

# Behaviour modifying chemicals for *Hylobius* management

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#### **Biocontrol & Natural Products (BANP) Group**



Goal: Develop environmentally friendly products and strategies for arthropod pest control.

https://www.swansea.ac.uk/bioscience/research-and-impact/banp/

#### **BANP** group work in different sectors.

• Agriculture, Horticulture, Forestry, Human and Animal Health

#### **BANP group work with different stakeholders**

- Industry developing new products
- Government agencies –regulatory matters
- Growers helping deal with specific issues



#### **BANP Group**

- Active in forestry
- Developing biopesticides (entomopathogenic fungi, semiochemicals) for pine weevil control





#### **Semiochemicals**

= behaviour modifying chemicals

- Pheromones and Kairomones
- Attractants, repellents
- Very little known about pine weevil pheromones



#### Pine weevil respond to

- Host plant volatiles (e.g. monoterpenes, ethanol)
- None host plant and fungal derived volatiles.



#### **BANP Strategy – reduce PW population**

#### Use EPF to control adults

#### Use EPF to control larvae and pupae

#### **Metarhizium use with semiochemicals**

- Attractants used in "Lure & Kill" Strategy luring pest to control agent (cost effective)
- Attractants also lure females to lay eggs on designated stumps.
- **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**

- Pine more attractive than spruce to Pine weevil
- GC-MS identified pine and spruce billet volatiles
- Several pine and spruce volatiles screened, including: αpinene, β-pinene, 3-carene, myrcene
- Several botanicals screened for repellent or antifeedant properties, including eucalyptus, garlic, peppermint
- PW avoid some fungi screened selected fungal volatile organic compounds (VOCs)\*

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.



### Mix3

- Patent (PCT/GB2013/000546) filed for a blend (Mix 3) which is attractive to pine weevil adults.
- Tested under a wide range of conditions, including different dispenser types e.g. vials, Hyalodor trap
- Gives encouraging results





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#### **Evaluation of Mix3 in field trials**

Garlic

Geraniol

Peppermint

- **Mix3 and Peppermint most attractive**
- **Garlic least attractive** •
- **Activity declines with time** •



#### Pipe trap plus Hylodor lure not as effective as conventional billets in field trials

- Mix3 and billets give best result
- Attractants appeared to enhance billets



## Attractants would enhance current monitoring systems.



#### Further evaluation of plant compounds

- Many more plant derived compounds screened alone and in blends
- Studies conducted in Scotland and Wales
- Biodegradable dispensers used for controlled release of the compounds
- Vials could be placed alongside billets or embedded in billets



- Attractant/repellent blends tested by FR (Scotland) and Swansea University (Wales)
- Randomised blocks.
- Treatments given blind codes
- Each treatment plot consisted of 8 billets
- Weevils counted and removed weekly.



David Edwards, Alan Wilson, Alun Jones, Dewi & Edgar



BLIND	Wales	Repellent ( <b>R</b> ) or		
CODE	EXPT TRT FULL	Attractan		
B13	Menthol	R		
L9	B-Pinene, Carene	Α		
S7	A-Pinene, B-Pinene, Carene, MyrceA			
C6	A-Pinene, B-Pinene	Α		
G2	CONTROL BILLET HOLE	С		
E5	A-Pinene	Α		
N1	Blank - Hole	С		
A8	CONTROL EMPTY VIAL	С		
I4	Carene	Α		
J15	A-Pinene, B-Pinene, Carene, Myrce	Α		
F10	A-Pinene, B-Pinene, Carene	Α		
H11	B-Pinene	Α		
D3	A-Pinene, Carene	Α		
M14	Sabinene	R		
K12	Myrcene	Α		

		Scotland		Repellent (R)
CODE	EXPT TRT FULL		(A) or	
I4	ABCD+ Sabinene+Ocimene			A
L9	Beta-Pinene			A
B13	Menthol			R
E5	Alpha-Pinene			Α
G2	BILLETCONTROL HOLE			С
M14	Sabinene			R
F10	A-Pinene, B-Pinene, Carene			Α
K12	ABCD+Sabinene+Ocimene+Terpinole			Α
A8	EMPTY VIAL ONLY		С	
S7	A-Pinene, B-Pinene, Carene, Myrcen		А	
H11	ABCD+Sabinene			Α
H7	Thymol		R	
F6	Carvone		R	



- Considerable variation in weevil captures between treatments
- Highest capture end Aug to early Sept
- Capture rate high initially but declines as billet ages
- 10 X more weevils collected in Scotland than Wales
- Menthol counts low at both sites
- Myrcene counts generally higher



#### Damage Topography – Heterogenous



#### "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.
- Mix 3 blend appears attractive to pine weevil adults.



### More weevils captured on billets with Mix-3 lure than billets without lure.





- Ultimate goal is to place *EPF* under billet with lure as part of "Lure & Kill" strategy for pine weevil control.
- PW adults attracted to billet would acquire fungus and die.



- Encouraging results obtained in field trials
- Results do not take into account insects which dispersed following inoculation

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



#### Challenge

#### EPF (*Metarhizium brunneum*) conidia germinate which reduces efficacy:

- Less conidia to infect insects (mortality is dose dependent)
- Mycelium (not conidia) repels PW adults (reduces attractiveness of billets/lures)



- FR studies showed that:
  - Although weevil capture was high in the control & lure treatments
  - All Met52 treatments had much lower catches
  - Met52 mycelium not conidia had repellent effect
- Similar observations made in Wales





### PW Count pooled and analyzed as a billet rate, of PW retained/billet/week (i.e. per billet log).



#### Metarhizium volatile organic compounds (VOCs)

- Over 40 *Metarhizium* VOCs identified [1]
- Several shown to influence behaviour of other invertebrates [2-4]
- Some (1-octen-3-ol and 3-octanone) repel several insect pest species [4]
- Hummadi, E.H., Cetin, Y., Demirbek, M., Kardar, N.M., Khan, S., Coates, C.J., Eastwood, D.C., Dudley, E., Maffeis, T., Loveridge, J. and Butt, T.M., 2022. Antimicrobial volatiles of the insect pathogen Metarhizium brunneum. *Journal* of *Fungi*, 8(4), p.326.
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- 3. Khoja, S., Eltayef, K.M., Baxter, I., Myrta, A., Bull, J.C. and Butt, T., 2021. Volatiles of the entomopathogenic fungus, Metarhizium brunneum, attract and kill plant parasitic nematodes. *Biological Control*, *152*, p.104472.
- 4. Bourdon, P.A., Zottele, M., Zafar, Z., Baxter, I., Midthassel, A., Myrta, A., Wechselberger, K.F., Strasser, H. and Butt, T.M., 2023. Behavioral response of three subterranean pests (Agriotes lineatus, Diabrotica virgifera virgifera, Phyllopertha horticola) to the fungal volatile organic compounds 1-octen-3-ol and 3-octanone. *Arthropod-Plant Interactions*, *17*(4), pp.473-483.

#### **Summary**

- Many potential PW attractants and repellents
- Most behaviour modifying chemicals are natural products
- Potential repellents include menthol
- Attractants include alpha and beta pine, carene, myrcene
- Mix3 very promising blend
- Metarhizium mycelium produces volatile organic compounds (VOCs) that repel PW.
- Several *Metarhizium* VOCs shown to repel other insects but still need to be tested against PW

- No doubt that semiochemicals will play a major role in future PW pest management programmes
- Many natural compounds
- To improve monitoring
- Use in lure and kill and push pull pest control programmes
- Manipulating PW behaviour



#### **Acknowledgements**



