



Natural β -triketone insecticides with novel mode of action for mosquito control

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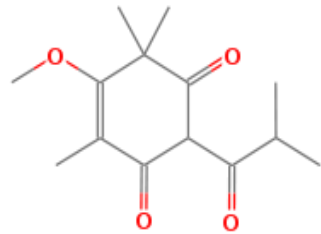
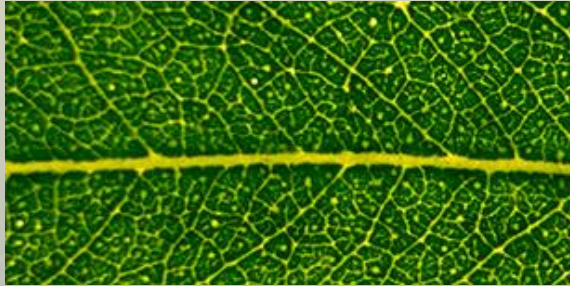
- ❖ Bio-Gene Technology
- ❖ Natural insecticide products
- ❖ Novel Mode of Action
- ❖ Research program – mosquito control
- ❖ Future directions



Bio-Gene's Technology Platform

Bio-Gene is developing two products for key insect control markets

Qcide® - tasmanone

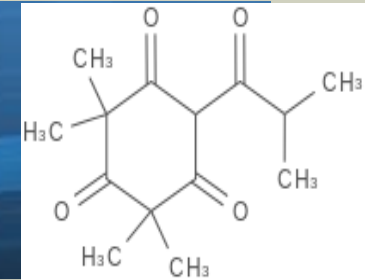
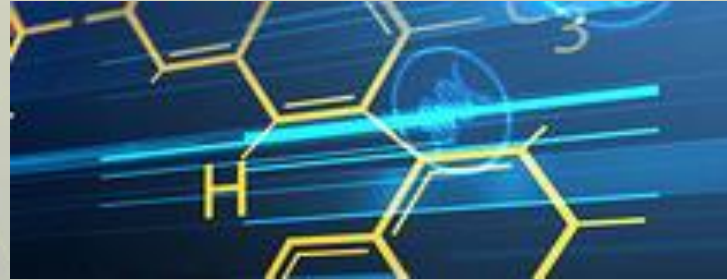


Natural Product

Extracted from the leaves of a chemotype of *Eucalyptus cloeziana* (Gympie messmate)

The leaves contain oil with high levels of tasmanone, a natural compound with insecticidal activity

Flavocide® - flavesone



Nature Identical Compound

Proprietary chemical process to synthesise the beta-triketone, flavesone.

Flavesone is a nature identical insecticidal compound able to be produced at commercial scale



IIIMPORTANCE OF NOVEL MODE OF ACTION IN MOSQUITO CONTROL

- ❖ Insecticide resistance is a significant challenge in all markets where insecticides are used.
- ❖ Adult mosquito control is heavily dependent on very few chemical classes, in particular synthetic pyrethroids.
- ❖ Resistance to these products undermines the effectiveness of product options for mosquito control
- ❖ A new Mode of Action is therefore a significant event in insecticide discovery
- ❖ The Mode of Action (MoA) of an insecticide defines the process of how a compound works on an insect at the molecular level





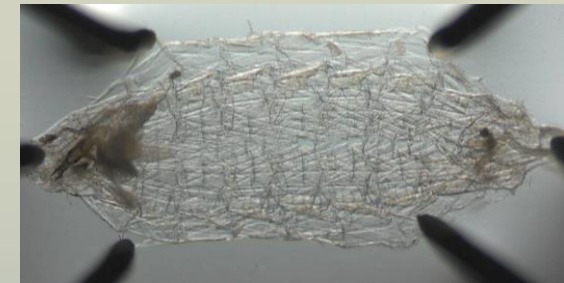
FLAVESONE NOVEL MODE OF ACTION

Bio-Gene has completed studies which confirm the definitive target site of β -triketone compounds in insects. These results provide critical information that creates validation data to support a “new class of chemistry” with IRAC (Insecticide Resistance Action Committee)

Studies to determine the MoA of using flavesone (Flavocide). have included:

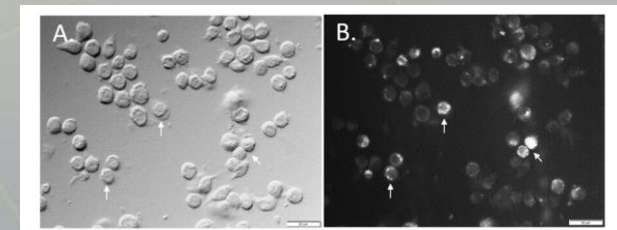
- ❖ Whole cell patch clamp electrophysiological testing initially on mammalian dorsal root ganglion (DRG) neurones confirmed an activity profile unique from MoA's of other known insecticide groups
- ❖ Electrophysiological testing on insect (*Drosophila*) DRG neurones identified the effects of Flavocide in invertebrates compared to vertebrates
- ❖ Results from testing Flavocide on *Drosophila* neurones (& mammalian cell testing) using specific target blockers confirmed the principal action of Flavocide
- ❖ Follow-up studies using heterologous expression further confirmed the specific sites of action of Flavocide transiently expressed in isolated HEK (mammalian) and S2 (*Drosophila*) cells

Electrophysiological testing on insect (*Drosophila*) DRG neurones



Dissected
Drosophila
larvae

Heterologous expression in isolated HEK (mammalian) and S2 (*Drosophila*) cells





Multiple laboratory and field efficacy studies contracted by Bio-Gene demonstrate activity of Flavocide and Qcide against mosquitoes

Flavocide and Qcide have been tested via contract research with:



Sydney, Australia



West Lafayette, IN, USA

Efficacy results vs. mosquito species confirm activity of Flavocide against the target mosquito vectors *Aedes*, *Anopheles* & *Culex spp.* including adult & larval stages and SP-resistant strains.





University of Technology, Sydney Australia -
Spray chamber tests vs. *Aedes* & *Culex*



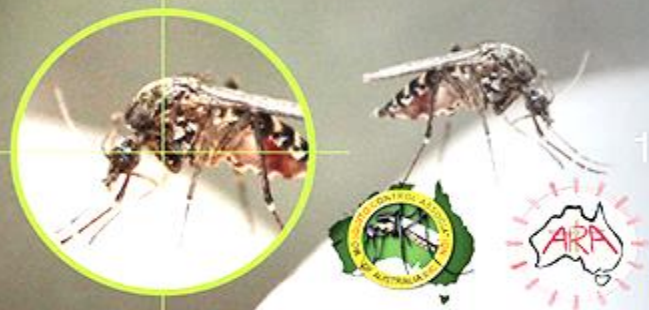
- A series of laboratory evaluations were undertaken to test the efficacy of Flavocide (technical and formulated) on mosquitoes under a range of conditions as follows:
 - ❖ Mosquitoes tested: *Aedes aegypti* (Dengue mosquito), *Culex quinquefasciatus* (Brown house mosquito)
 - ❖ Technical grade Flavocide & Flavocide 500EW tested.
 - ❖ Permethrin, pyrethrins/PBO used as positive controls.
 - ❖ Direct knockdown & residual efficacy assessed.
 - ❖ Combination treatments tested in knockdown studies
 - ❖ Aerosol metered sprays (lab)



Flavocide - Direct (spray chamber) and residual summary data for mosquito vs. adults (2-5d females)

Mosquito	Test	Material	Rate	KD50 (secs)	KD90 (secs)	KD100 (secs)	24hr Mortality (%)
<i>Aedes aegypti</i>	Direct spray*	Flavesone	50mg/ml	488	633		100
<i>Aedes aegypti</i>	Direct spray*	Flavesone	25mg/ml	570	788		100
<i>Culex quinquefasciatus</i>	Direct spray*	Flavesone	50mg/ml	1025.1	1431		100
<i>Culex quinquefasciatus</i>	Direct spray*	Flavesone	25mg/ml	1606	1932		100
<i>Aedes aegypti</i>	Residual tile	Flavesone	50mg/ml	-	-	900	100
<i>Aedes aegypti</i>	Residual tile	Flavesone	20mg/ml	-	-	900	100
<i>Culex quinquefasciatus</i>	Residual tile	Flavesone	50mg/ml	-	-	900	100
<i>Culex quinquefasciatus</i>	Residual tile	Flavesone	20mg/ml	-	-	1800	100

- Flavocide technical & EW formulation **provides rapid knockdown of both *Aedes* and *Culex* spp.** at rates as low as 25mg/mL; as well as **100% mortality at 24h.**
- Flavocide EW formulation applied to glazed tiles can provide **100% knockdown of both *Aedes* and *Culex* species** after 15-30 minutes at rates of 20mg/mL.



Flavocide + Permethrin Combination – Direct Spray* Knockdown vs. Adults (2-5d females)

Combo	Mosquito	Conc'n mg/mL	KD50 (sec)	KD90 (sec) Q-test	KD100 (sec)	Mortality @24h	Combo
F+P	<i>Aedes</i>	25+2.5	240	410 a	600	100%	F+P
F+P	<i>Aedes</i>	25+1.25	360	444 b	720	100%	F+P
Permethrin	<i>Aedes</i>	1.25	300	498 b	840	100%	Permethrin
Permethrin	<i>Aedes</i>	2.5	360	510 b	840	100%	Permethrin
Flavocide	<i>Aedes</i>	25	600	788 e	1080	100%	Flavocide
F+P	<i>Culex</i>	25+2.5	600	800 a	1080	100%	F+P
F+P	<i>Culex</i>	25+1.25	840	1068 b	1500	100%	F+P
Permethrin	<i>Culex</i>	1.25	840	1047 b	1800	100%	Permethrin
Permethrin	<i>Culex</i>	2.5	1500	1745 d	2100	100%	Permethrin
Flavocide	<i>Culex</i>	25	1800	1933 e	2100	100%	Flavocide

- Flavocide in combination with Permethrin can provide **more rapid knockdown** than when used alone vs. *Aedes* and *Culex* species; with higher KD90's for *Culex*.



PURDUE

UNIVERSITY

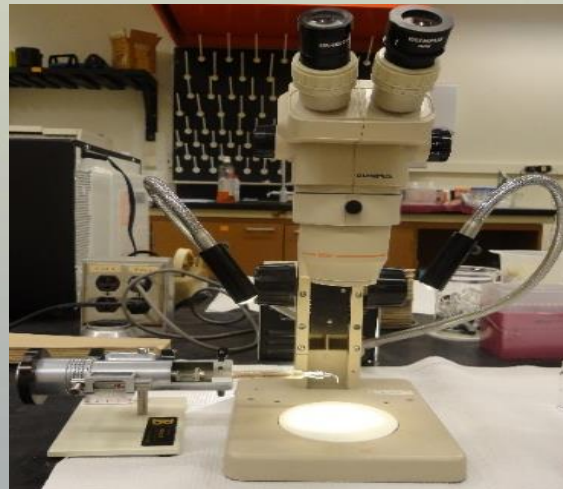
Purdue University, Department of Entomology,
Vector Biology Program

- SP-susceptible & resistant strains
- SOPs, controlled environments, positive/negative controls, technical/biological replicates, statistical analyses

Larval Lethal Dose/Time Assays



Adult Lethal Dose Assays





Purdue University (USA)
Department of Entomology,
Vector Biology Program

- Larval topical dose, larval lethal time & adult dose-mortality assays vs. *Ae. aegypti*
- Flavocide tested in adult topical dose-mortality vs. *Anopheles gambiae*

Target Species	Strain	Life Stage	Test	Flavocide	Control (Permethrin)
<i>Ae. aegypti</i>	LVP*	L3 Larvae	LC ⁵⁰ (24h)	40.9 µg/mL	25.9 ng/mL
	PRS**	L3 Larvae	LC ⁵⁰ (24h)	38.7 µg/mL	164.2 ng/mL
	LVP*	L3 Larvae	LT ⁵⁰	2.3h	5.4h
	PRS**	L3 Larvae	LT ⁵⁰	1.4h	14.7h
	LVP*	Adult	LD ⁵⁰ (24h)	2.6 mg/mL	0.18 µg/mL
	PRS**	Adult	LD ⁵⁰ (24h)	1.6 mg/L	13.6 µg/mL
<i>An. gambiae</i>	Kisumu #	Adult	LD ⁵⁰ (24h)	0.5mg/mL	
	RSP ##	Adult	LD ⁵⁰ (24h)	0.7mg/mL	

*LVP = Liverpool strain (susceptible); ** PRS = Puerto Rico strain (resistant); # = SP susceptible; ## = SP resistant

- ❖ Flavocide controls larvae & adults of susceptible & resistant strains of *Aedes aegypti*
- ❖ Flavocide controls adults of resistant & susceptible strains of *Anopheles gambiae*



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Flavocide 500EW - adult tarsal contact assay
Anopheles gambiae

- Tarsal assays with Flavocide 500EW vs. *Anopheles gambiae* KISUMU (SP susceptible) & RSP (SP resistant) strains 3-5 days old adult female mosquitoes in tarsal contact assay
- Lethal dose (LD₅₀) of Flavocide at 24 & 48 hrs post exposure to an inert surface coated with test material

	LD ₅₀ Adult KISUMU (SP susceptible) Strain	LD ₅₀ Adult RSP (SP resistant) Strain
Time post exposure	Flavocide (mg/m ²)	Flavocide (mg/m ²)
1 hour	177.0	203.9
24 hours	315.3	214.5

Flavocide controls susceptible & resistant strains of *Anopheles* at comparable rates



Future Directions

❖ Future research:

- Potential for other life stage effects including as a larvicide
- Potential for post-exposure effects for population & disease suppression
- Formulation optimisation & field testing

❖ Product registration

- Safety in use, ecotoxicity/environmental impact

❖ Commercialisation

- Engagement with industry & refine marketing strategy





Summary

- ❖ **Beta-triketones** - new class of chemistry to key insecticide markets including public health
- ❖ **Novel Mode of Action** that provides a means to control **resistant** mosquito populations - alone or in combination treatments
- ❖ **Qcide natural & Flavocide nature identical products** provide flexibility in use & markets
- ❖ **Flavocide is active against major vectors** of arboviral diseases including resistant mosquito strains
- ❖ **Future research** opportunities to improve scope of use and performance
- ❖ **Registration data packages** are being developed to support both local & international use
- ❖ **Commercial collaborations** have been established to facilitate market access



Acknowledgements

- ❖ Dr James Wade, Program Manager – Bio-Gene Technology Ltd, Melbourne
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