Prospects for application

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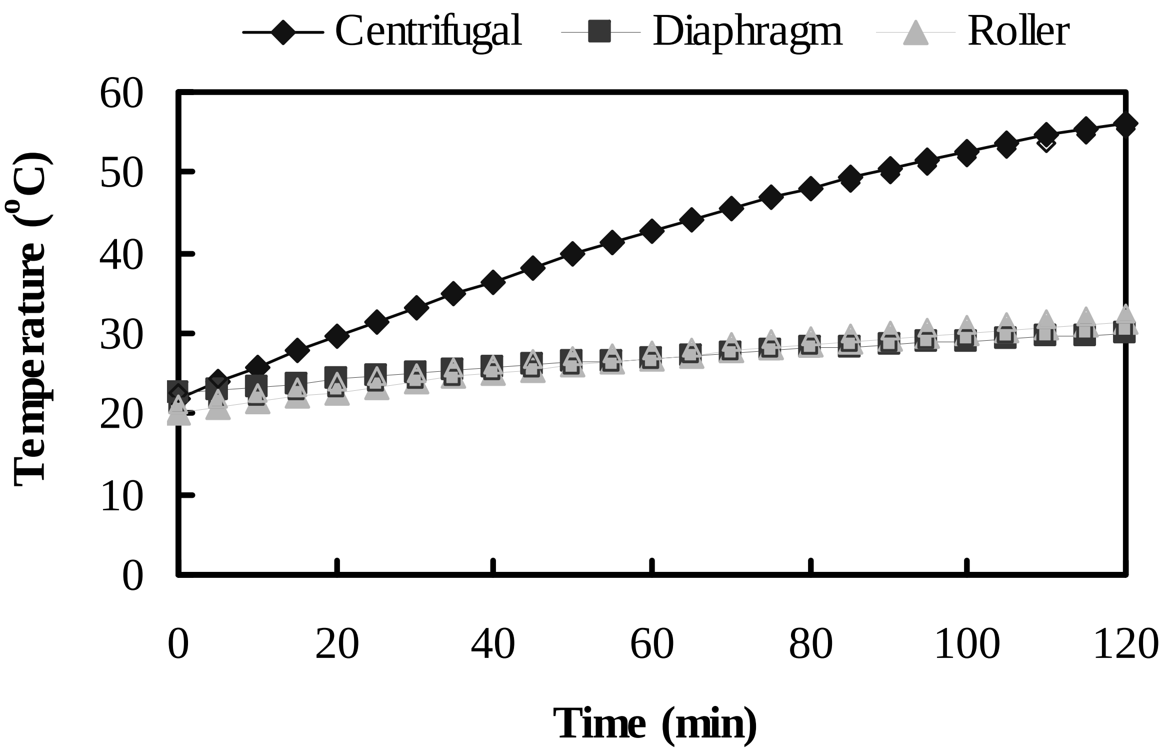
Consider three aspects:

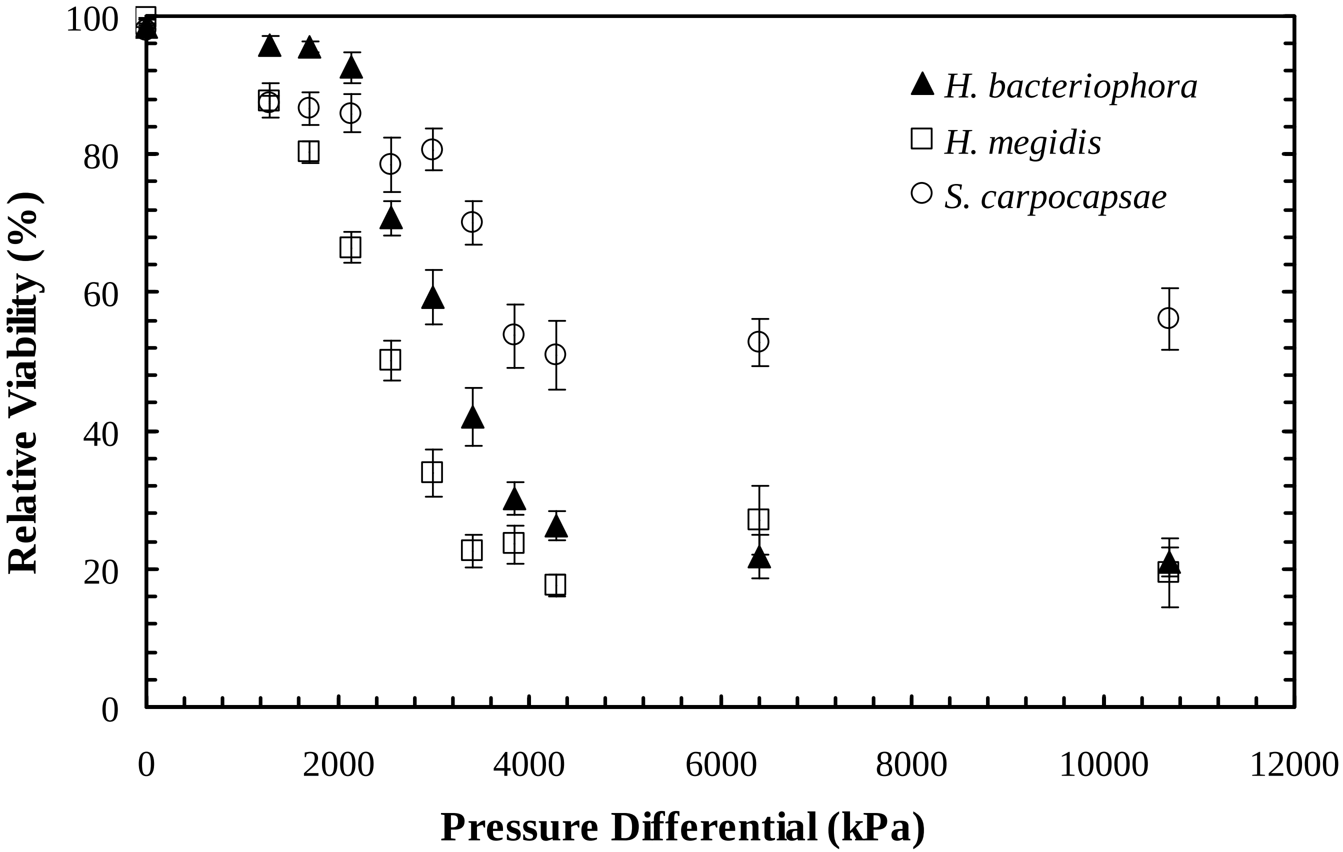
- equipment - effects on EPN viability and application efficiency
- formulation - improvements in EPN efficacy on foliage
- nematode quality and shelf life

Temperature during recirculation of 45.4 l of water at a volumetric flow rate of 15.1 l/min using centrifugal, diaphragm and roller pumps



Fife *et al.*, 2003

Relative viability of *Heterorhabditis bacteriophora*, *H. megidis* and *Steinernema carpocapsae* after pressure differential treatments.



Fife *et al.*, 2003

Operating pressures within a spray system should not exceed 20 bar (2000 kPa; 295 p.s.i.) for *S. carpocapsae* and *Heterorhabditis bacteriophora*, and 13.8 bar (1380 kPa; 204 p.s.i.) for *H. megidis*

The smaller the volume of spray liquid the more times the liquid will pass through the pump, causing the temperature to increase at a greater rate

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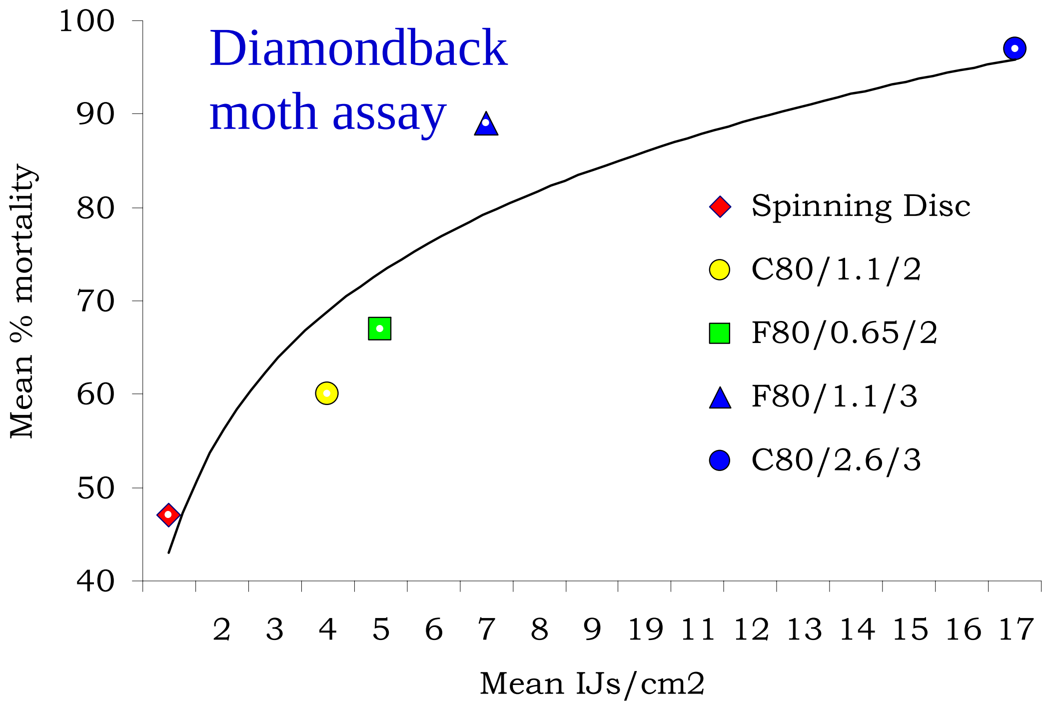
Filters and sieves should be at least 300 µm wide (50 mesh) or they should be removed before application (Klein and Georgis, 1994)

Nozzle apertures >500 µm are generally recommended for nematodes applications

Can foliar spray systems for nematodes be made more robust?



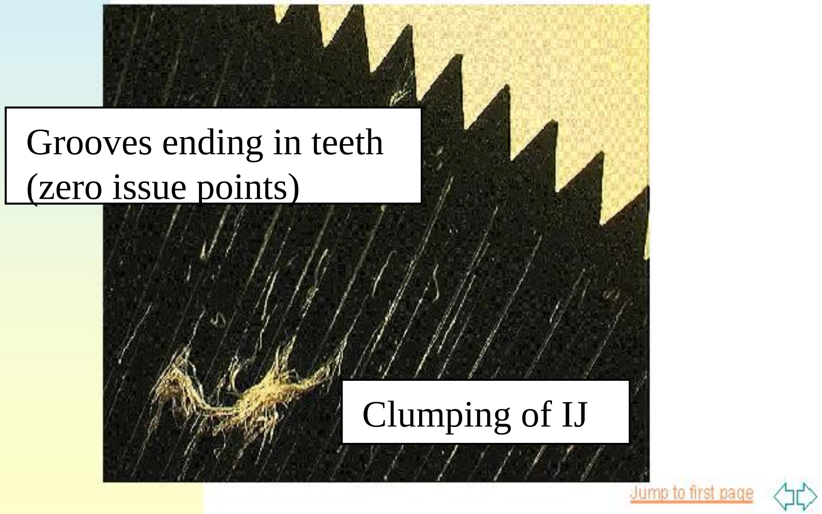
Hydraulic sprayers relatively inefficient unless nozzle type and pressure are optimised [Lello *et al*., 1996]

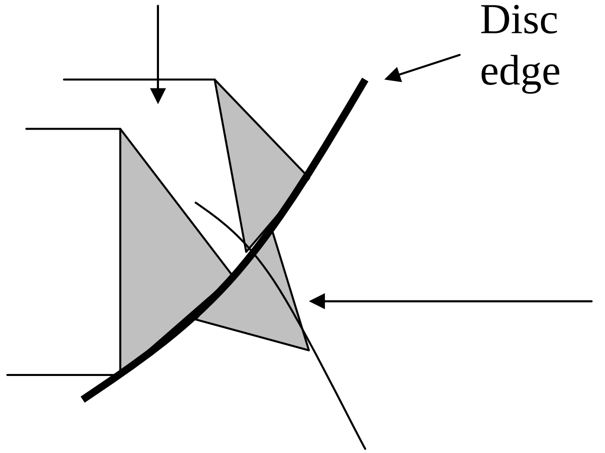
BUT… misting systems used with some success in against thrips & leafminers on ornamentals

Spinning discs (SD) improve efficiency (c. 10% IJ used compared with hydraulic systems) but conventional SD show a trade-off between coverage of foliage and droplet spectrum for carrying IJ (>90% too small) [Mason *et al*., 1998]

Ulva- spinning disc

Clumping caused by centrifugation

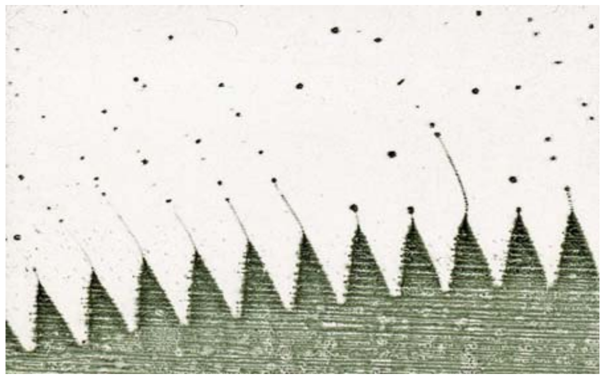




Major drawbacks of Ulva+

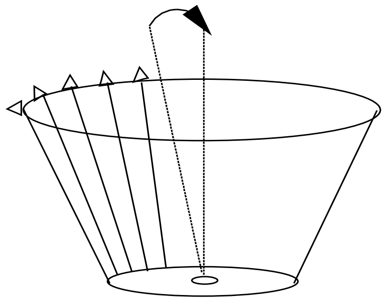
Emission of many IJ in clumps

Large no. of (narrow) grooves and ‘zero issue points’ (teeth) designed to produce very small droplets (too small to carry IJ)

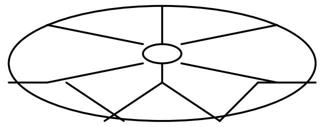


Redesigning spinning discs for nematodes

Ulva+ disc



Prototype disc



Flatter with no teeth

Prototype spinning disc (PSD):

Flatter
reduces residence time,
increases droplet size

Fewer, larger grooves
increases liquid flow/drop size,
reduces clumping of IJ

No teeth
release of IJ from grooves in
larger droplets

PSD gives greater deposition of IJ on foliage:

PSD = 5.5 IJ cm-2

Ulva+ = 0.6 IJ cm-2

[Piggott *et al* (2003) *Pest Management Science*]

In reality - growers are unlikely to change whole spray systems to apply nematodes

…..but they might consider changing the nozzle type or operating conditions (?)

One possibility might be redesigning hydraulic SDs?

Need to close the nematode efficacy gap between experimental crops and commercial crops (see Rob Jacobson's talk) with improved protocols/label instructions

Improved formulations for nematode sprays

e.g. - a combination of surfactant and polymer (Schroer & Ehlers) can enhance efficacy against DBM

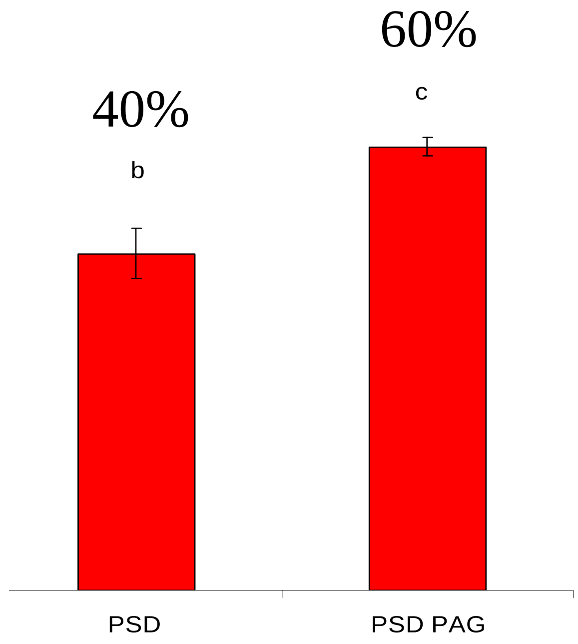
Polymers can give improved control in both hydraulic and SD spray systems

For example:

% mortality of *Spodoptera exigua* on tomato under glass.

Steinernema feltiae IJ were applied by PSD ± polymer (PAG)

(Piggott & Wright, unpublished)

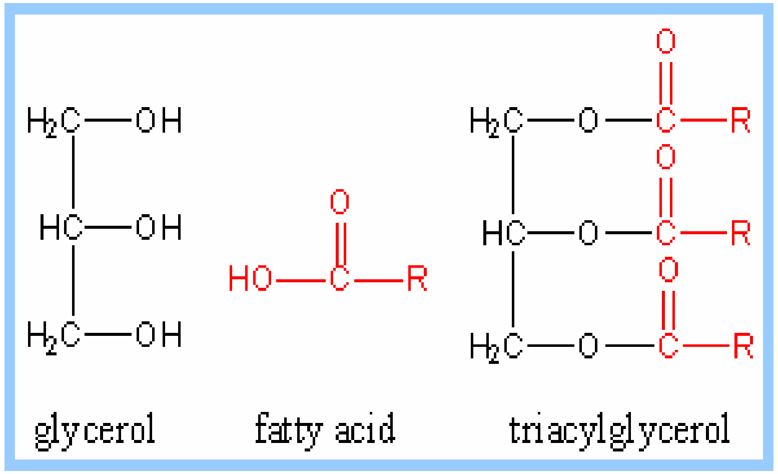


Nematode quality and ‘shelf-life’

Storage lipids in IJs

Neutral lipids primary energy stores

- >25% of dry wt in freshly-emerged IJ.
- triacylglycerols: the most abundant form

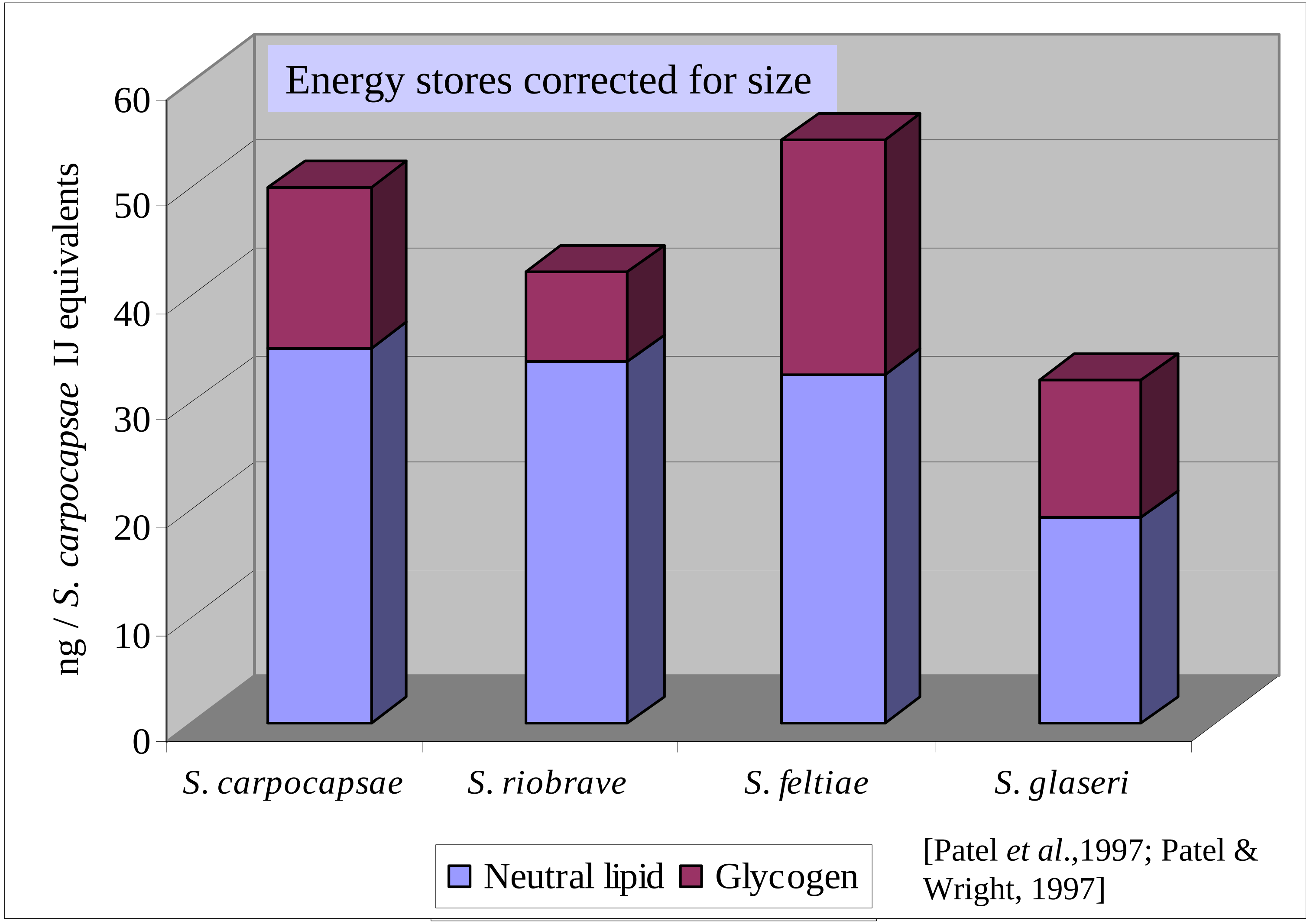


Neutral lipid stored in intestine and epidermis (oil red 0 stained)

Infectivity is usually low by level 4 or 3

[Patel *et al.*, 1997]

Energy stores in freshly emerged IJ from *Galleria*

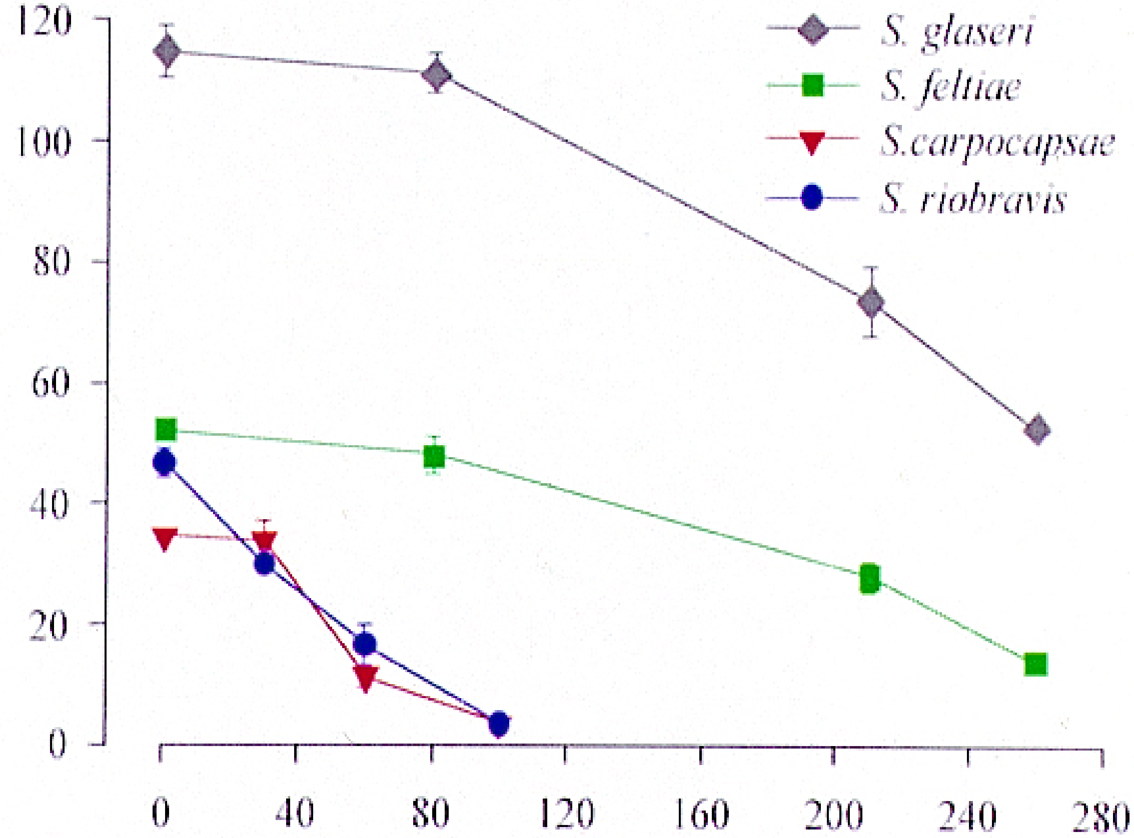


[Patel *et al*.,1997; Patel & Wright, 1997]

Neutral lipid consumption in water at 25°C

Slower rate in *S. glaseri* & *S. feltiae* correlates with greater survival time

Neutral lipid (ng/IJ)



Storage (days)

[Patel *et al.*, 1997]

Energy stores, metabolic rate and ‘shelf-life’ of commercial products

Within a species there is a good correlation

✓ initial lipid level has a positive correlation with shelf-life

✓ shelf-life greater at lower temperatures.

✓ shelf-life generally increases with decreasing nematode mobility

Between species the correlation can be less obvious, e.g.

Actively moving (sponge/vermiculite) / high or low temp:

✓ shelf-life of *S. carpocapsae* > *H. bacteriophora*
? shelf-life of *S. carpocapsae* ≥ *S. feltiae*

Reduced mobility (fluid gels) / high or low temp:
? shelf-life of *S. carpocapsae* > *S. feltiae*
? shelf-life of *S. carpocapsae* > *S. glaseri* [Grewal, 2002; Georgis, 2002]

Quantity and composition of lipids in EPN

The amount of lipid per IJ can vary with the physical and chemical conditions (temperature, dissolved oxygen…) during *in vitro* production

Culture temperature can also influence composition:

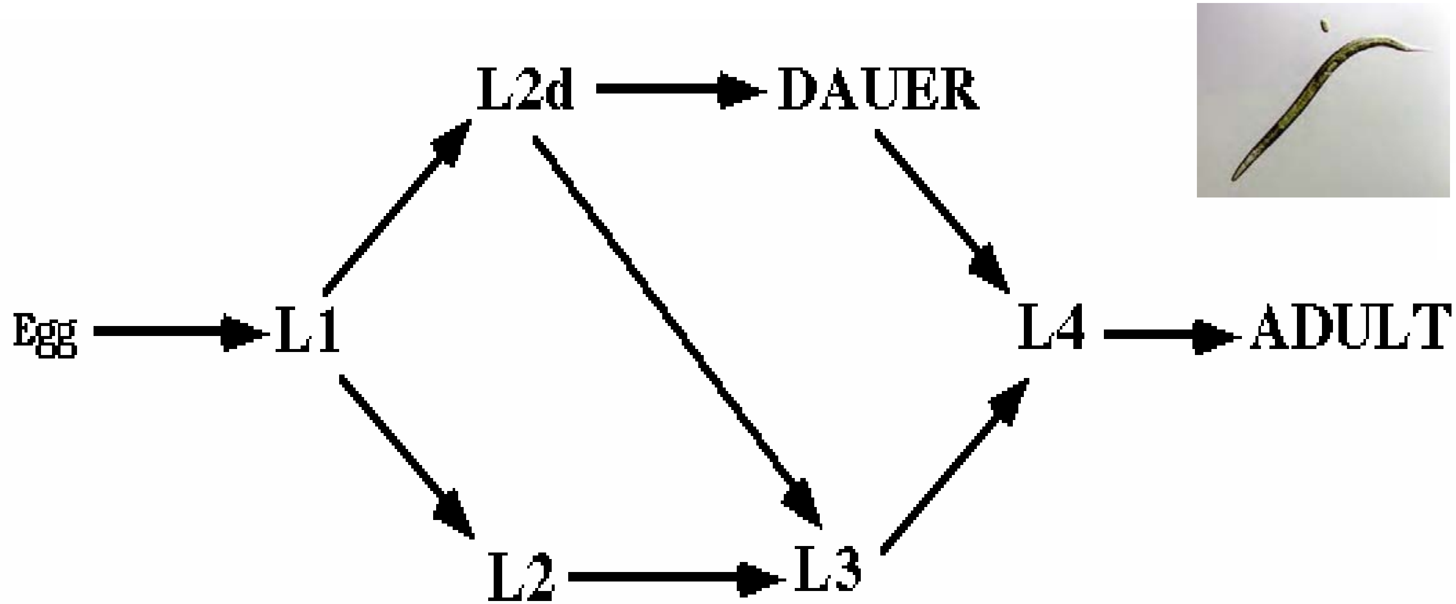
- greater proportion saturated fatty acids at high temp. in *Steinernema*

Dietary effects:

- *S. glaseri* from *Popillia japonica* (natural host) accumulate more total lipid compared with IJ from *Galleria*
- insect lipids (rich in mono-unsaturated fatty acids / low in saturated fatty acids) optimal for *H. bacteriophora* growth *in vitro*
- *H. bacteriophora* grown *in vitro* with insect lipids have greater lipid reserves (major fatty acid = 18:1), similar to *in vivo*

Dauer formation and metabolism in nematodes

Caenorhabditis elegans dauer stage has slower metabolism & larger energy stores than non-dauer juveniles.

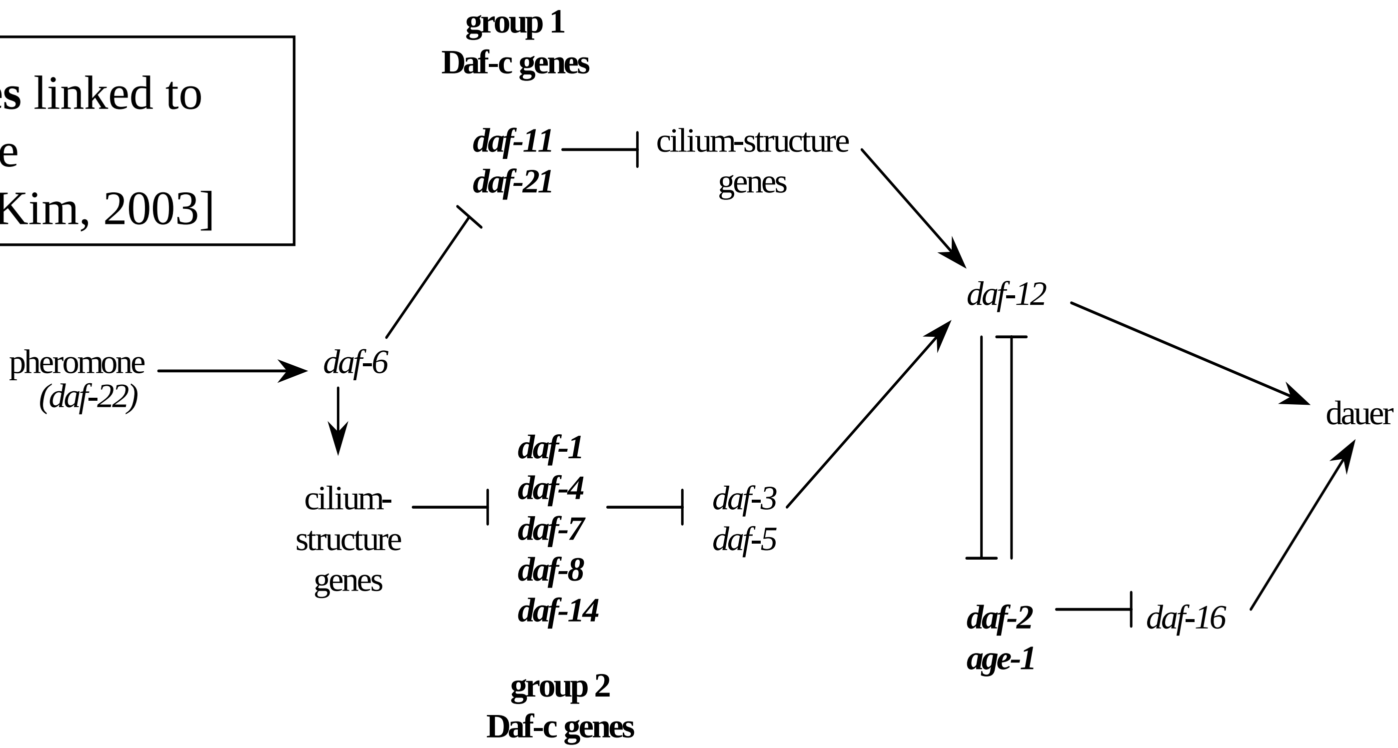


Presumably the same is true for IJ (dauer) of EPN?

Dauer formation and metabolism

Signalling pathways for dauer larvae: *Caenorhabditis elegans*

1984 genes linked to dauer stage
[Wang & Kim, 2003]



RNAi analysis of genome has identified **305 and 112 genes involved in reduced and increased lipid storage** respectively (many have mammalian homologues) [Ashrafi *et al*., 2003]

Energy reserves and EPN

- Correlation between energy stores per IJ, infectivity and survival but other factors can also influence shelf-life of products
- The fatty acid composition of the medium influences lipid content and fatty acid composition in *in vitro* IJ
- Saturated fatty acids best energy stores but max.lipid content and nematode growth associated with mono-unsaturated fatty acids
- Knowledge of genes enriched in *C. elegans* dauer stage may assist in understanding EPN biochemistry and quality of IJ *in vitro*