The New Zealand Institute for Plant & Food Research Limited

DEVELOPING A METHYL BROMIDE SCIENCE-BASED FUMIGATION SCHEDULE FOR EXPORT LOGS

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Alternatives for phytosanitary treatment of NZ export logs

- A comprehensive literature review of fumigants and disinfestation strategies, methods & techniques (2014)

- Identified two fumigation treatment strategies with potential to reduce MB use:

  - Reduction of MB dose rates

  - Ethanedinitrile (EDN)


PFR SPTS No 10678

Comprehensive literature review of fumigants and disinfestation strategies, methods and techniques pertinent to potential use as quarantine treatments for New Zealand export logs

Armstrong JW, Brash DW, Waddell BC

October 2014

Identifying the most MB tolerant species life stage

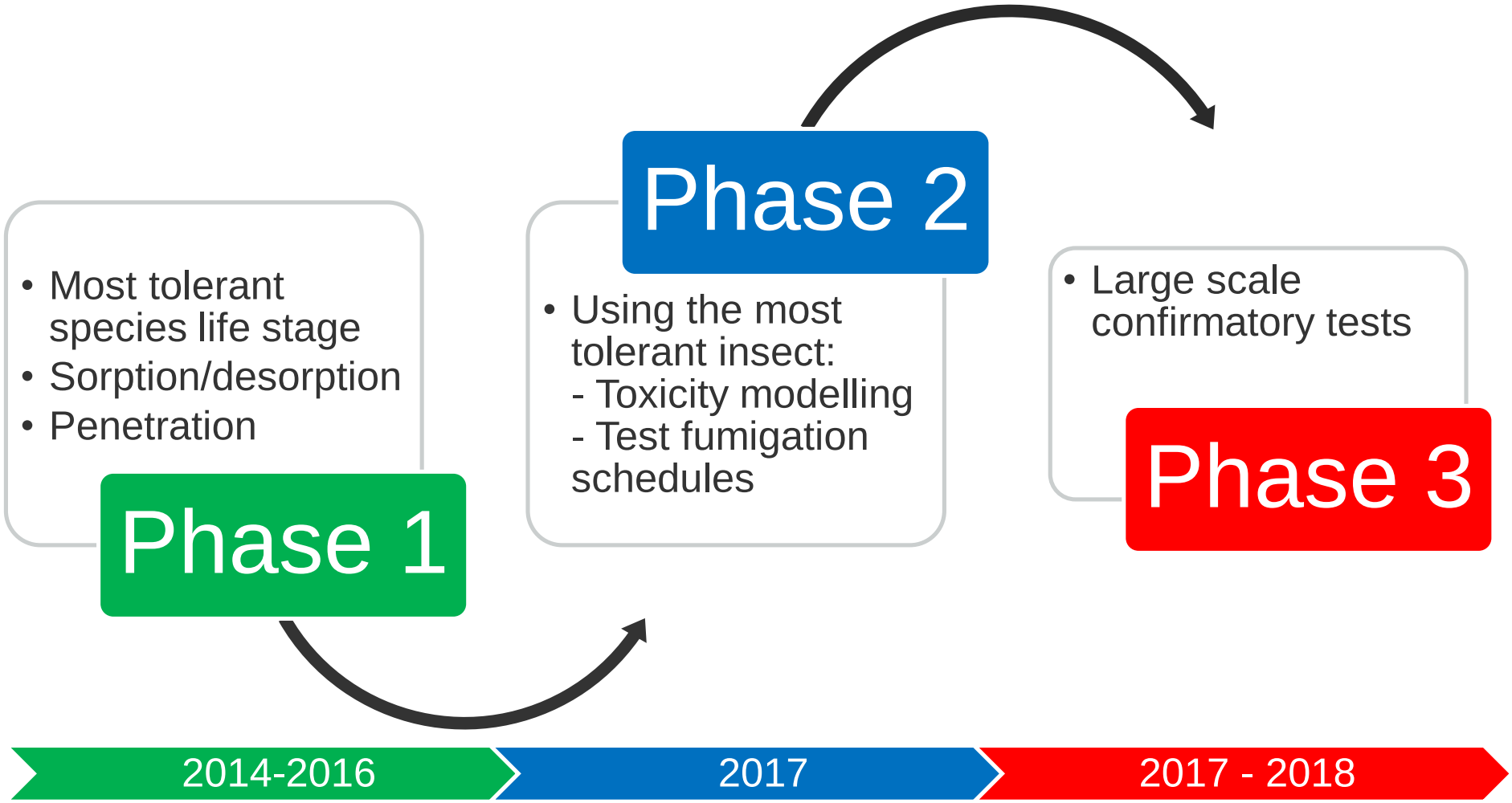
- Datasets are needed to understand the toxicity of reduced doses of MB under a range of fumigation conditions
- Key forest insects associated with New Zealand log exports:
  - Burnt pine longhorn beetle, *Arhopalus ferus*
  - Golden-haired bark beetle, *Hylurgus ligniperda*
  - Black pine bark beetle, *Hylastes ater*

A. Ferus
15-27 mm long

H. Ligniperda
5-7 mm long

H. Ater
3-4 mm long

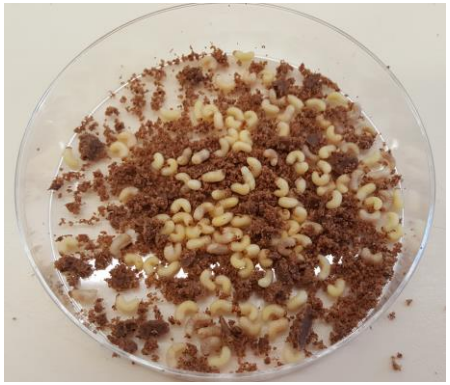
MB Research Plan



Phase 1 - Insect breeding

- **Breakthrough** mass-rearing methods were developed

- Many issues were solved for all species and life stages including:
  - » Artificial diets
  - » Rearing conditions
  - » Oviposition substrates
  - » Cool temperature storage
  - » Microbial contamination



- **Result**: Mass production of high quality, healthy, developmentally synchronised forest beetles for fumigation trials with reduced control mortalities.

>100,000 insects

Phase 1 - Insect breeding

- Massive effort
- PFR staff plus **14** wage workers
- Over **100,000** insects supplied for fumigation trials to date
- Now producing **12,000** *H. ligniperda* and **4,500** *H. ater* eggs each month
- Each year more than **1.5 tons** of artificial diet produced and consumed
- Over **400 kg** of fresh pine bark collected

Phase 1: Identifying the most MB tolerant species life stage

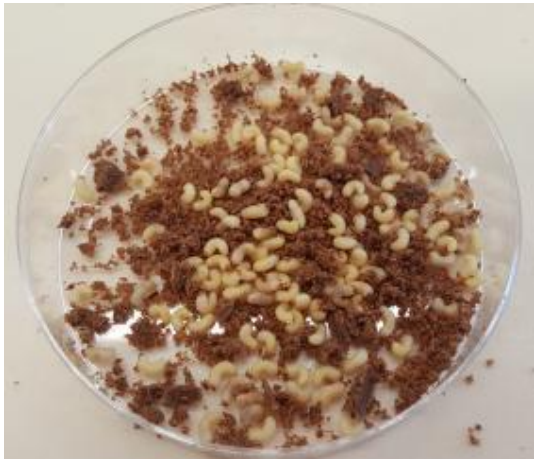
- **Factors tested**:
  - Fumigation time – 4 h
  - Temperature – 10 and 20^{o}C
  - Three species and their respective life stages (excluding *A. ferus* pupae)
  - Eight doses per life stage/species/temperature
  - Fifty insects per life stage/species/temperature/dose
  - Mortality assessed at 24, 48, 72 and 96 h or adult emergence (pupae)
- Mortality analysed using Probit analysis to calculate lethal dose (LD_{99})
- An unbalanced multi-factorial ANOVA to compare LD_{99} across species/ life stages
- Total number of insects = **79,200** – lab colonies

Phase 1: Identifying the most MB tolerant species life stage

- Identified the MB doses which were toxic to all species/life stages tested

- Tolerance to MB varied significantly with **species**, **life stage** and **temperature**

- Greater tolerance typically observed at 10ºC than at 20ºC



Ranking of tolerance to MB for selected forest insects – 24 h after a 4-hour fumigation

Temperature (°C)	Species	Life stage	LD_{99} (g/m^3)		CI[a]
10	*H. ligniperda*	Pupae	64.25 ± 3.64	a	58.15-75.21
	H. ater	Pupae	61.90 ± 2.70	a	57.53-68.08
	H. ligniperda	Larvae	53.08 ± 2.32	ab	50.03-59.08
	H. ater	Larvae	48.70 ± 2.20	bc	45.33-53.19
	H. ater	Adults	40.44 ± 2.27	c	36.78-45.63
	H. ligniperda	Adults	31.46 ± 2.28	d	28.06-36.90
	A. ferus	Larvae	23.01 ± 0.82	de	21.49-24.4
	H. ater	Eggs	21.31 ± 1.02	e	19.65-23.63
	A. ferus	Eggs	18.25 ± 0.89	ef	16.78-20.27
	H. ligniperda	Eggs	14.43 ± 0.89	f	16.78-20.27
	A. ferus	Adults	9.29 ± 0.39	g	8.66-10.17
20	*H. ater*	Pupae	32.26 ± 1.52	a	29.87-35.80
	H. ligniperda	Larvae	26.81 ± 0.79	a	25.52-28.60
	H. ligniperda	Pupae	25.34 ± 1.67	ab	22.88-29.78
	H. ater	Larvae	23.61 ± 1.17	bc	21.76-26.32
	H. ater	Adults	18.66 ± 0.93	cd	17.24-20.85
	H. ater	Eggs	16.62 ± 0.68	cde	15.48-18.14
	H. ligniperda	Adults	14.92 ± 0.85	def	13.72-17.04
	A. ferus	Larvae	13.82 ± 0.34	ef	13.29-14.60
	A. ferus	Eggs	11.25 ± 0.41	f	10.94-12.53
	H. ligniperda	Eggs	10.98 ± 0.97	fg	9.68-14.28
	A. ferus	Adults	7.36 ± 0.34	h	6.82-8.14

[a] Values represent the 95% confidence interval (CI) around the mean.

Ranking of tolerance to MB for selected forest insects – 48, 72 and more h after fumigation

Time after fumigation (h)[a]	Temperature (ºC)	$LD_{99} \pm SE$ (g/m³)[b]					
		H. ligniperda				*H. ater*	
		Larvae		Pupae		Pupae	
48	10	35.07 ± 1.44	b	56.68 ± 3.28	a	48.28 ± 1.61	a
	20	17.14 ± 0.85	d	21.60 ± 1.48	cd	30.31 ± 2.03	bc
72	10	31.70 ± 1.65	bc	49.87 ± 2.73	a	43.01 ± 1.55	ab
	20	14.03 ± 0.64	d	17.26 ± 0.72	d	27.15 ± 1.24	c
96 or at adult emergence	10	26.35 ± 1.66	bc	48.95 ± 4.50	a	37.12 ± 1.53	ab
	20	13.79 ± 0.60	c	18.59 ± 1.63	c	21.88 ± 0.80	c

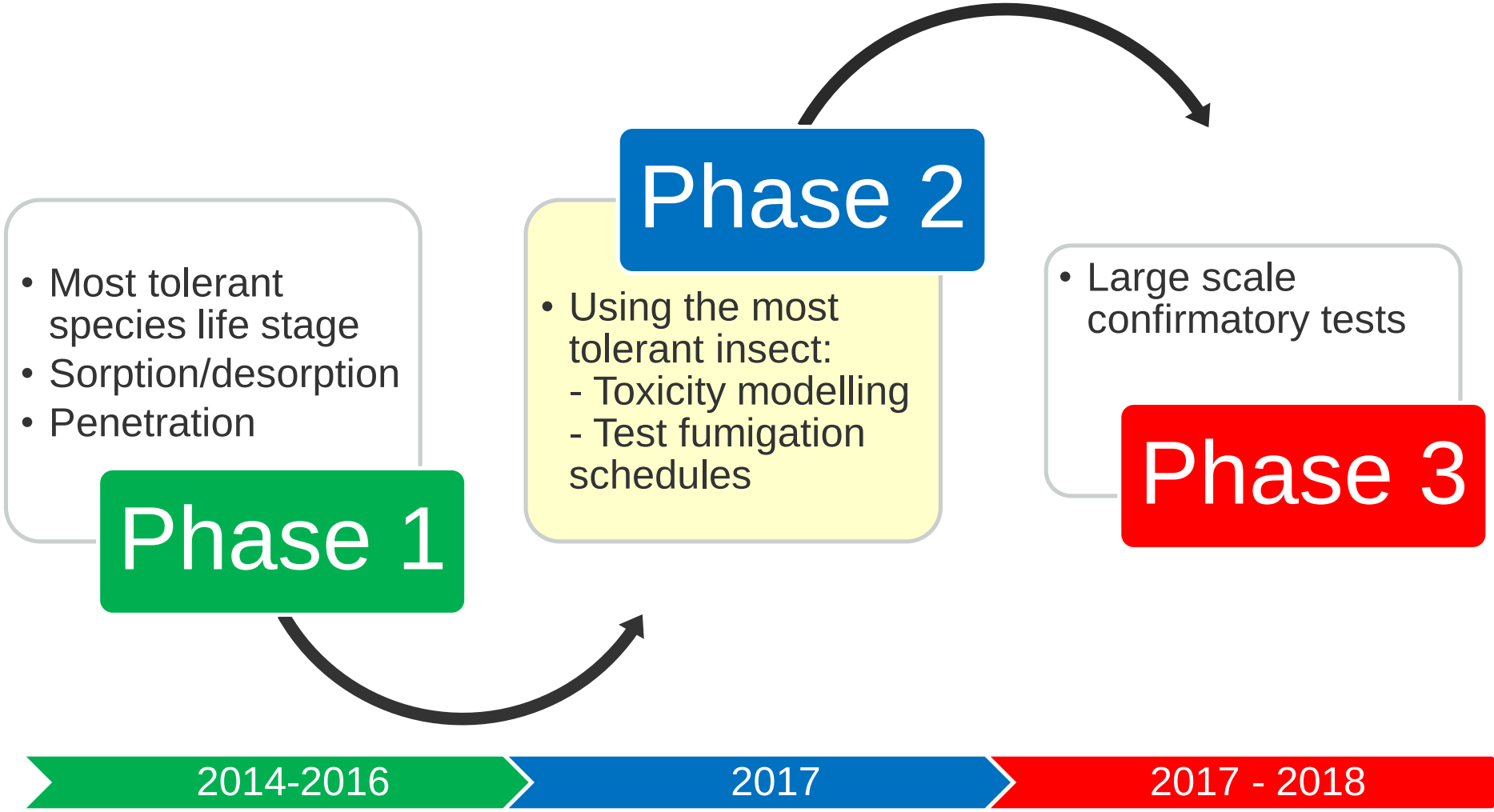
Toxicity modelling

- Based on the results:

  - *H. ligniperda* pupae were selected as the most MB-tolerant species and life stage

  - This insect/life stage was then used in the subsequent phases of the programme

MB Research Plan



Phase 2 - Toxicity modelling

- **Factors**:
  - Six durations – 2, 4, 8, 12, 14 or 16 h
  - Three temperatures – 5, 10 or 20^{o}C
  - Eight MB doses– different for each duration and temp
  - Fifty insects per dose/duration/temp
  - 3 reps/duration/temperature
  - Mortality assessed at 24, 48, 72 and adult emergence

- Mortality data analysed using Probit analysis to calculate lethal dose (LD_{99})
- A multifactorial ANOVA to test for effects of **dose**, **duration** and **temperature** on MB toxicity
- Total number of insects = **21,600**

Phase 2: Toxicity of MB at 20°C

Example

	2 h		4 h		8 h		12 h		14 h		16 h	
Dose (g/m^3)	CT	Mortality %	CT	Mortality %	CT	Mortality %	CT	Mortality %	CT	Mortality %	CT	Mortality %
0	0	0	0	0	0	0	0	2	0	0	0	3
3	5	13	10	9	20	15	32	33	35	19	41	26
6	8	4	17	10	37	35	52	73	67	62	79	76
9	14	10	27	55	47	69	88	97	113	100	115	100
12	16	7	35	58	91	92	114	100	168	100	192	100
18	30	38	58	89	134	100	216	100	252	NT	288	NT
24	40	95	81	100	192	100	288	NT	336	NT	384	NT
31	51	97	124	100	248	NT	372	NT	434	NT	496	NT
37	67	100	148	NT	296	NT	444	NT	518	NT	592	NT

***H. ligniperda* pupae fumigated with MB at 20°C**

Toxicity modelling

- Toxicity of MB varies with **dose**, fumigation **time** and **temperature**, with all three factors having a significant effect
- Doses (g/m^3) needed to control 99% of *H. ligniperda* pupae decreased as the temperature and fumigation times increased:

Temperature (°C)	Time (h)					
	2	4	8	12	14	16
5	246	148	98	74	74	74
10	96	64	32	24	24	24
20	37	24	18	12	9	9

[1] Values represent the dose in g/m^3 needed to kill 99% of insects.

Toxicity modelling

- Modelling is under way to develop **equations** that would allow interpolation and/or extrapolation of CTs, e.g. 15ºC for 24 h

- Methyl bromide CT products needed to control *H. ligniperda* pupae at 5, 10 or 20ºC and for 8 and 16 hr fumigations:

Temperature (ºC)	Time (h)[1]					
	2	4	8	12	14	16
5	492	592	784	888	1036	1184
10	192	256	256	288	336	384
20	74	96	144	144	126	144

Phase 1- Determining MB sorption

- Refers to the absorption and adsorption of molecules to the material treated

- Sorption rates vary with the fumigant used, material treated and load factor

- Dose, bark cover, end-grain sealing and temperature might also influence sorption

- Higher rates generally undesirable because less of the applied fumigant is available in the treated space for respiratory uptake by insects

Phase 1 - MB sorption by logs

Research Article

SCI

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Sorption and desorption characteristics of methyl bromide during and after fumigation of pine (*Pinus radiata* D. Don) logs

Matthew Hall,* Adriana Najar-Rodriguez, Anthony Adlam, Alistair Hall and Don Brash

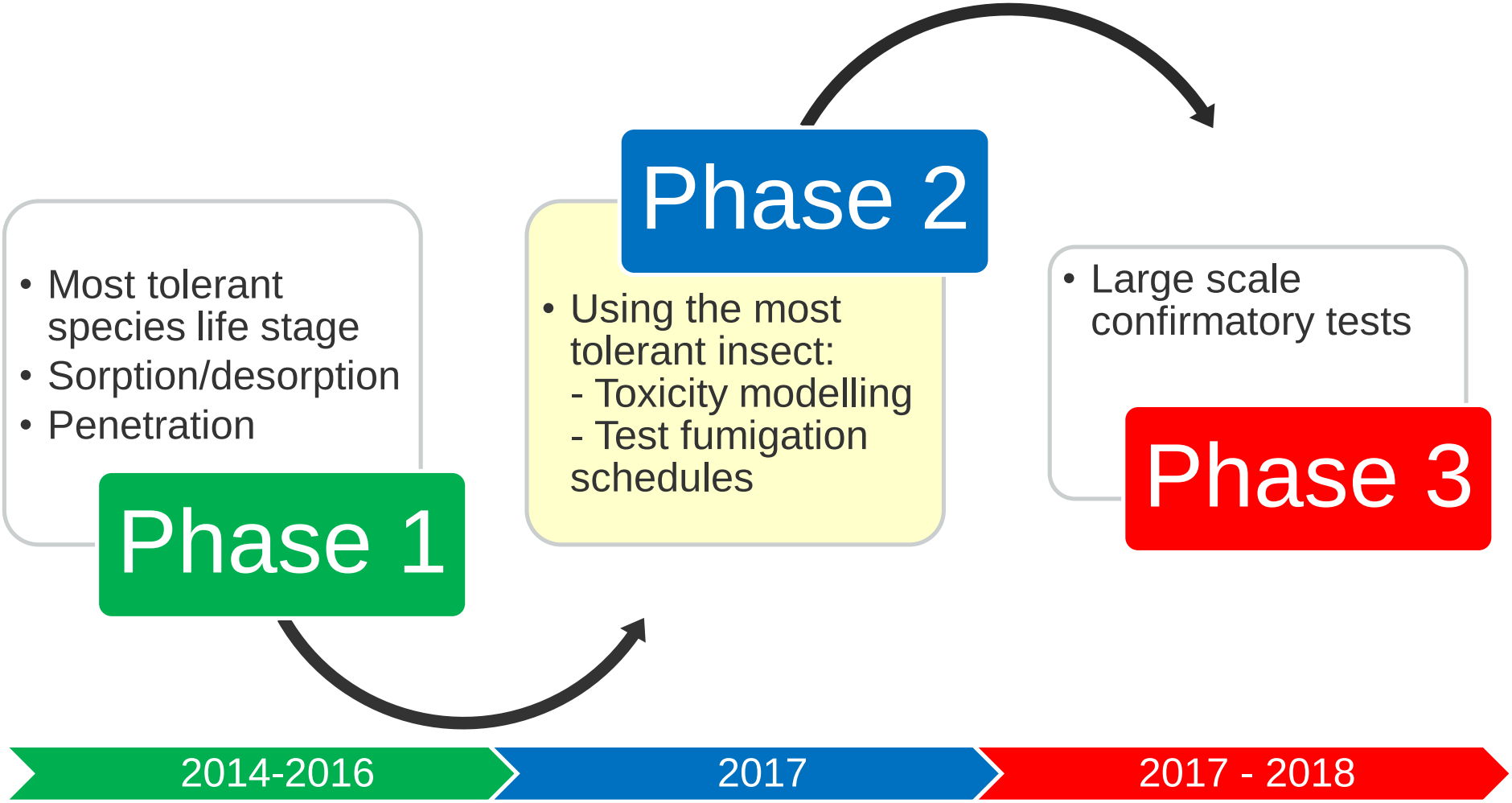
Abstract

BACKGROUND: The sorption and desorption characteristics of methyl bromide (MB) were determined during and after fumigation of recently harvested pine (*Pinus radiata* D. Don) logs. The effects of dose (48 or 120 g m^{-3}), degree of bark cover (0, 50 or 100%) and end-grain sealing (sealed or unsealed) on sorption and desorption were determined over time.

RESULTS: Sorption of MB was proportional to the dose applied and dependent on the amount of end-grain sealed. After 16 h, an average of 70.7 $\pm$ 2.5% of the initial concentration remained in the treated space when end-grains were sealed, whereas only 47.3 $\pm$ 2.5% remained when unsealed. During aeration, MB was released from logs, initially ranging from 2.8 to 8.8 g·h m^{-3}, depending on the treatment. The rate of desorption quickly decreased during aeration.

CONCLUSION: The surface area of a log is the most important factor influencing MB sorption and desorption rates, with greater surface area resulting in greater (de)sorption rates. Sorption data can now be combined with insect toxicity data to estimate a minimum effective dose of MB for further evaluation, while desorption data can be combined with fumigant plume modelling to assess worker safety.

MB Research Plan



Infested logs – 28 L chambers

- Combining the results of:
  - MB toxicity
  - Sorption studies
  - Toxicity modelling
- Evaluation of the efficacy of MB to *H. ligniperda* pupae in artificially-infested logs
- Mortality assessed for other life stages - excluding eggs

Infested logs – 28 L chambers

- Logs infested for approximately 6-8 weeks – larvae and pupae
- **Factors**:
  - Two times: 8 and 16 h
  - Three temperatures: 5, 10 or 20^{o}C
  - Eight MB concentrations: different for each time & temp
  - 3 reps/time/temperature
  - Insects per log: ~200
  - Mortality assessed at 24, 48 and adult emergence

Infested logs – 28 L chambers

- Mortality data analysed using Probit analysis to calculate lethal dose (LD_{99})
- Effects of MB **dose**, **fumigation time** and **temperature** tested with a multi-factorial ANOVA

Infested logs – 28 L chambers

- Effective doses (g/m^3) that result in control of *H. ligniperda* pupae under different treatment conditions:

Time (h)	5^{o}C	10^{o}C	20^{o}C
8	44.4 (41.3-48.4)	17.1 (16.2-18.2)	9.0 (8.5-9.6)
16	17.6 (16.7-18.9)	9.9 (9.5-10.5)	7.9 (7.3-8.5)

Values represent the dose in g/m^3 and the 95% confidence interval around the mean.

Current doses:

120 g/m^3
5-15^{o}C

80 g/m^3
>15^{o}C

Verification tests – 500 L chambers

- Logs infested for approximately 8 weeks – pupae
- **Factors**:
  - Two times: 8 and 16 h
  - Three temperatures: 5, 10 or 20°C
  - Eight MB concentrations: different for each time & temp
  - Three reps/time/temp
  - Insects per log: ~200
  - Mortality assessed at 24, 48 and adult emergence

  ➢ 3 logs per chamber plus filler logs
- Total number of insects = **220,000**

Preliminary results – verification tests 500 L

Dose (g/m^3) resulting in 99% mortality of *H. ligniperda* pupae

Temperature (°C)	Larvae		Pupae		Adults	
	8 h	**16 h**	**8 h**	**16 h**	**8 h**	**16 h**
5	44.9* (42.78 - 48.06)	23.49 (22.67 - 24.41)	140.33* (125.86 - 161.90)	98.69 (84.67 - 123.05)	38.04* (35.33 - 61.46)	18.20 (14.32 - 21.08)
10	22.61 (23.67 - 27.02)	14.53 (17.58 - 18.79)	31.76 (30.15 - 33.73)	17.35 (16.41 - 18.57)	22.01 (21.10 - 23.39)	10.24 (9.19 - 11.84)
20	15.14* (14.33 - 16.16)	6.85 (6.83 - 7.91)	20.76* (19.64 - 22.11)	10.28 (9.70 - 11.06)	10.78* (9.35 – 45.18)	5.86 (4.47 - 9.91)

* Based on 2 instead of 3 reps

Preliminary results – verification tests 500 L

28 L chambers vs 500 L chambers

Temperature (°C)	Pupae		Pupae	
	8 h	**16 h**	**8 h**	**16 h**
5	44.4 (41.3-48.4)	17.6 (16.7-18.9)	140.33* (125.86 - 161.90)	98.69 (84.67 - 123.05)
10	17.1 (16.2-18.2)	9.9 (9.5-10.5)	31.76 (30.15 - 33.73)	17.35 (16.41 - 18.57)
20	9.0 (8.5-9.6)	7.9 (7.3-8.5)	20.76* (19.64 - 22.11)	10.28 (9.70 - 11.06)

* Based on 2 instead of 3 reps

What next?

» Verification of effective MB doses derived from the 500 L chamber experiments through simultaneous fumigations of logs infested with *H. ligniperda* or *H. ater*

- **Factors**:
  - Two times – 8 and 16 h
  - Three temperatures – 5, 10 or 20°C
  - 1 MB concentration and 1 control
  - 3 logs per species/chamber plus filler logs
  - 3 reps/time/temperature
  - Mortality assessed at 24, 48 and adult emergence

Expected completion date: early 2018

Conclusions

- Our data supports a **reduction** in MB doses while **maintaining** quarantine security and, ultimately, the reduction of both MB **use** and **emissions**
- The most MB-tolerant species and life stage tested are:
  - *H. ligniperda* pupae
  - *H. ligniperda* larvae
  - *H. ater* pupae
- Toxicity of MB to *H. ligniperda* pupae, and by extrapolation to other species and life stages, is the result of interactions between **dose**, fumigation **time** and **temperature**

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