (<10 Gy). Insects get 50 to 400 Gy.
Also, tend to be expensive, complicated, and specific.

Inspiration from Food Irradiation

Long history of developing tests for irradiated foods.
Inspiration from Food Irradiation

Long history development, but few accepted tests.
10 in Codex Alimentarius, only 1/2 radiation specific.
- Thermoluminescence or photostimulated luminescence of charge states trapped in inorganic constituents.
- Oxidative lipid byproducts – 2-alkyl-cyclobutanones and other oxidized hydrocarbons.
- Electron Spin Resonance.
- Comet assay for DNA Damage.

Biological effects of irradiation - Generalized

Direct damage
- DNA

Indirect damage
- Water

DNA damage and damage to other biomolecules

DS break mutations disrupt mitosis and drive sterility/death.

Tests for DNA damage already exist.

But, are complicated and can take 4+ hours.

Comet assay reliably detects breaks in dsDNA.
**Comet assay on irradiated Caribfly larvae.**

Comet assay an accurate indicator of irradiation. 

**Good dose response – but long lead time, finicky**

Comet assay lab standard for detecting DNA damage.

Need an easy, inexpensive diagnostic

Molecular markers of DNA damage.

Oxidative DNA damage – Guanine to 8oxodG.
DNA repair proteins – H2AX.

Antibodies and test kits available.
DNA repair proteins

DNA is wound around histone proteins.

Phosphorylation of histone H2AX—occurs rapidly after irradiation.

Occurs at dsDNA break creates a binding site for repair proteins.

γH2AX as a biomarker for DNA repair.

γH2AX signal is proportional to IR exposure.

Histone Protein H2A Control.

Signal fades with time.

Antibody may not apply broadly to insects.
Alternative methods: Biomedical research
Detection of tumor irradiation in radiotherapy.

DNA damage signaling proteins
ROS detection.

Two major uses for irradiation in Entomology.
Phytosanitary Irradiation
Sterile Insect Technique

Kill/Sterilize insects in Food! Sex kills offspring!

Sterile Insect Technique
medfly example, but representative

SIT and Trapping
Dye can wear off or fade!
Transgenic caribflies overexpressing markers and target genes.

- Created 12 independent lines carrying MnSOD transgene (6 single autosomal, 2 multiple chromosome, 3 X-linked, 1 Y-linked)

**Challenges for biomarker development:**

1. **Accurate** indicator of sterility in live insects.
2. **Broadly** effective to insect pests (mostly).
3. **Fast** and performed away from the lab.
4. **Persist** several weeks after irradiation.
5. **Dose range** of 50 to 400 Gy (maybe 1kGy)

"Oxygen Effect" increases radiotolerance of tumor cells
The Oxygen Effect

- Modified Atmosphere Packaging (MAP) is widely used for fresh fruits and vegetables.
- MAP can include low $O_2$ and/or high $CO_2$.
- Anoxia — no $O_2$ — can affect radiotolerance.
Anoxia had greatest effect, some effects of intermediate O₂.

Probability of adult emergence from 5th instar larvae.

- Males
- Radiation (Gy) – by X-ray

Thank you.

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- The Hahn Lab
- Sabrina White (looper wrangling)
- Rob Meghar and Amy Rowley (providing loopers)
- Carl Gillis, FDACS-DPI (irradiation)
- Lyle Buss (Looper pictures)
What level of O₂ affects insect physiology and thus potential for direct damage versus indirect oxidative damage?

Is 18% O₂ too high or too low?
Effects of ROS – mitochondria especially important
Mitochondria are still running fairly well >4% O₂
Is 18% too high for a restriction on IPT?

What level of O₂ affects insect physiology?
Is 18% O₂ too high or too low?
What about CO₂?
THE OXYGEN EFFECT

- Oxygen is a powerful oxidizing agent and therefore acts as a radiosensitizer if it is present at the time of irradiation (within μsecs).
- The magnitude of the OER is critically dependent upon oxygen tension. The greatest increase occurs between 0-20 mm Hg with further modest increases to air (155 mm Hg) and above (760 mm Hg=100% oxygen).
- Its effects are measured as the oxygen enhancement ratio (O.E.R.)
  - O.E.R. = the ratio of doses needed to obtain a given level of biological effect under anoxic and oxic conditions = D(anox)/D(ox)
  - For low LET radiation the O.E.R. is 2.5-3.0 and in the higher range at higher doses.
  - For neutrons, O.E.R is about 1.6

Dose (Gy) vs. O.E.R.

Inspiration from Food Irradiation

Don’t Nuke Our Food

Stop Food Irradiation

Weaponized Food

Anoxia greatest affected emergence, other mod. atmospheres had little effect on emergence.
Does Hypoxia Affect Irradiation Sensitivity of Female Reproduction?

- Female pharate adults treated with 0, 200, 400, 600 & 800 Gy in anoxia or normoxia in e-beam.
- All doses delivered within 5% of target dose with DUR = 1.05.
- Treated females individually mated with untreated virgin males.
- Pairs held individually and scored for female fecundity and fertility.

Atmospheric treatment affected probability of producing larvae at 200 & 400 Gy doses.

- Only anoxia diff from normoxia
- 15 & 21 kPa O₂ atmospheres diff. from anoxia at 200 & 400 Gy
- Some fertility at 400 Gy even in normoxia

Atmosphere did not affect fertility when no irradiation.

Atmosphere affected fertility @ 200 Gy.
Implications for 400 Gy

- Still had substantial fertility at 400 Gy
- Most lepidopteran SIT programs use ~200 Gy and get very good F₁ sterility.
- Is F₁ sterility an acceptable treatment outcome for phytosanitary irradiation?

What is Next?:

- 2nd hypoxic linear series of O₂, 0-15%?
- 3rd does critical PO₂ predict where O₂ effect kicks in?
What level of O₂ affects insect physiology?

Does critical PO₂ predict O₂ effect levels?

What is Next?:
• 2nd hypoxic linear series of O₂, 0-15%?
  – There were some effects of 5 & 10% O₂.
• 3rd does critical PO₂ predict where O₂ effect kicks in?
• 4th reciprocal series of modified atmospheres, trade O₂ for CO₂.

Inspiration from Food Irradiation

Long history of developing tests for irradiated foods.

The Problem - Male Quality

• Irradiation effectively sterilizes insects by damaging genomic DNA in the nucleus.
• Irradiation also has unwanted side effects
  1) Direct energy transfer damages DNA and others.
  2) Indirect damage from free radicals and other oxidants – continues after irradiation.
Oxidative stress is pervasive – critical for life histories. Male signals indicate ox-stress resistance - good genes.

Boosting Antioxidants
- Cells have natural antioxidant defenses
- Increasing antioxidant enzymes may lower oxidative damage & improve performance
- Ischemia-reperfusion response?

Mating success is increased, while maintaining sterility.

Pharate adults exposed to 1h anoxia and irradiated. Performance scored 10 days later at sexual maturity.

Anoxia also improves flight & longevity.

Mating success at 10 days is improved by anoxia!

Anoxia-treated flies have greater lifespan... what about healthspan?

Can they be effective longer?
Mating success at 30 days is still improved by anoxia.

Basic mechanisms in performance & Economic impacts for SIT programs!

Anoxia reduces oxidative damage at time of sexual maturation

Anoxia elevates antioxidant defenses

Trolox-equivalent antioxidant capacity (TEAC)

Antioxidants are up!

Groupings based on multivariable ANOVA followed by Tukey’s HSD test