Novel technological solutions – the potential role and limitations of fungi in insect pest control programmes.

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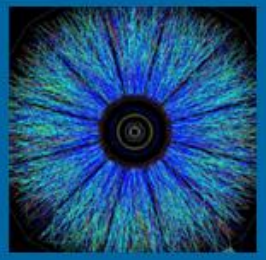
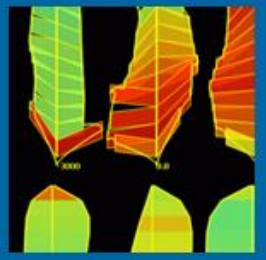



ICF National Conference 2015 –
Tree Health, Resilience and Sustainability.
Angel Hotel, Cardiff. 22-23 April 2015

Outline of Presentation

- Introduction to Entomopathogenic fungi (EPF)
- Why develop EPF?
- EPF Limitations
- Enhancing EPF efficacy



Introduction to EPF

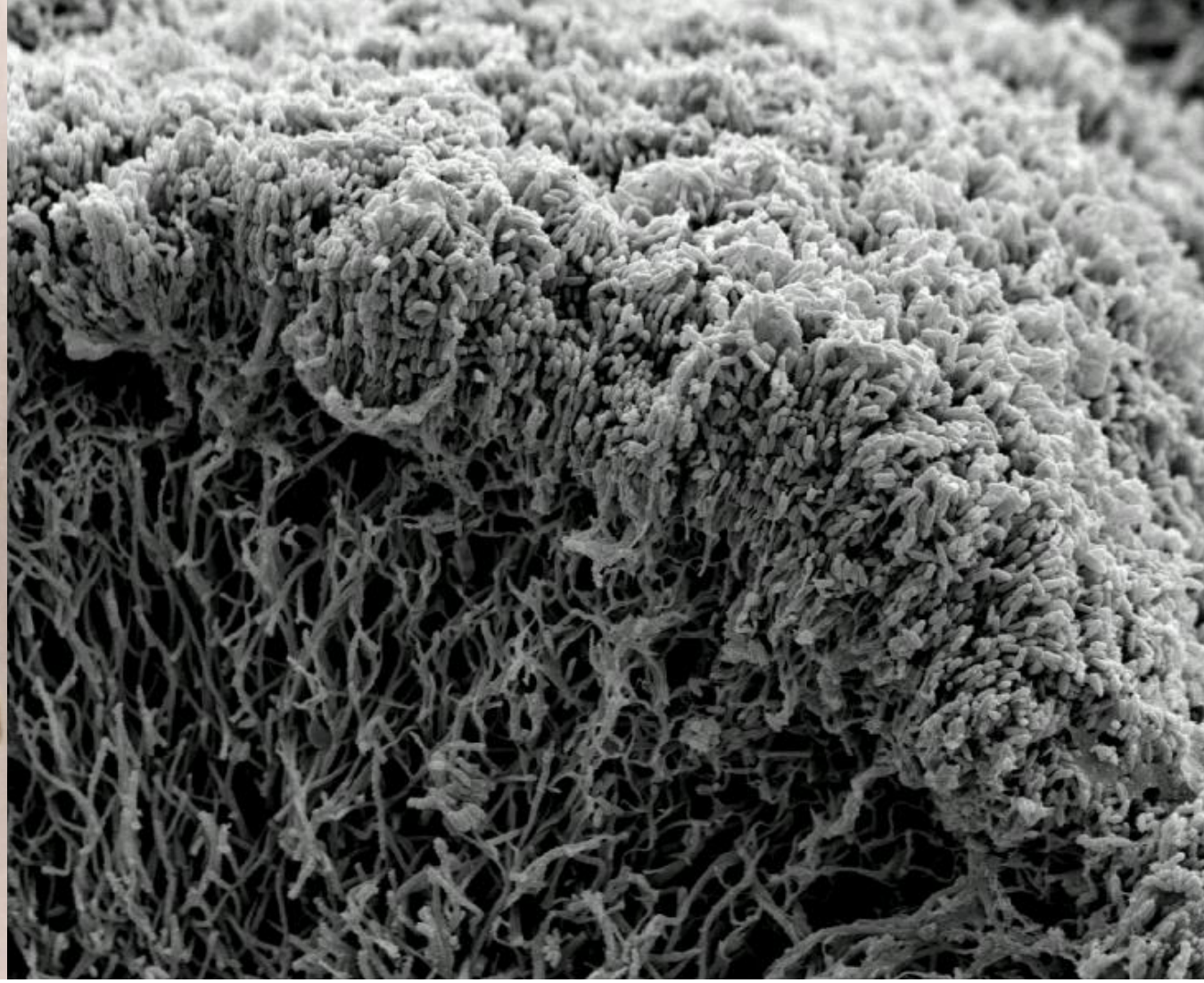
- Over 700 different species of EPF.
- Important genera: *Metarhizium*, *Beauveria*, *Isaria* and *Lecanicillium*.
- Present in soil.
- Worldwide distribution.



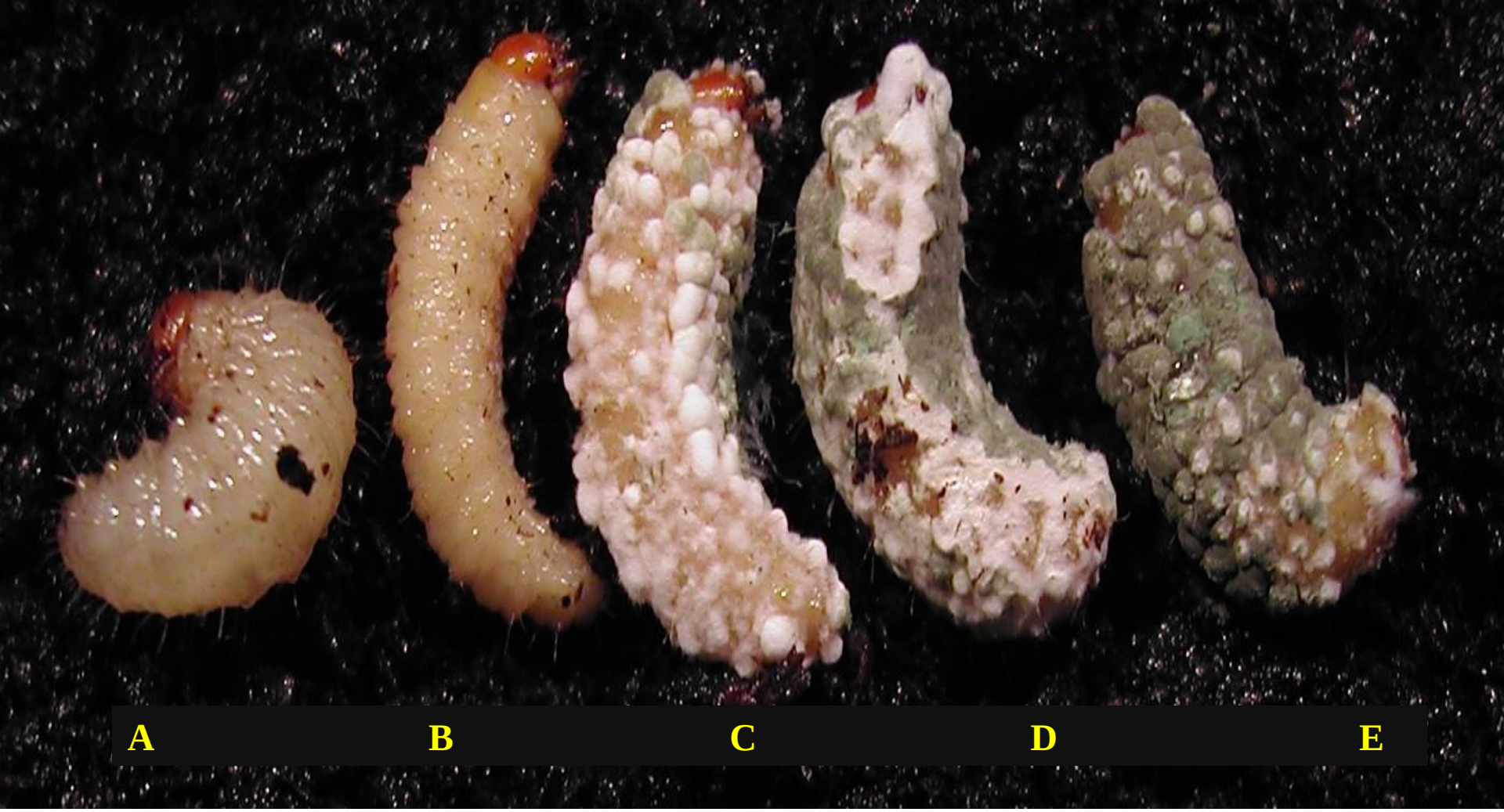

Metarhizium life cycle

Conidium = infective unit (active ingredient)

Conidia germinate and penetrate the host cuticle using a combination of enzymes and mechanical force



After colonising the host, the fungus emerges and sporulates.



BVW larvae at different stages of *M. anisopliae* infection

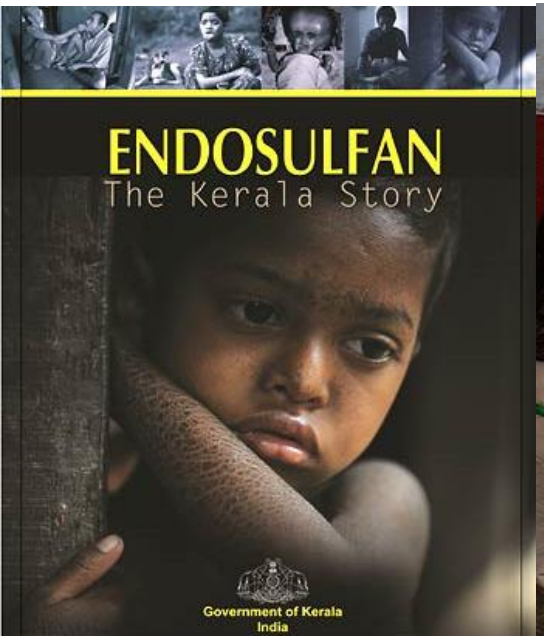
A. Healthy larva

B. Dead larva 3-5 days post inoculation

C-E. Cadaver 2-3 post mortem

Why develop fungi for pest control?

- **More specific - restricted to target or specific arthropods. Pose little risk to humans (unlike chemical pesticides).**



- **Alternative** - where pests are resistant to chemical pesticides
- **Environmentally friendly** - no pollution like chemicals

One tablespoonful of spilled pesticide concentrate could pollute the water supply of 200,000 people for a day. (MAFF)

Source: http://www.pan-uk.org/waste-pesticides/pesticide-disposal

- **Replacement for chemicals being phased out (ca. 67% withdrawn in EU 2009)**

- **EC Legislation - EC regulation 1107/2009 & Directive 2009/128/EC:**

  - *Obliges EU Member States to implement principles of IPM with priority to be given to non-chemical methods of pest control.*

http://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides/index_en.htm
http://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides/ipm/index_en.htm

Challenges using EPF

Chemical pesticide	EPF
• Wide host range	• Narrow host range. Need several strains.
• Fast acting (1-3 days)	• Slow acting (>3 days)
• Effective over wide temperature range	• Less effective at extreme temperatures

Enhancing EPF efficacy

- Use with low dose (1-10% recommended rate of chemical pesticide).
- Exploit synergies with entomopathogenic nematodes (EPN).
- Use with botanicals and semiochemicals (natural insect behaviour modifying chemicals).

Metarhizium to control black vine weevil

- Black vine weevil (BVW) are polyphagous – >200 host plants.
- Major pest of ornamentals, nursery stock and soft fruit
- Adults feed on foliage
- Larvae feed on plant roots - most damaging.
- Global costs of BVW ca. $1 billion

Untreated plant infested with BVW larvae

Untreated – media removed

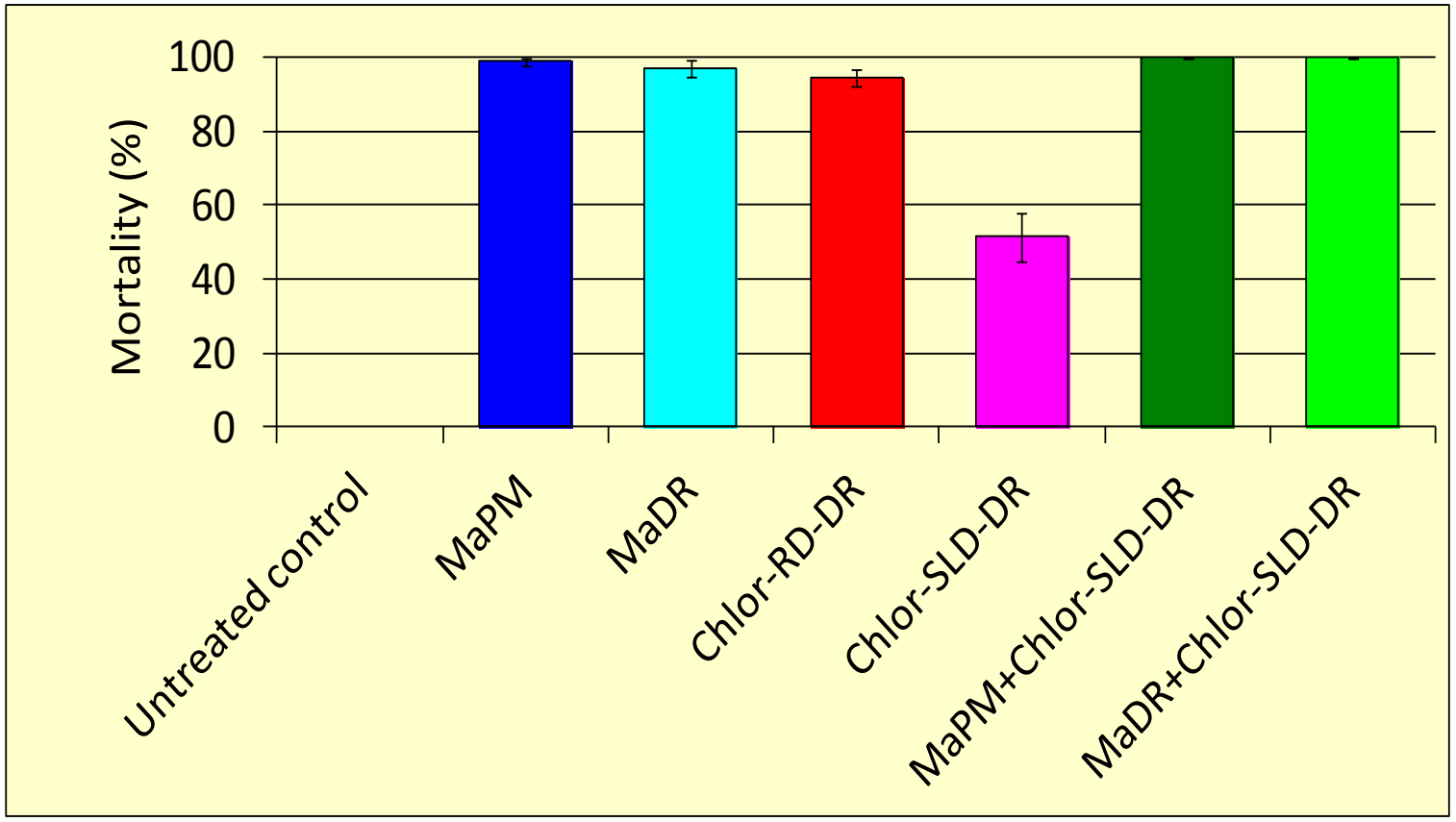
***M. anisopliae* treated – media removed**

- **Criticism of EPF – act slow, less efficacious**

EPF + low dose insecticide

- *Metarhizum* used with sublethal dose (SLD) of insecticide.
- One of several **“Stress & Kill” Strategies** being developed at Swansea University.
- Chemical stresses the insect and increases it susceptibility to the fungus.
- Tested on ornamentals and strawberry plants.

- “Worse case scenario” - 20 BVW eggs per strawberry plant.
- Destructively assessed 6 weeks post-infestation to determine number of live larvae per pot.



Chlor = chlorpyrifos, SLD = 10% RR = 90% reduction in pesticide

Benefits of using EPF with SLD of pesticide

1. SLD chemicals enhance efficacy of *Metarhizium*
2. Pest stops feeding - gives immediate protection
3. Gives fungus more time to kill its host
4. Control similar to recommended rate of chemical
5. Reduces pesticide inputs by 90%
6. Reduced residues allows for continuous cropping (more money for growers)
7. Safer for humans and the environment

"Stress and Kill" Strategy

Excellent results when *Metarhizium* is used with:

- Other insecticides used at low doses *e.g.* 1% (RR) imidacloprid or 10% (RR) fipronil [1]
- Botanicals *e.g.* spent neem cake [2]

1. Shah, F.A., Ansari, M.A., Prasad, M. & Butt, T.M. 2007. Evaluation of black vine weevil (*Otiorhynchus sulcatus)* control strategies using *Metarhizium anisopliae* with sublethal doses of insecticides in disparate horticultural growing media. Biological Control. 40: 246-252

2. Shah F. A., Gaffney, M., Ansari, M. A., Prasad, M. &. Butt, T. M. 2008. Neem seed cake enhances the efficacy of the insect pathogenic fungus *Metarhizium anisopliae* for the control of black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Biological Control 44: 111-115

Exploiting synergies between BCAs

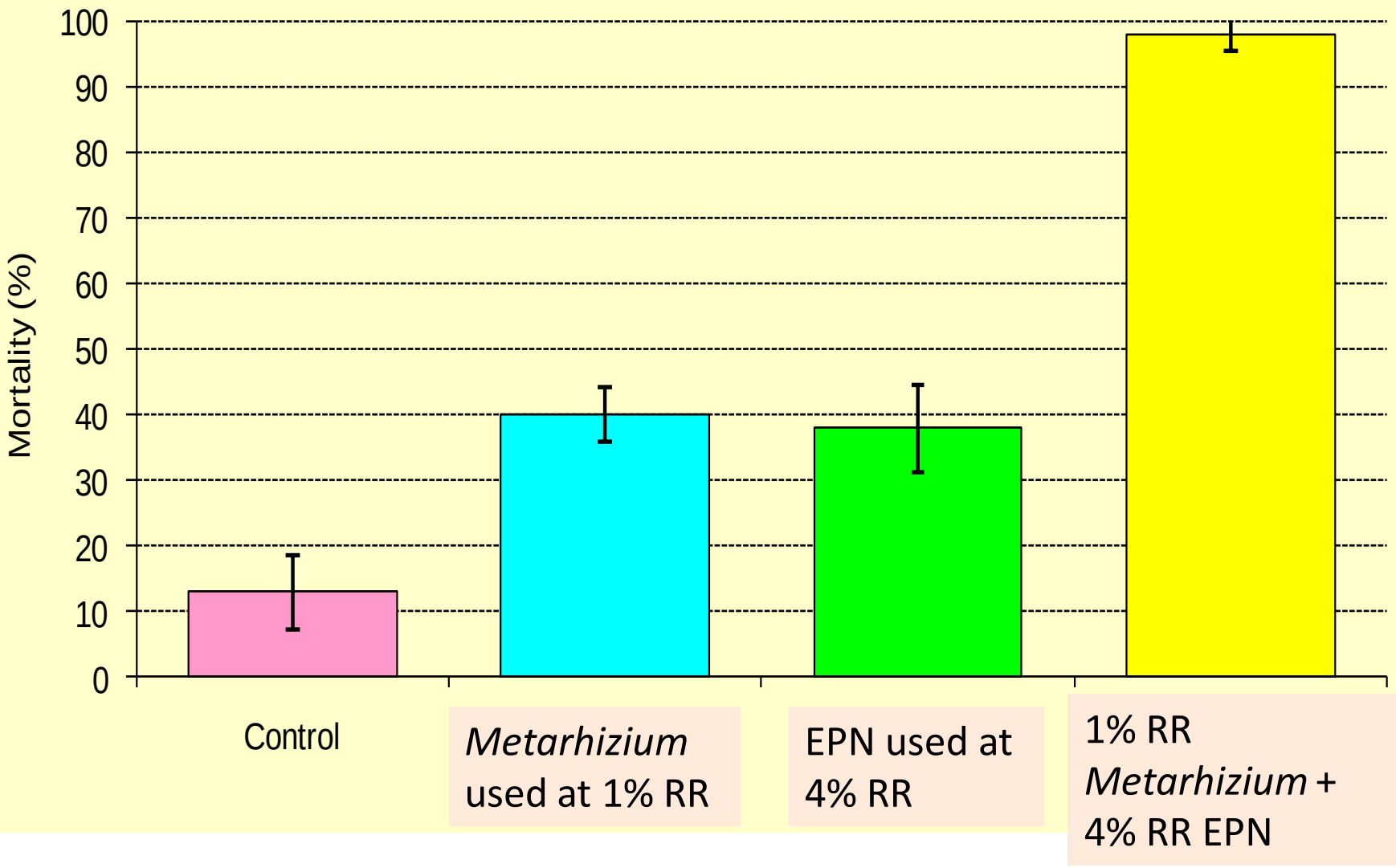
- *Metarhizium* works synergistically with entomopathogenic nematodes (EPN) in killing BVW larvae [1].
- Allows each agent to be applied at rates significantly lower than the recommended rate (RR)
- Growers save money
- Totally organic

1. Ansari, M.A., Shah, F.A. & Butt, T.M. 2008. Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) control. *Entomologia Experimentalis et Applicata*, 129: 340-247.

Metarhizium and EPN work synergistically

- *Metarhizium* recommended rate (RR) = 10^{10} conidia/l of soil-less plant growing medium
- EPN RR = 50 IJs per cm2
- Synergy between these BCAs allows
  - *Metarhizium* used at 10^8 conidia/l = 1% RR.
  - EPN (*Heterorhabditis bacteriophora*) used at 2 IJs/cm^2 = 4% RR.
- This strategy offers potential savings for growers because less is product used.

Synergy between *Metarhizium* and EPN (*H. bacteriophora)* against 3rd instar BVW larvae



Metarhizium to control pine weevil

- Pine weevil (*Hylobius abietis*) – major forest pest.
- Adults damage/kill young plants.
- Eggs laid on stumps of recently felled trees.
- Larvae feed behind bark.

- Adults controlled using alpha cypermethrin
- Derogation for cypermethrins ends 2017.
- Forest Stewardship Council certified companies must find alternatives to chemical control.
- EPN - *Steinernema carpocapsae* - used to control pine weevil larvae – can give inconsistent results.

Metarhizium + EPN

- *Metarhizium* kills pine weevil adults, larvae and pupae [1].
- 80-90% control achieved.
- *Metarhizium* works slowly at low temperatures.
- Persists behind bark (>7 months).
- EPN only kill larvae and are short-lived (few weeks).

1. Ansari, M. & Butt, T. (2012). Susceptibility of different developmental stages of large pine weevil Hylobius abietis (Coleoptera: Curculionidae) to entomopathogenic fungi and effect of fungal infection to adult weevils by formulation and application methods. Journal of Invertebrate Pathology 111(1), 33-40.

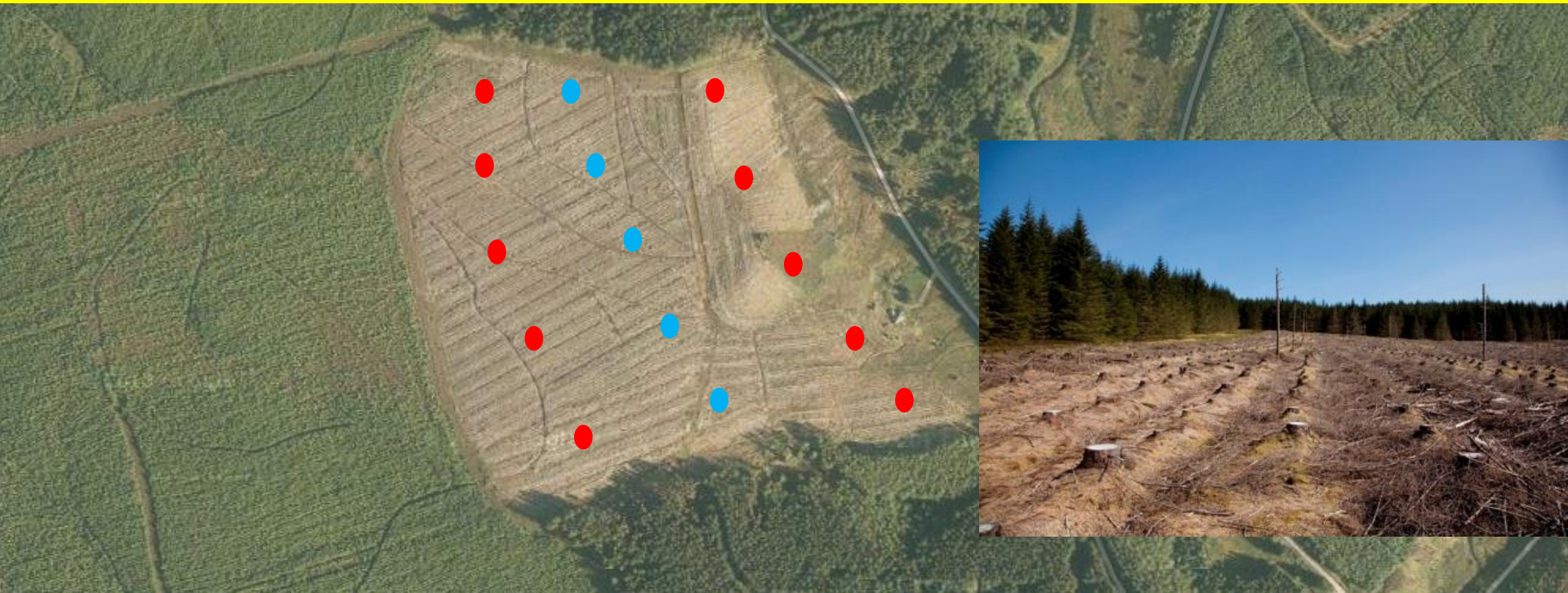
- EPF + EPN combinations give more consistent control even at reduced rates.
- Reduced application rates offer potential savings.

Metarhizium use with semiochemicals

- Other biopesticides to use with *Metarhizium* include attractants and repellents.
- Attractants used in **"Lure & Kill" Strategy** - luring pest to control agent (cost effective).
- Repellents used to prevent:
  - Oviposition
  - Feeding damage of saplings
- Attractants and repellents could be used in **"Push-Pull"** pest control programmes.

“PUSH PULL”

- **Attractants** used to get adults to lay eggs on treated stumps.
- **Repellents** used to deter egg laying.
- Concentrates pests – requires less control agent.



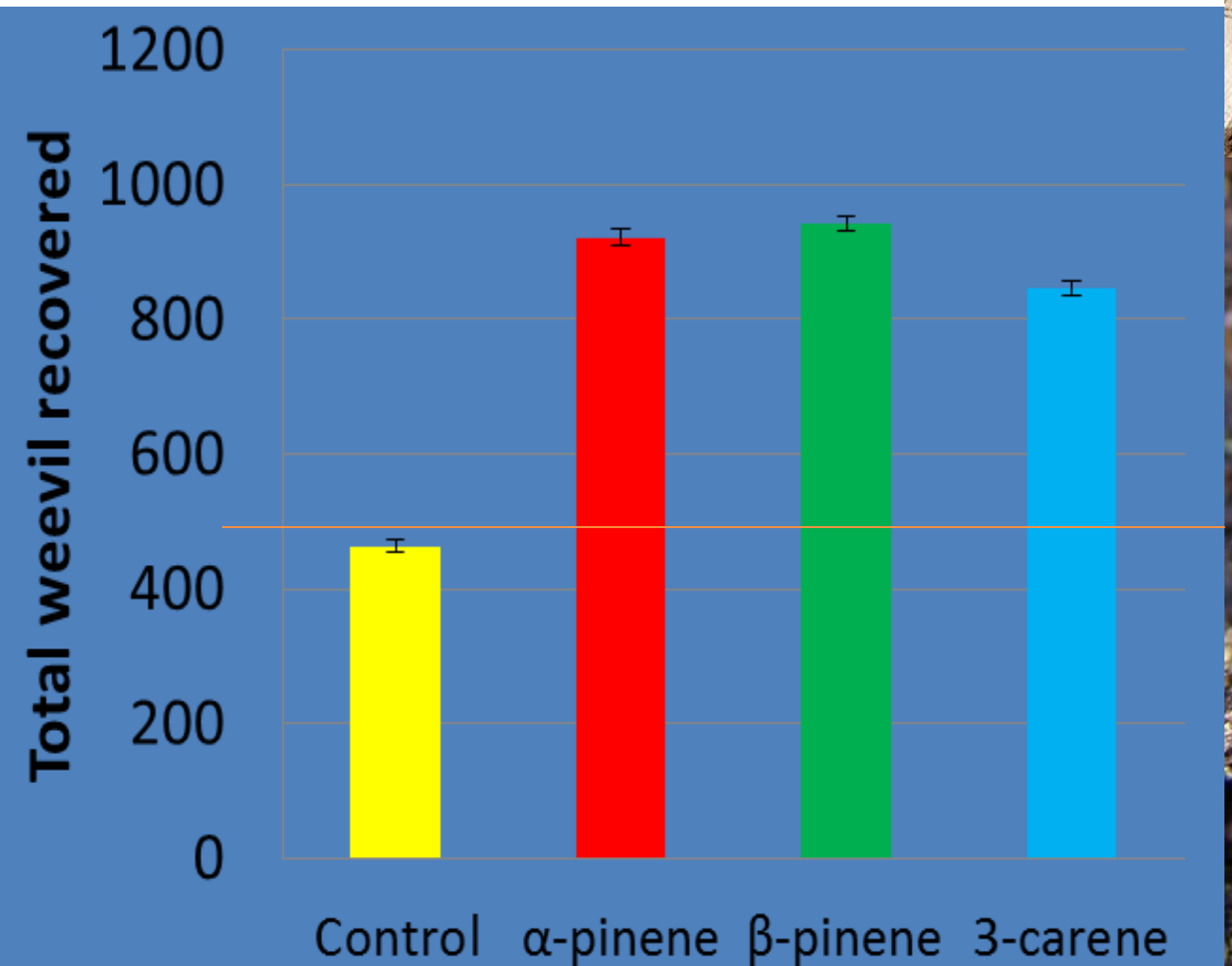
Identification of attractants and repellents

- Large number of compounds screened.
- Compounds included:
  - Pine/spruce volatiles (e.g. α-pinene, 3-carene)
  - Botanicals (e.g. eucalyptus, garlic)
- Compounds deployed in:
  - Polymer string wrapped around billet/stump/sapling.
  - Waterproof glue painted onto billet/stump.

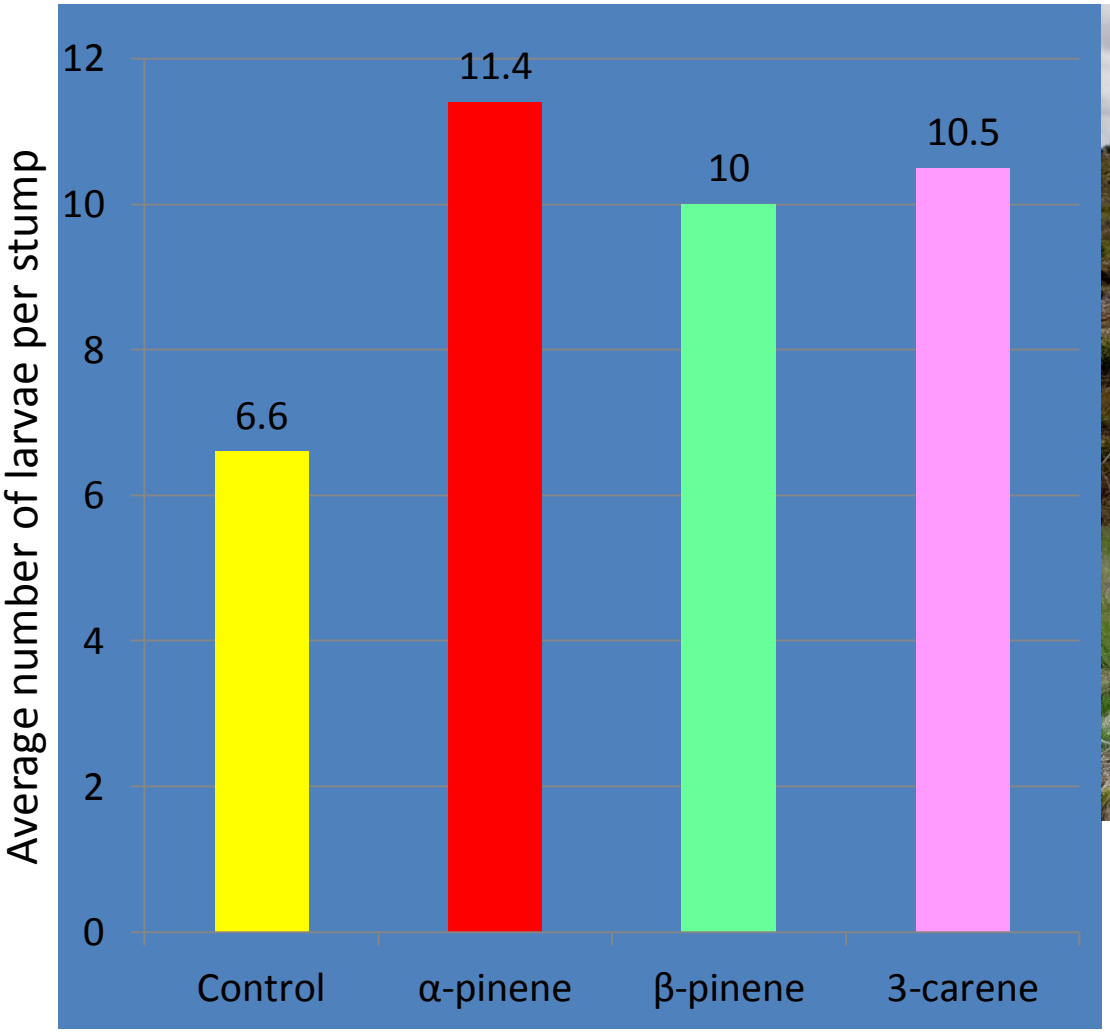



- Trials conducted in Scotland and Wales.
- Several hundred billets and stumps used in trials.
- Assessment:
  - Number of adults on billet + feeding damage
  - Number of larvae recovered from stumps.

- Attractants – encourage females to lay eggs on treated stumps.
- More larvae recovered from treated than control plots.



- Average of the average number of larvae recovered per stump was higher **(34-42%)** in treated than control plots.



Stump before and after assessment.

“Lure & Kill” Strategy

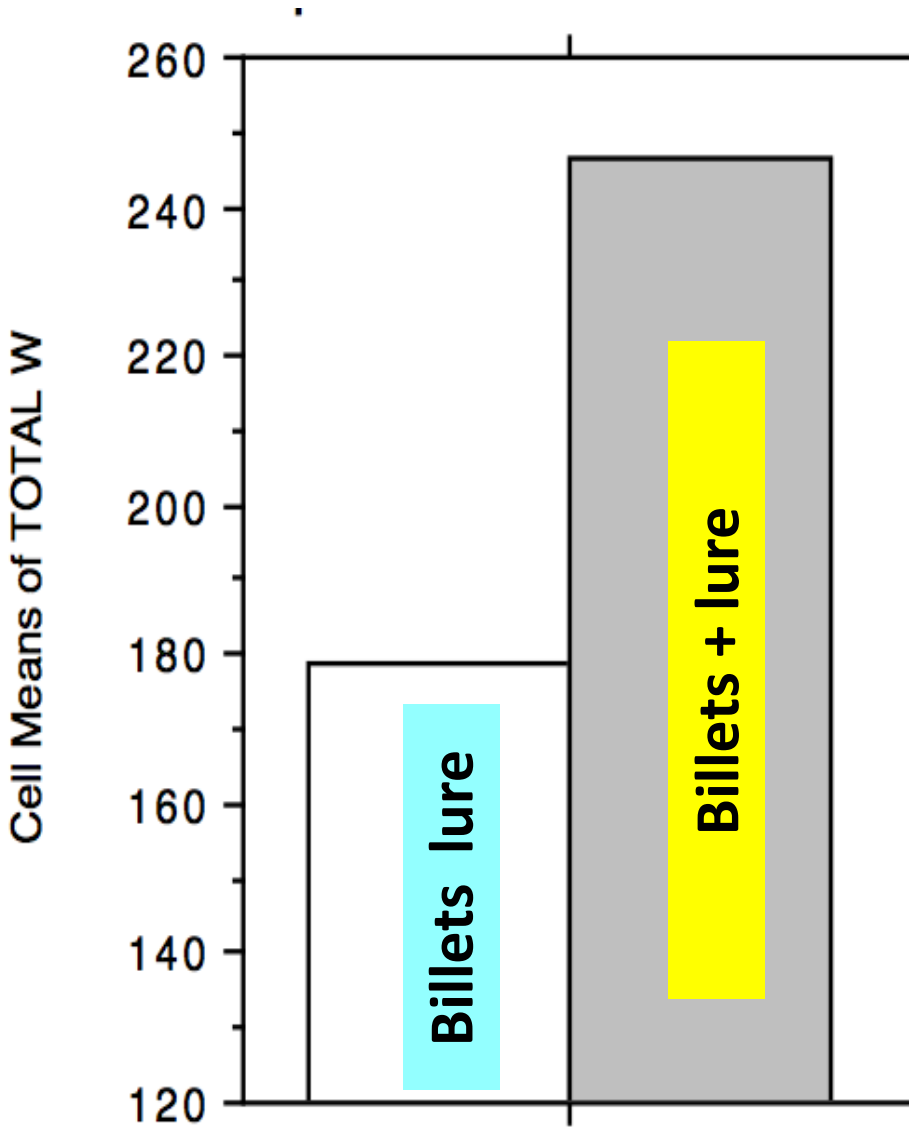
- Concentrating the weevils will make it easier and cheaper to control.
- Less control agent will be required.
- Billets attract pine weevil and are a feeding station.
- Several billets more attractive than single billet.



- Potential exists to reduce number of billets or billet stacks by using lures in biodegradable dispensers.
- Patent (PCT/GB2013/000546) filed for a blend (Mix 3) which is attractive to pine weevil adults.

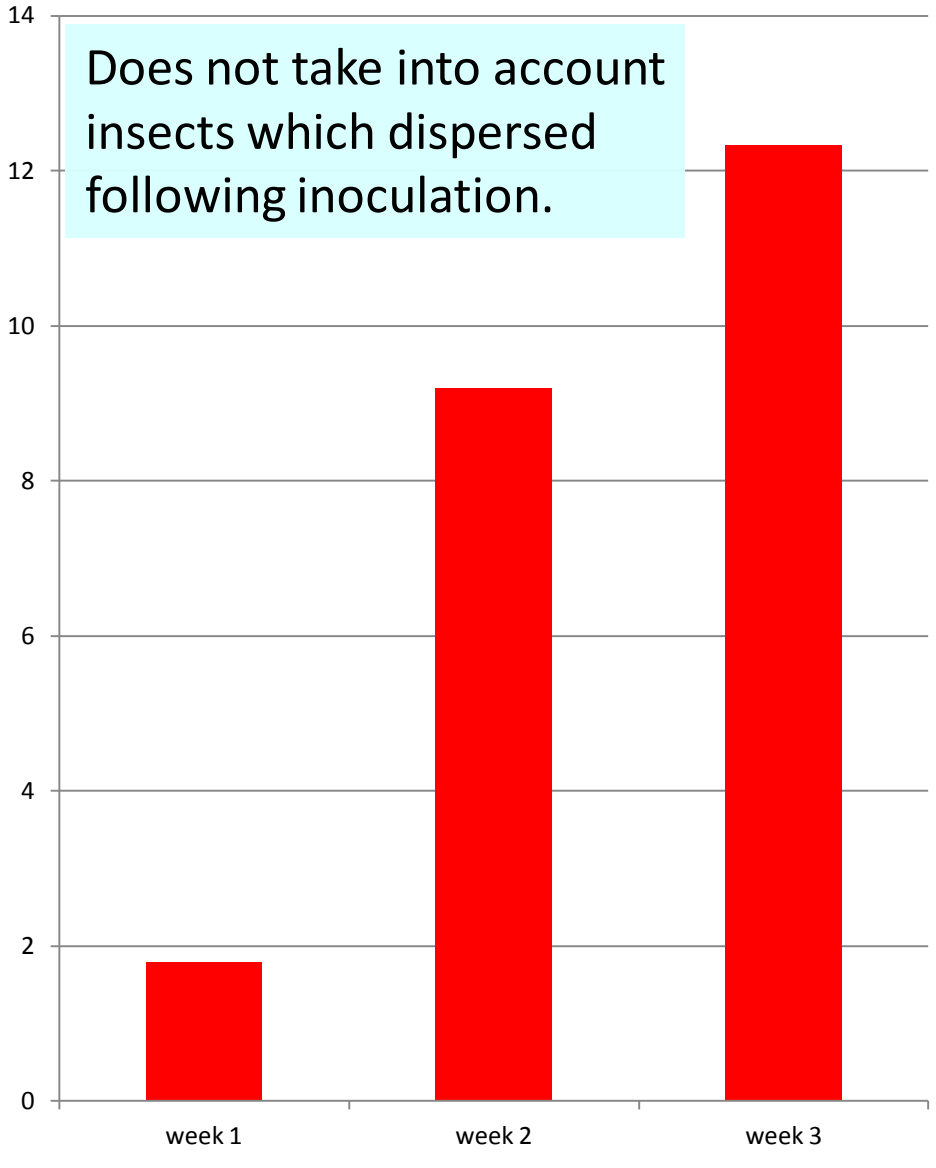


More weevils captured on billets with patented lure (Mix-3) than billets without lure.



- Ultimate goal is to place *EPF* under billet with lure as part of "Lure & Kill" strategy for pine weevil control.
- Field trials conducted in 2014 - results are encouraging.

% Mortality of PW collected at 3 time points (billet + lure + EPF)



- “Lure & Kill” Strategy being optimised.
- Serendipitous discovery of new source of powerful attractants and repellents.
- Compounds currently being isolated and characterized.



Summary

- EPF show much promise for pest control.
- *Metarhizium* efficacy enhanced when used with:
  - EPN (EPF-EPN "**synergy**")
  - Low dose insecticides ("**Stress & Kill**").
- Botanicals and semiochemicals show much promise for use in pest control programmes.
- Attractants can be used to:
  - Influence pine weevil oviposition
  - Lure the pest to the control agent (**"Lure & Kill"**).

Acknowledgements

Prof Hugh Evans
Mr McAllister
Mike Harvey
Imam Sayyed
Richard Parsons
David Edwards
Graham Chalk
Paul Sopp
Dr Nadeem Kardar
Dr Shah
Dr Ansari
Dr Piasecki
Dr Harper
Dr Owen Jones
David Laughlin
Mustafa Muntazir
Mr Richard Massy
Mr Yogendra Gaihre
Dr Roger Moore
Dr Greenfield
Dr Piasecka
Mr Taylor
Ms Woodhouse
Andrew Shearer
John Flaherty
Prof Rory Wilson
Dr Shazia Khan

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