

How can we tell insects were irradiated?

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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.



E-beam irradiator at
Fla Dept Ag
Gainesville, FL



A Need for Standards:

Development of generic doses of irradiation that can be used across commodities and pests.

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



Fourth Research Coordination Meeting of the Coordinated Research Project 06206
on the
Development of Generic Irradiation Doses for Quarantine Treatments
IAEA Headquarters, Vienna, Austria
2 – 8 June 2014

A Need for Standards:

Development of generic doses of irradiation that can be used across commodities and pests.

- 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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Insects can be **alive** post irradiation, but sterile.

Commodities with live insects can be held or even rejected.



"Wigglers"!



Irradiated or not?



Irradiated pupae, almost all emerged adults.

Irradiated or not?



0 Gy



400 Gy



400 Gy

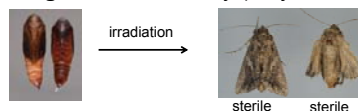
Irradiated insects can still look healthy



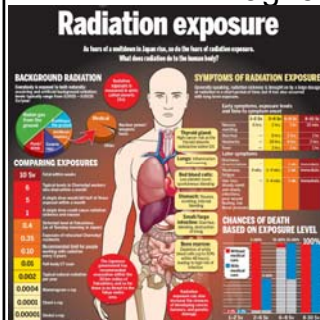
We need a test to verify irradiation!

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



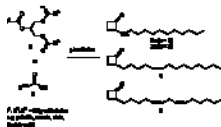
Long history of developing tests for irradiated foods.

Inspiration from Food Irradiation

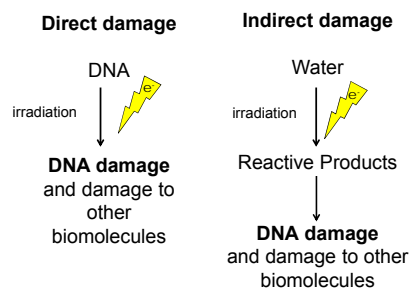
Long history development, but few accepted tests.

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.

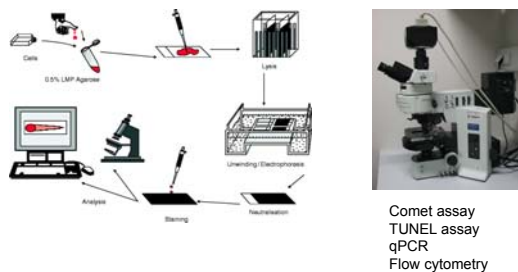


Biological effects of irradiation - Generalized



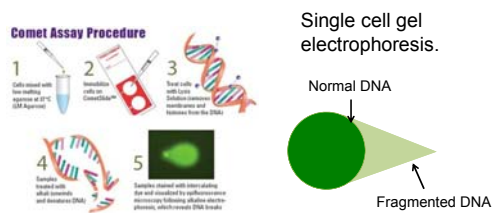
DS break mutations disrupt mitosis and drive **sterility/death**.

Tests for DNA damage already exist.

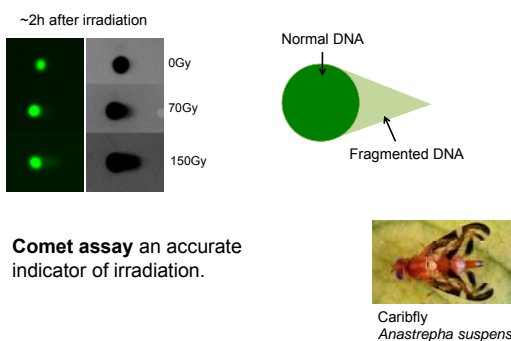


But, are complicated and can take 4+ hours.

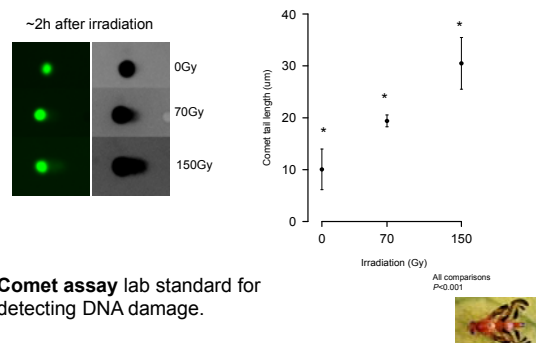
Comet assay reliably detects breaks in dsDNA.



Comet assay on irradiated Caribfly larvae.



Good dose response – but long lead time, finicky



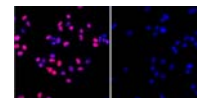
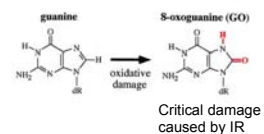
Need an easy, inexpensive diagnostic



Molecular markers of DNA damage.

Oxidative DNA damage – Guanine to 8oxodG.

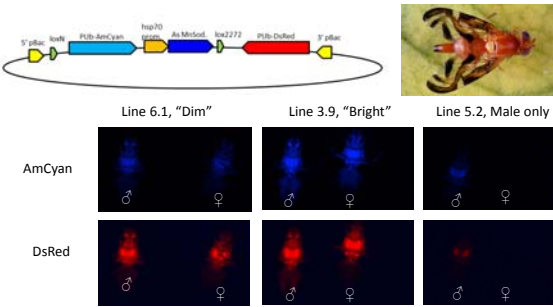
DNA repair proteins – H2AX.



Antibodies and test kits available.

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)

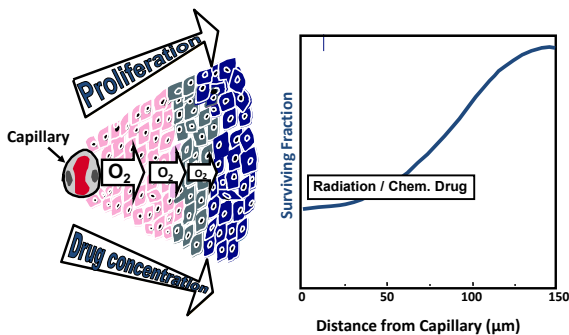


Challenges for biomarker development:

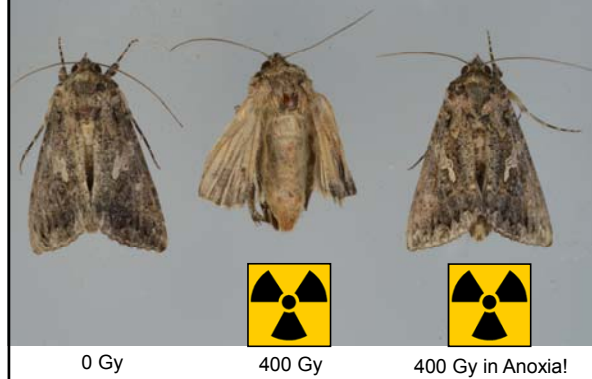
1. **Accurate** indicator of sterility in live insects.
2. **Broadly** effective to insect pests (mostly).
3. **Fast** and performed away from the lab.
4. **Persist** several weeks after irradiation.
5. **Dose range** of 50 to 400 Gy (maybe 1kGy)



"Oxygen Effect" increases radiotolerance of tumor cells



Irradiated or not?



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

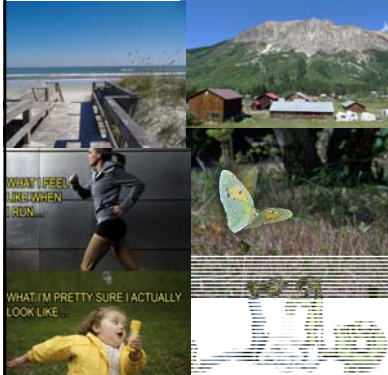


Sea level
21 kPa O_2



Sea level
21 kPa O_2

Crested Butte CO
2,500m = 16 kPa O_2



Sea level
21 kPa O_2

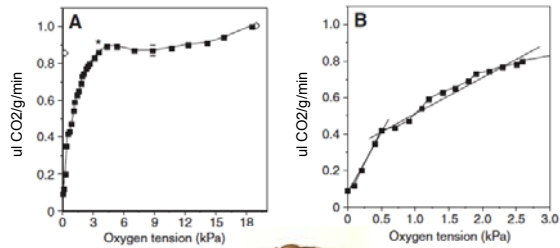
Crested Butte CO
2,500m = 16 kPa O_2

Mt. Everest
8,000m = 8 kPa O_2



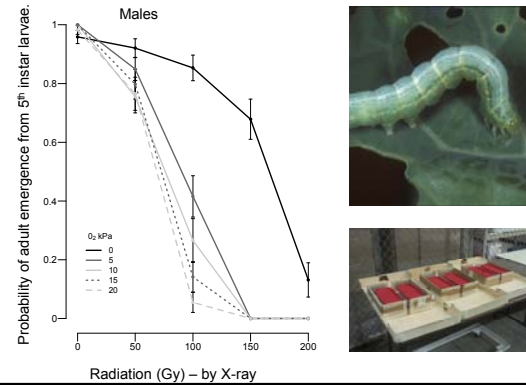
What level of O₂ affects insect physiology?

Respiratory rates not affected above 4%O₂

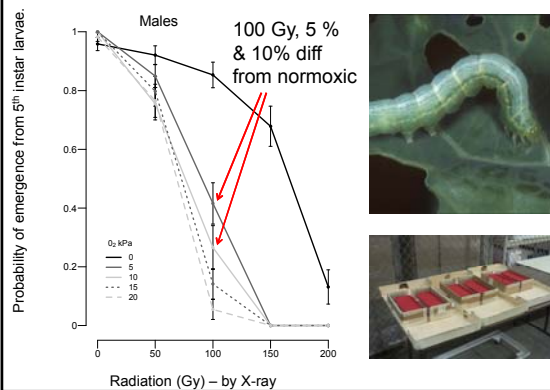


VanVoorhies 2009
J. Exp. Biol.

Anoxia had greatest effect, some effects of intermediate O₂.



Anoxia had greatest effect, some effects of intermediate O₂.

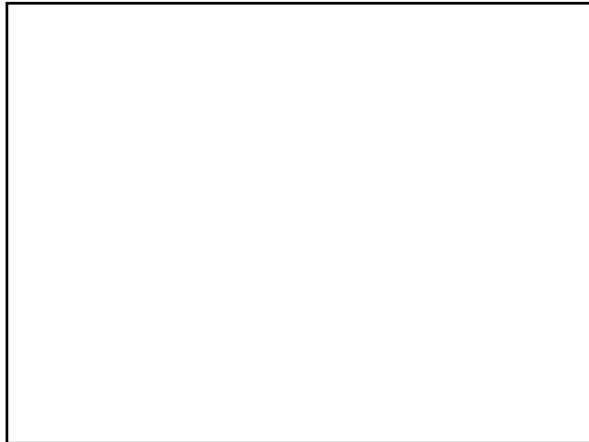


Thank you.



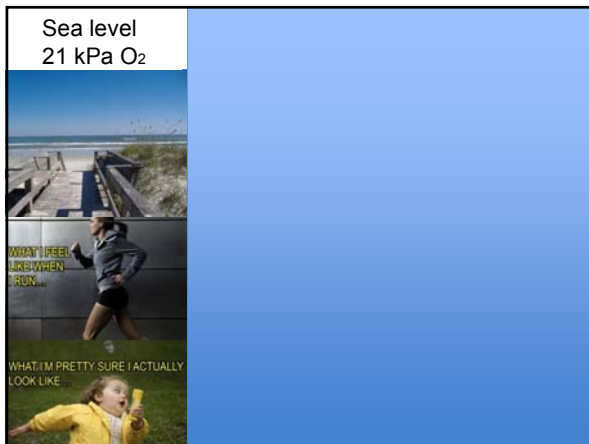
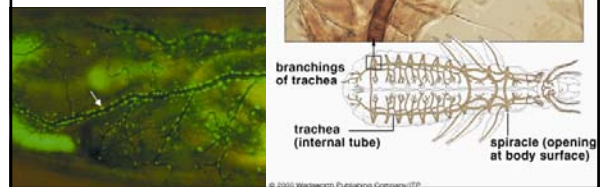
- USDA-APHIS co-op agreement to DH
- The Hahn Lab
- Sabrina White (looper wrangling)
- Rob Meghar and Amy Rowley (providing loopers)
- Carl Gillis, FDACS-DPI (irradiation)
- Lyle Buss (Looper pictures)





What level of O_2 affects insect physiology and thus potential for direct damage versus indirect oxidative damage?

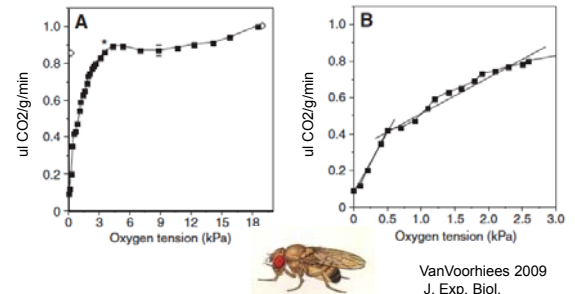
Is 18% O_2 too high or too low?





What level of O₂ affects insect physiology?

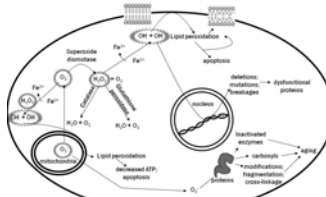
Respiratory rates not affected above 4%O₂



Effects of ROS – mitochondria especially important

Mitochondria are still running fairly well >4% O₂

Is 18% too high for a restriction on IPT?

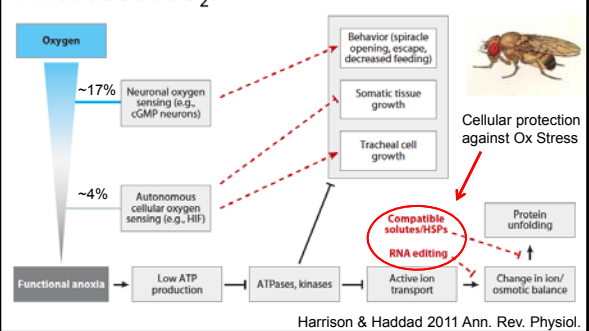


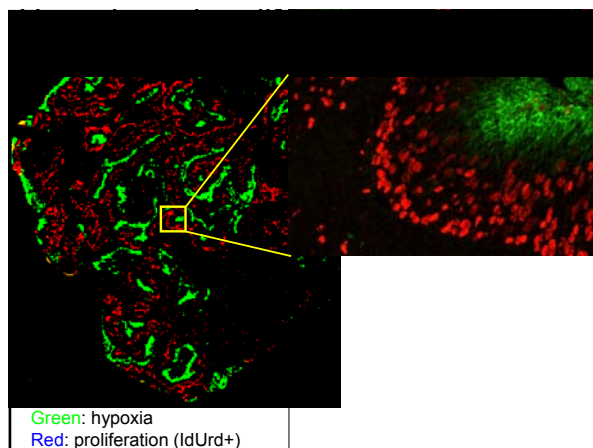
Benoit and Lopez-Martinez 2011

What level of O₂ affects insect physiology?

Is 18% O₂ too high or too low?

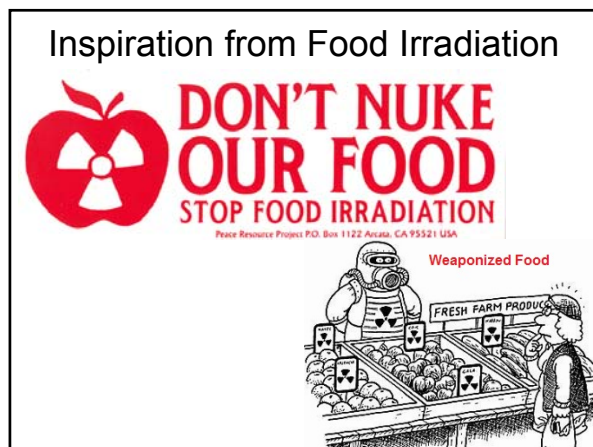
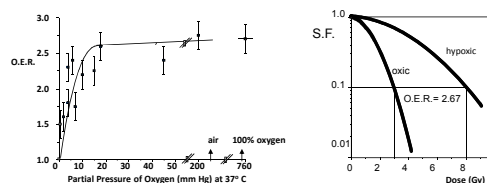
What about CO₂?



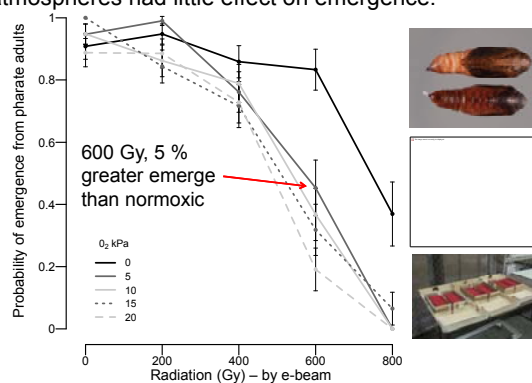


THE OXYGEN EFFECT

- Oxygen is a powerful oxidizing agent and therefore acts as a radiosensitizer if it is present at the time of irradiation (within μ secs)
- 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 = $D(\text{anox})/D(\text{ox})$
 - 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



Anoxia greatest affected emergence, other mod. atmospheres had little effect on emergence.

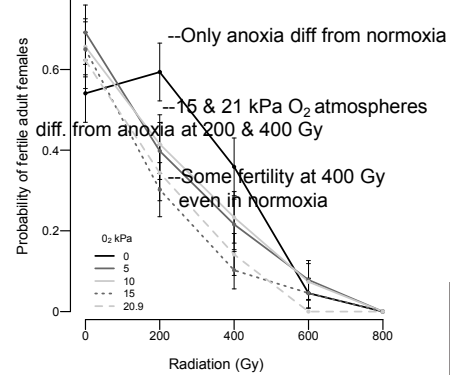


Does Hypoxia Affect Irradiation Sensitivity of Female Reproduction?

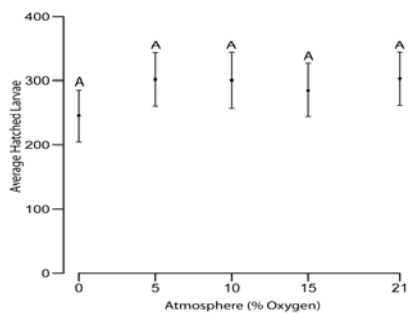


- Female pharate adults treated with 0, 200, 400, 600 & 800 Gy in anoxia or normoxia in e-beam.
- All doses delivered within 5% of target dose with DUR = 1.05.
- Treated females individually mated with untreated virgin males.
- Pairs held individually and scored for female fecundity and fertility.

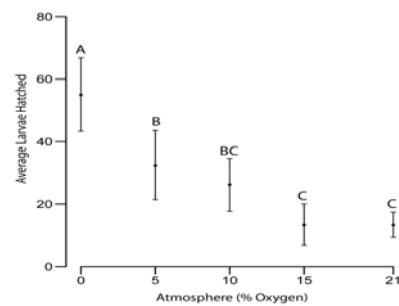
Atmospheric treatment affected probability of producing larvae at 200 & 400 Gy doses.



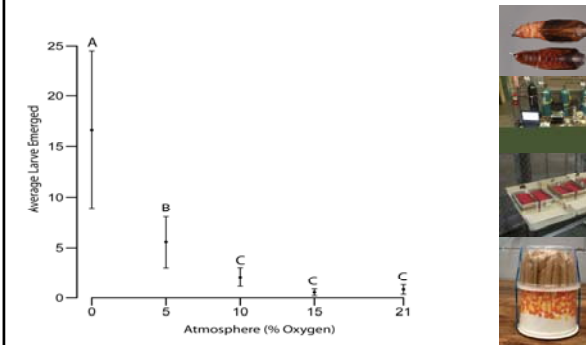
Atmosphere did not affect fertility when no irradiation.



Atmosphere affected fertility @ 200 Gy.



Atmosphere affected fertility @ 400 Gy.



Anoxia Increases Fertility of Cactus moths too.

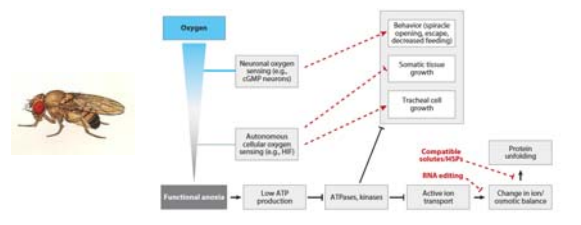


Implications for 400 Gy

- Still had substantial fertility at 400 Gy
- Most lepidopteran SIT programs use ~200 Gy and get very good F_1 sterility.
- Is F_1 sterility an acceptable treatment outcome for phytosanitary irradiation?

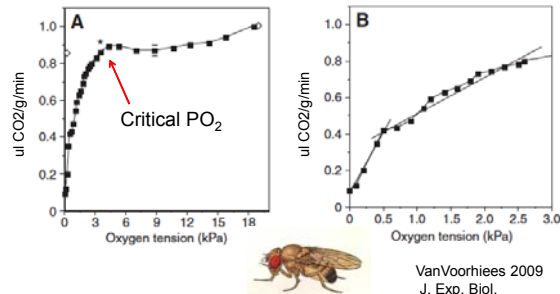
What is Next?:

- 2nd hypoxic linear series of O_2 , 0-15%?
- 3rd does critical PO_2 predict where O_2 effect kicks in?



What level of O_2 affects insect physiology?

Does critical PO_2 predict O_2 effect levels?



What is Next?:

- 2nd hypoxic linear series of O_2 , 0-15%?
– There were some effects of 5 & 10% O_2 .
- 3rd does critical PO_2 predict where O_2 effect kicks in?
- 4th reciprocal series of modified atmospheres, trade O_2 for CO_2 .



Inspiration from Food Irradiation

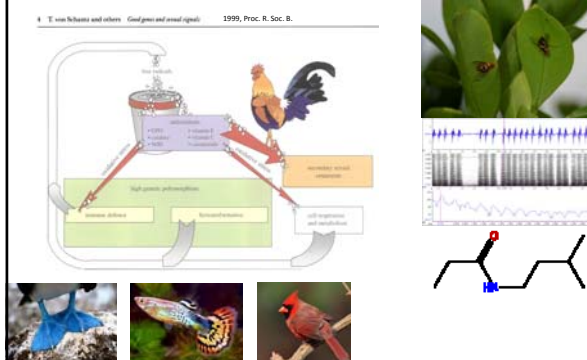


The Problem - Male Quality

- 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.

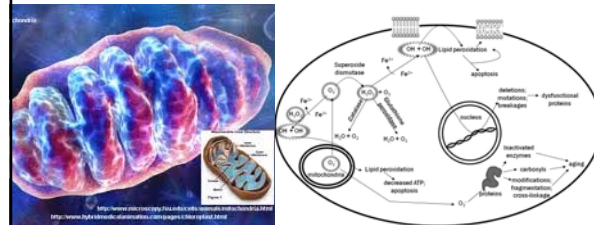


Oxidative stress is pervasive – critical for life histories.
Male signals indicate ox-stress resistance - good genes.

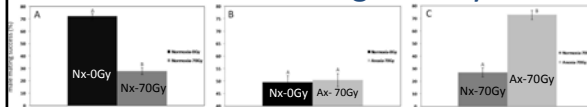


Boosting Antioxidants

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



Mating success is increased,
while maintaining sterility.



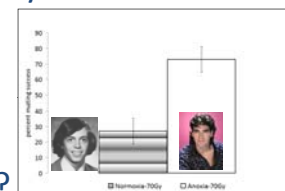
Pharate adults exposed to 1h anoxia and irradiated.
Performance scored 10 days later at sexual maturity.



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?



