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Biopesticide-based products and strategies for control of tree pests

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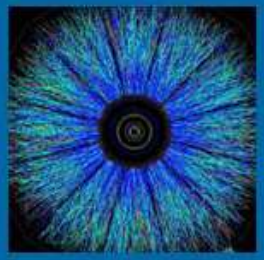


INNOVATION FOR CHANGE
New drivers for tomorrow's forestry
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Outline of Presentation

- Biopesticides
- Products and strategies for control of:
  - Chestnut weevil and chestnut tortrix
  - Pine processionary moth (PPM)
  - Pine weevil (PW)




Biopesticides

- **Biopesticides** are naturally occurring plant protection products and include:
  - Microbial biological control agents (fungi, viruses, bacteria)
  - Semiochemicals (e.g. pheromones)
  - Botanicals (plant extracts, essential oils)
- Global biopesticides market growing rapidly (CAGR 16%) - will reach US$7.6 billion by 2022.

Many drivers:

- Withdrawal of many pesticides (>67%, 2009)
- EU directives e.g. Directive 2009/128/EC-sustainable use of pesticides
- Supermarkets/consumers want zero residues
- Insecticide resistance in pest populations
- Environmental protection - promote biodiversity

- **Biopesticides weaknesses** - slow acting, inconsistent results, narrow host range.
- It can take 2-7 days for entomopathogenic fungi (EPF) to kill – speed depends on fungal virulence, dose and target host.

- **Biopesticides have their strengths** - in some instances they are the **best** or **only alternative** to chemical pesticides.
- **Efficacy enhanced using innovative strategies** e.g. “Lure & kill”, “Stress & kill”



Metarhizium for control of chestnut pests

- Sweet chestnut (*Castanea sativa*) source of timber and nuts
- UK Chestnut production - 18,000 ha (Kent, Sussex)
- Nuts have high retail value

- Vast sweet chestnut forests in Turkey.
- Turkey 2nd largest producer in world – ca. 64,750 tonnes (FAO Statistics, 2016)
- Trees and nuts attacked by various insect pests
- Growers do not want to use chemical pesticides – impacts on health and biodiversity

Chestnut weevil, (*Curculio elephas)* and **Chestnut tortrix** (*Cydia splendana)* lay eggs on nuts of **oak** and **sweet chestnut**

Curculio elephas

Cydia splendana

Metarhizium – best solution for pest control

- Fruit placed in pile on soil then covered with leaf litter.
- Heat generated causes fruit to dehisce and nuts fall out – easy to harvest.
- Weevil and moth larvae leave nuts and pupate in soil.
- Pests are highly concentrated – allows for targeted control.

Fruit (burrs)

Metarhizium – “top-dress” application

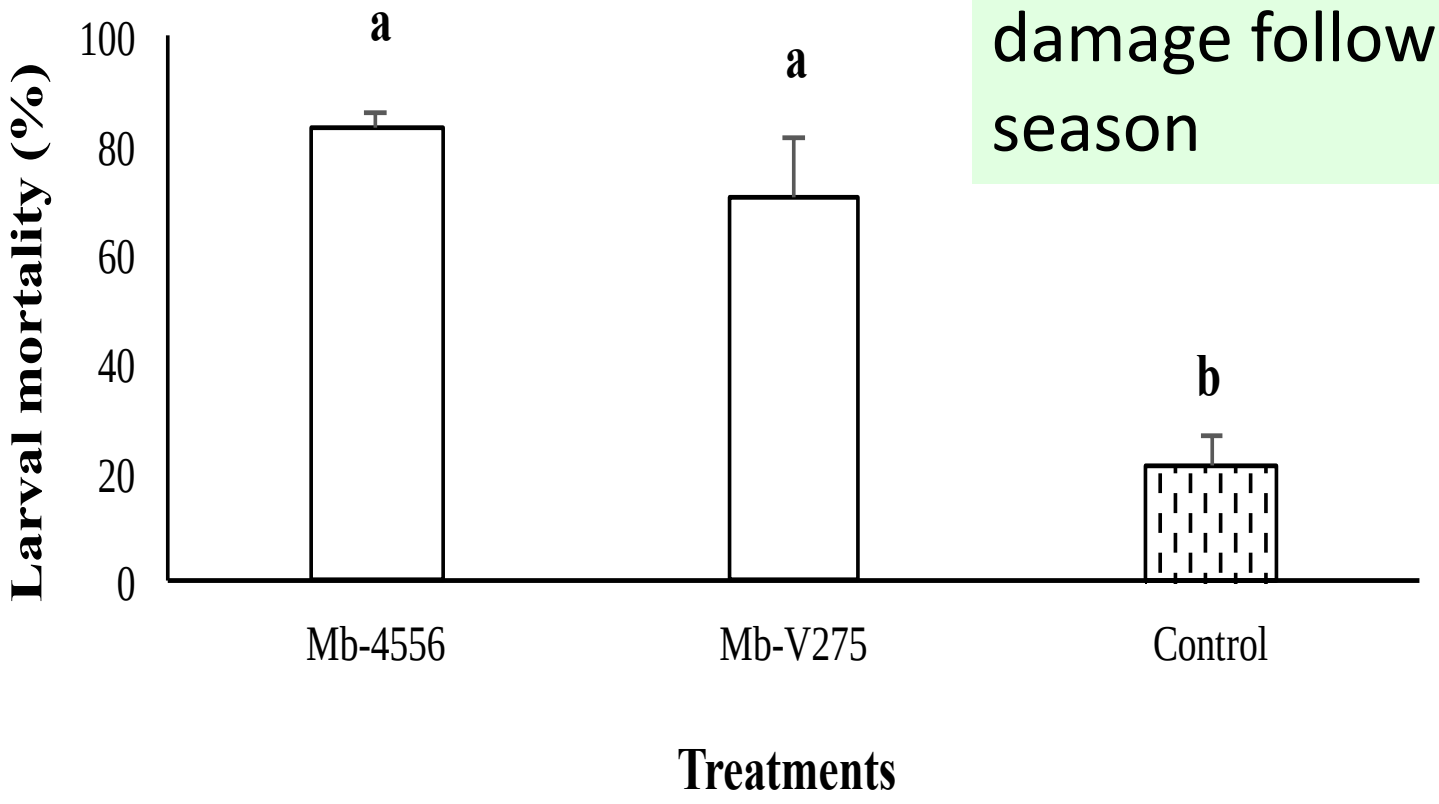
Larvae pupate in top 5-10cm of soil. Highly concentrated – easier to control.

Asan, C., Hazir, S., Cimen, H., Ulug, D., Taylor, J., Butt, T. & Karagoz, M. (2017). An innovative strategy for control of the chestnut weevil *Curculio elephas* (Coleoptera: Curculionidae) using *Metarhizium brunneum. Crop Protection* 102, 147-153.

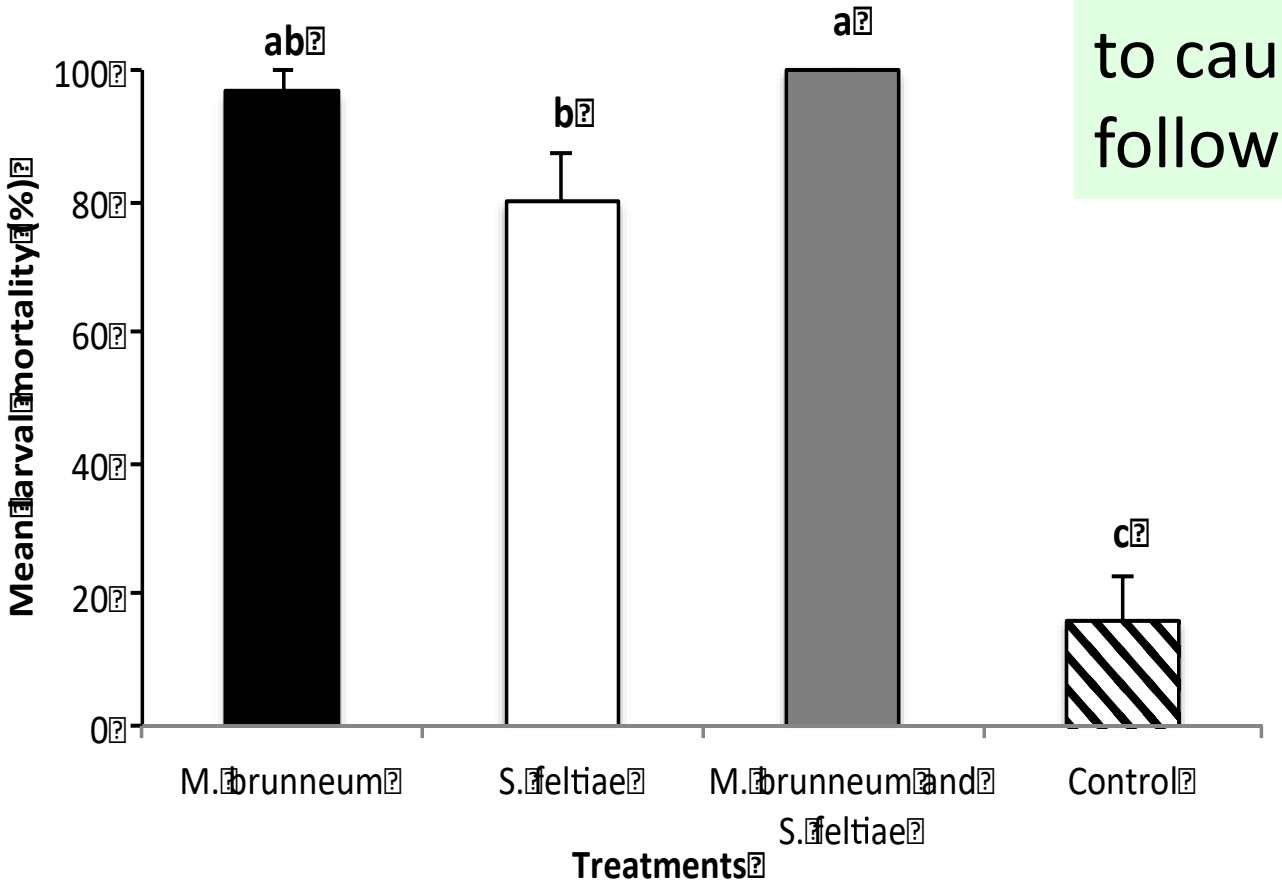
Mean % mortality of *Curculio elephas* larvae following "top-dress" application of *M. brunneum* strains V275 and 4556.

Different lower case letters above the bars indicate significant differences at P<0.05.

Fewer adults emerge to cause damage following season



Mean % mortality of *Cydia splendana* larvae by *M. brunneum 4556* and the EPN *Steinernema feltiae* used alone or together.



Fewer adults emerge to cause damage following season

Pine processionary moth (PPM)

- *Thaumetopoea pityocampa, T. wilkinsoni*
- Invasive species – spreading North from Mediterranean
- Major pest of pine – feed on needles
  - Stunt or kill trees
  - Reduce pine nut yield

- Urticating hairs – pose a serious risk to humans and animals.
- Toxin (thaumetopoein) - can cause extreme skin irritation, respiratory problems, conjunctivitis, anaphylaxis (allergic reaction) and even blindness.

- Portero et al., 2012. Corneal Inflammation From Pine Processionary Caterpillar Hairs. *Cornea, June 5* http://www.ncbi.nlm.nih.gov/pubmed/22673853
- Battisti, A., Larsson, S., & Roques, A. (2017). Processionary moths and associated urtication risk: global change–driven effects. *Annual review of entomology*, *62*, 323-342.
- Costa, D., Esteban, J., Sanz, F., Vergara, J., & Huguet, E. (2016). Ocular lesions produced by pine processionary caterpillar setae (Thaumetopoea pityocampa) in dogs: a descriptive study. *Veterinary ophthalmology*, *19*(6), 493-497.

Procession

Pupation in soil

Moth emergence

Nests 3-5th instar

Urticating hairs

Mar-Apr

May-June

July-Aug

Sept-Oct

Nov-Dec

Jan-Feb

1st -2nd instar

Egg laying

Biopesticide-based solutions for PPM control

- Solutions for all stages of development
- Mating disruption pheromone - targets adults, reduces oviposition
- Plant derived oviposition repellents
- *Metarhizium* strains – kill eggs, larvae, pupae
- Botanicals – kill larvae

- Aydin, T., Branco, M., Guven, O., Goncalves, H., Lima, A. Karaca, I., Butt, T.M. 2018. Significant mortality of eggs and young larvae of two Pine Processionary Moth species due to the entomopathogenic fungus *Metarhizium brunneum*. *Biocontrol Science and Technology*. 28, 317–331
- Sönmez, E., Demir, İ., Bull, J., Butt, T. & Demirbağ, Z. (2017). Pine processionary moth (*Thaumetopoea pityocampa,* Lepidoptera: Thaumetopoeidae) larvae are highly susceptible to the entomopathogenic fungi Metarhizium brunneum and Beauveria bassiana. *Biocontrol Science and Technology*, 1-12

PPM eggs, larvae, pupae are susceptible to EPF

- PPM egg masses are conspicuous, accessible (i.e. easy to spray with EPF)
- Early instar (1st, 2nd) larvae have no urticating hairs – no risk to operators

- *M. brunneum* (V275/ARSEF4556) conidia adhere to surface of egg mass
- Reduces egg hatch and kills emergent larvae (>90%)
- 98-100% mortality of 2^{nd} instars achieved 2-4 days pi, using dose 1 X 10^6 conidia ml^{-1}

Aydin, T., Branco, M., Guven, O., Goncalves, H., Lima, A. Karaca, I., Butt, T.M. 2018. Significant mortality of eggs and young larvae of two Pine Processionary Moth species due to the entomopathogenic fungus *Metarhizium brunneum*. *Biocontrol Science and Technology*. 28, 317–331

- Older (3rd -5th) instar larvae are also susceptible to EPF
- Excellent control when EPF injected into nest: 100% dead 3-6 days pi, using dose 1 X 10^8 conidia ml^{-1}.
- Larvae rest inside nest during day and feed at night. No risk to operator.
- Good horizontal transfer of conidia from treated to untreated larvae inside the nest.

Sönmez, E., Demir, İ., Bull, J., Butt, T. & Demirbağ, Z. (2017). Pine processionary moth (*Thaumetopoea pityocampa,* Lepidoptera: Thaumetopoeidae) larvae are highly susceptible to the entomopathogenic fungi Metarhizium brunneum and Beauveria bassiana. *Biocontrol Science and Technology*, 1-12

- Excellent results obtained both in lab and field trials when 10ml conidial suspension injected inside nest.

M. brunneum strain	Mean % mortality of 3rd instar PPM	
	Lab	**Field**
V275	100	86.9 ± 6.4
ARSEF 4556	100	--
Control	0.8 ± 0.4	3.7 ± 1.6

- High mortality of last instar larvae and pupae
- Pre-treating pupation soil with *M. brunneum* (V275/ARSEF4556) gives good control
- 70-90% mortality using dose of 1.4 X 10^{11} conidia kg^{-1} soil
- 20-40% die as larvae remainder die inside pupal “cell”
- Inoculum available to infect larvae following year




Botanicals offer alternative to EPF

- Large number of botanicals (plant extracts, essential oils) available
- Most botanicals used in cosmetic sector – considered safe
- Not all botanicals have insecticidal properties.

Mortality of PPM larvae 7 days pi following injection of nest with 10ml 1% aq. v/v plant oil.

Treatment	Mean % mortality L3-L4			
	Ginger*	Eucalyptus	Rosemary	Control
Laboratory	71.2 ± 8.5	24.2 ± 6.7	30.0 ± 4.4	1.3 ± 0.8
Field	87.4 ± 6.7	86.7 ± 4.6	76.1 ± 5.5	2.3± 0.9

- 100% mortality, faster control using 10% v/v
- Larvae stop feeding (less frass), stunted growth, stressed, eventually die.
- Higher mortality in field than lab .

- EPF and essential plant oils could be used to control oak processionary moth (OPM, *Thaumetopoea processionea*)
- OPM often forms nest on tree trunk – easy to treat with EPF/botanical
- Biopesticides safer than chemical (deltamethrin) alternative.

Semiochemicals

- Behaviour modifying chemicals – include pheromones and kairomones.
- Species specific sex pheromones widely used for:
  - Pest monitoring
  - Pest control e.g. mating disruption, mass trapping

- **Pests use plant volatiles (kairomones) to**:
  - locate suitable hosts for food or oviposition
  - avoid unsuitable hosts
- **“Attractant” compounds could be used for**
  - pest monitoring
  - mass trapping
  - “lure & kill” pest control

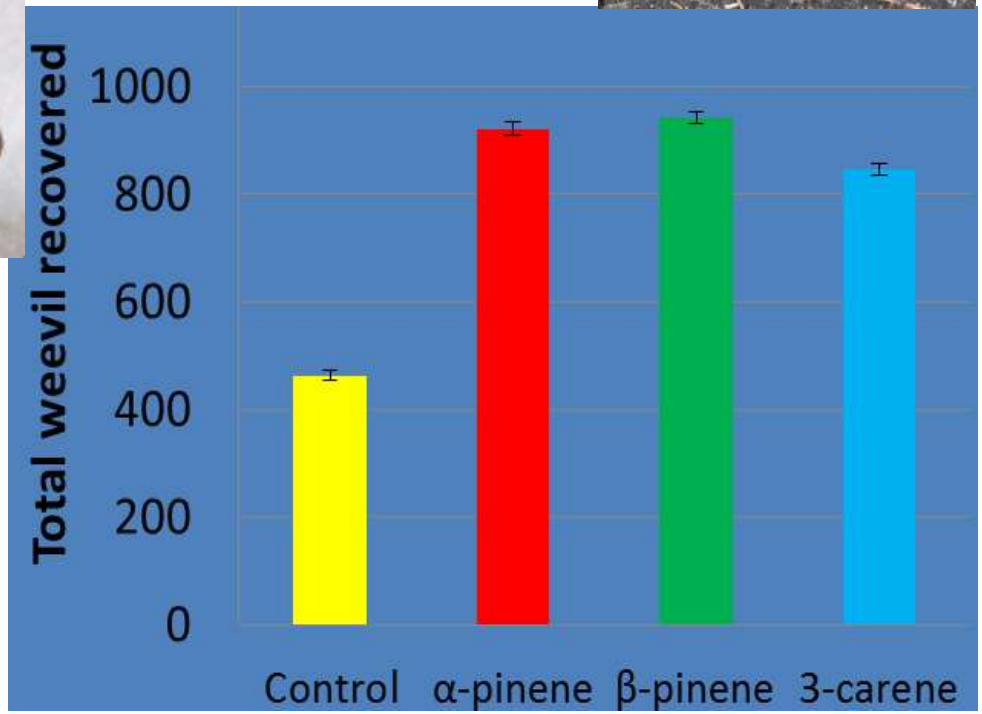
Benefit of “Lure & Kill” pest control strategy

- Attractants lure pests to control agent.
- Targeted control (reduces area to be treated).
- Reduces application rates and cost of control.
- Useful in environmentally sensitive or difficult terrain

Progress to date

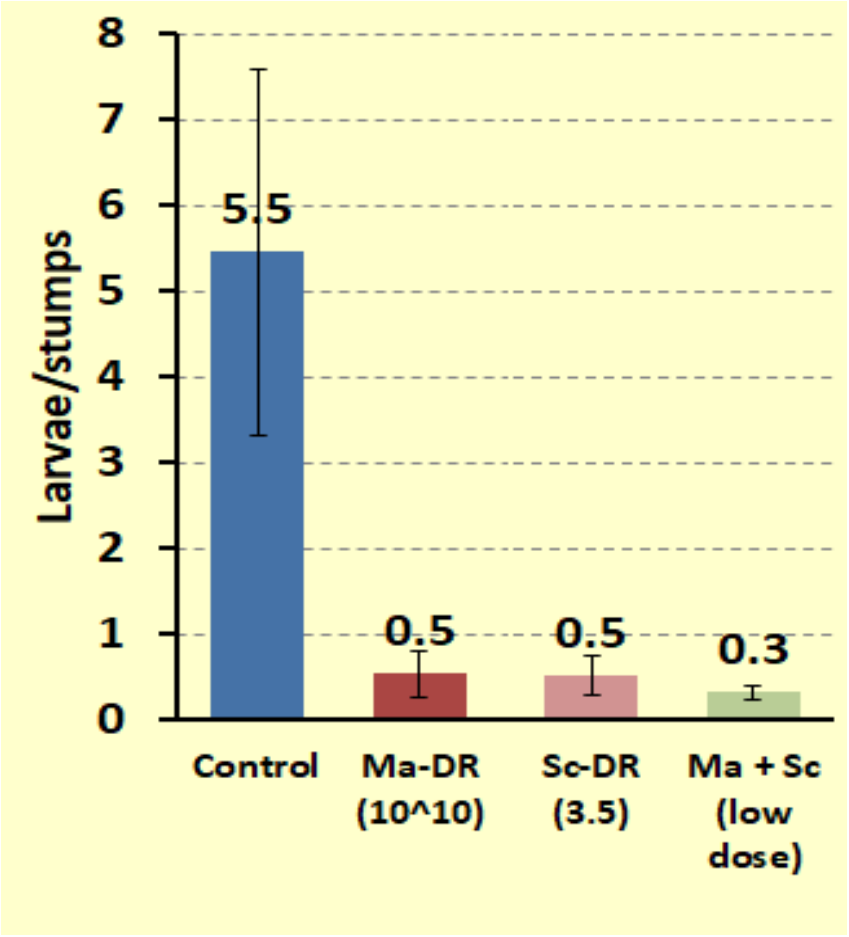
- PW highly damaging to saplings
- PW lay eggs on freshly cut stumps
- Attractants encourage oviposition on treated stumps

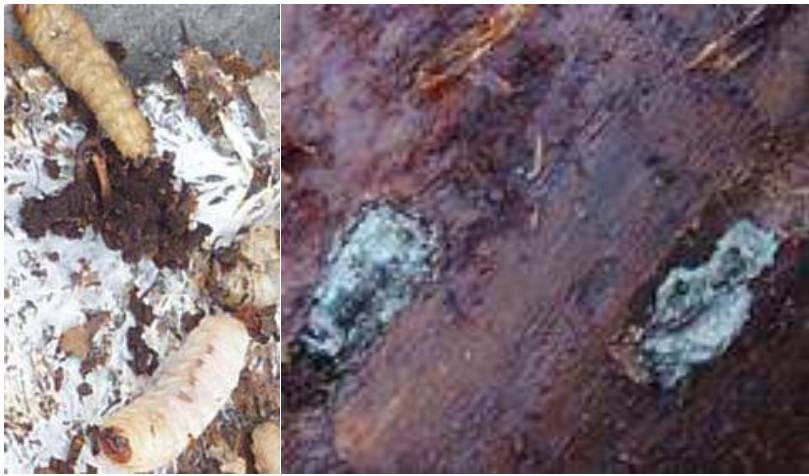
Attractants infused in polyvinyl acetate string



More PW larvae recovered from treated than control plots.

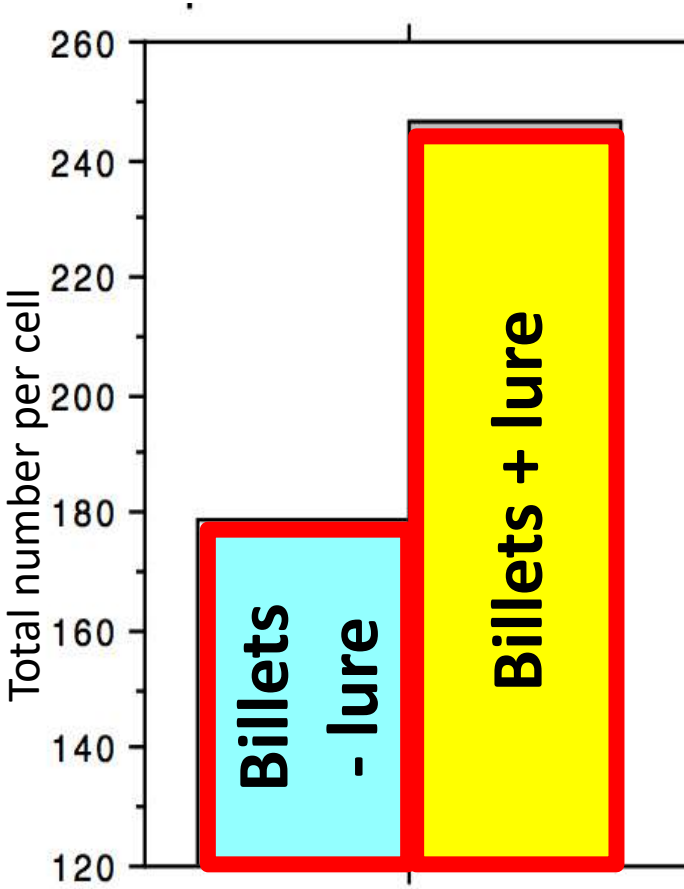
- EPF-EPN combinations give >90% control.
- Encouraging oviposition on selected stumps allows for targeted control – reducing application rates and reducing costs.
- Fewer adults emerge to cause damage to saplings





Lure & Kill for PW control

- Billets release volatiles that attract adult PW. They also act as feeding station.
- Several billets are more attractive than single billet.
- Number of billets reduced using lures in biodegradable dispensers.



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

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

Summary

- EPF, plant oils and kairomones have been identified for the control of PPM, PW and other tree pests.
- These “biopesticides” offer an environmentally friendly alternative to conventional chemical pesticides.
- EPF kill both adult and juvenile stages of most pest species

- Botanicals show much promise for the control of PPM larvae – without exposing operators to the urticating hairs.
- Semiochemicals will play an increasingly important role in pest control programmes.
- Attractants could be used to lure pests to control agents – reducing the area to be treated and help reduce cost.

- Some of the products and strategies could be used to control other pest species that threaten tree and human health.
  - Oils and EPF developed for PPM could be used to control larvae of OPM

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