Biorational Larvicides and Larviciding for Mosquito Control with Notes on Resistance Management

Western US Floodwater Summit

Solutions for Floodwater Mosquito Control

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Sponsored and hosted by Valent BioSciences

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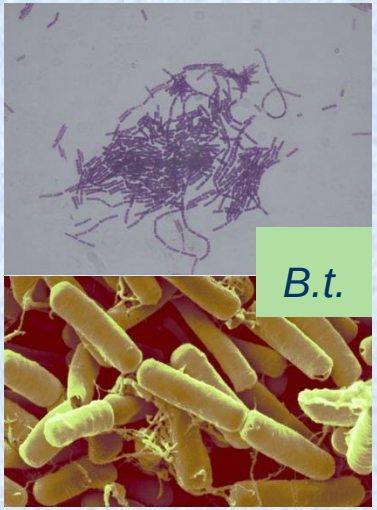
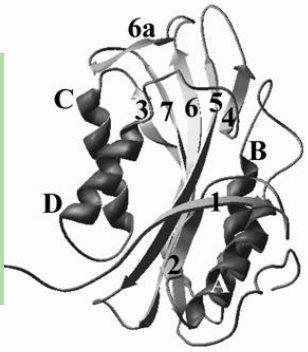
B.t. and *B.t. israelensis*

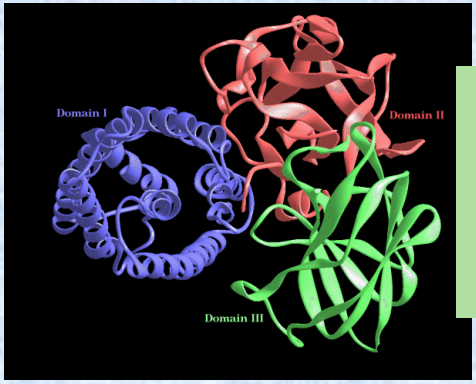
B.t.

- First discovered in 1901 in silkworm (Japan) – *Bacillus soto* (Shigetane Ishiwata)
- Rediscovered in 1911 in Mediterranean flour moth larvae (Thuringia, Germany) – *B. thuringiensis* (Ernst Berliner)
- 70 serotypes, > 80 subspecies (Lecadet 1999)
- 14 serotypes, 16 subsp. against mosquito larvae and other dipteral insects (Delecluse et al. 1995)

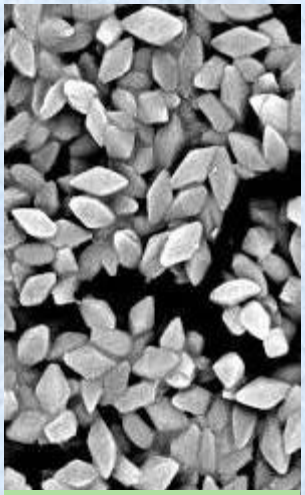


Domain A, B C and D of Cyt1A





Domain I, II and III of crystal toxins



Toxin crystals

Sub-species of *Bacillus thuringiensis* active against Dipteran larvae (Delecluse et al., 1995)

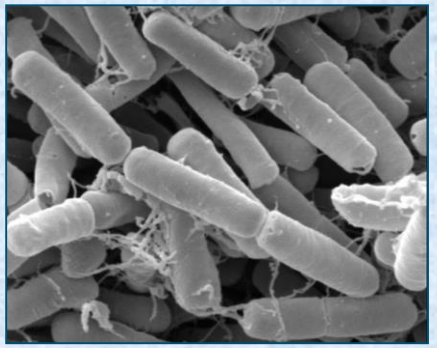
Subsp. of *B. thuringiensis*	Serotypes	Activities against			Proteins presented in crystals (kDa)*
		Ae. aegypti	*An. stephensi*	*Cx. pipiens*	
israelensis	H14	+++	+++	+++	135, 125, 68, 28
morrisoni	H8a8b	+++	+++	+++	144, 135,1 25, 68, 28
canadensis	H5a5c	+++	+++	+++	135, 125, 68, 28
thompsoni	H12	+++	+++	+++	135, 125, 68, 28
malaysiensis	H36	+++	+++	+++	135, 125, 68, 28
AAT028 K6		+++	+++	+++	135, 125, 68, 28
AAT021 B51		+++	+++	+++	135, 125, 68, 28
jegathesan	H28a28b	++	+++	++	80, 70-72, 65, 37, 26, 16
medellin	H30	++	+++	++	94, 68-70, 28-30
kurstaki	H3a3b3c	±	+	UD	130-135, 66
fukuokaensis	H3a3d3e	-	±	-	90, 86, 82, 72, 50, 48, 37, 27
galleriae	H5a5b	±	±	UD	130-135, 61
aizawai	H7		UD	UD	130-135
darmstadiensis	H10a10b	±	+	+	125, 83, 79, 69, 50, 27
kyushunsis	H11a11c	±	+	±	140, 8, 80, 70, 66, 50, 27, 15, 14
Higo	H44	ND	±	±	98, 91, 78, 61, 50, 44, 36, 27

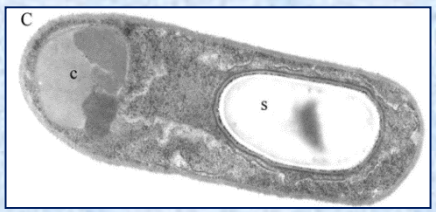
B.t.i.

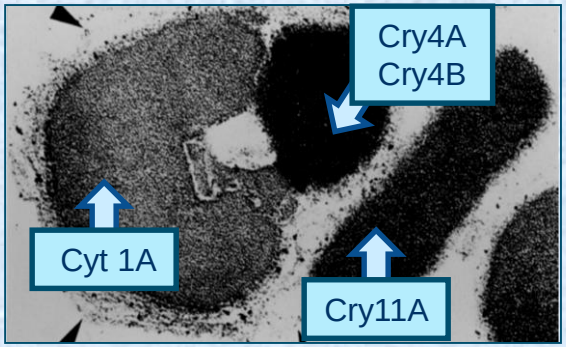
- Serotype: *Bt* H-14 (Israel, 1976)
- Toxins: Synergistic 4 toxins:

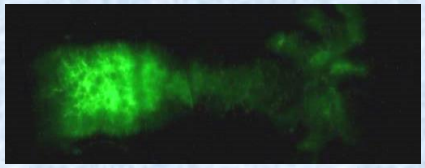
  Cyt1A (27 kDa), Cry4A (134 kDa),
  Cry4B (128 kDa), Cry11A (66 kDa)

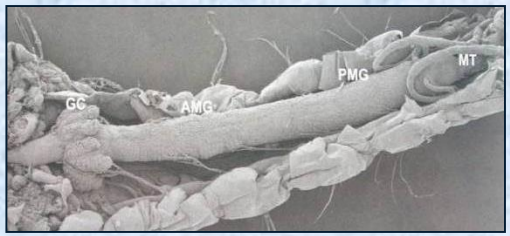
- Mode of action (MoA): Gut toxins
- IRAC: Group 11
- Targets: Nematoceran Diptera
- Non-targets: Safe at label rates
- Resistance concern:
  - Whole *B.t.i.*: **No**
  - Individual toxins: **Yes**







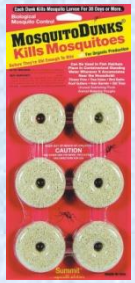




B.t.i. Products

Product	Formulation	Potency (ITU/mg)	Rate (unit/ac)	Application	Residual Efficacy (d)
VectoBac 12AS	AS	1,200	0.25-2 pt	Mix w/ water, spray	7
Teknar SC	SC	1,200	0.25-2 pt	Mix w/ water, spray	7
AquaBac XT	AS	1,200	0.25-2 pt	Mix w/ water, spray	7
FourStar SBG	SG	150	3-20 lb	Aerial, hand or ground equipment	7-14
VectoBac CG/G	Corncob G	200	2.5-20 lb	Aerial, hand or ground equipment	7-14
VectoBac WDG	WDG	3,000	0.1-0.9 lb	Mix w/ water, spray (ground/aerial)	7-14
AquaBac 200G/400G	Corncob CG/G	200/400	2.5-20 lb/ 1.25-8 lb	Mix w/ water, spray (ground/aerial)	7-14
Summit Bti Dunks	Dunk	700	1 ea/100 sq. ft	Hand	30



Resistance to *B.t.i.* – Laboratory selections

Whole *B.t.i.*

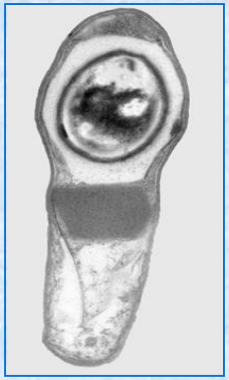
Species	Gen's.	Resistance Ratios (RR)	Ref.
Cx. pipiens complex	11-60	4-17	Vasquez-Gomez 1983
	20	2-3	Mittal 2005
	20	3	Saleh et al. 2003
Ae. aegypti	15	1	Goldman et al. 1986
	15	2	

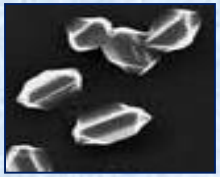
B.t.i. toxins

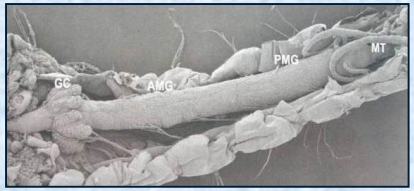
Species	Toxins for selection	Gen's	RR	Ref.
Cx. quinque-fasciatus	Cry11Aa	28	> 900	Georghiou & Wirth 1997
	Cry4Aa, Cry4Ba	25	> 120	
	Cry4Aa, Cry4Ba Cry11Aa	28	91	
	B.t.i. (all toxins)	28	3	

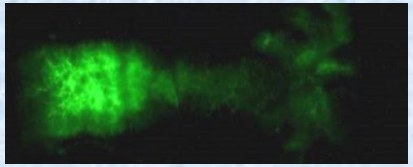
Lysinibacillus sphaericus

- 49 serotypes identified;
- 9 serotypes /16 entomopathogenic strains to mosquitoes（H1, H2, H3, H5, H6, H9, H25, H26, H48）;
- Active strains:
  - 2362, Nigeria, *Simulium damnosum* (Weiser 1984);
  - 1593, Indonesia, *Cx. fatigans* (1974);
  - 2297, Sri Lanka, *Cx. pipiens* (1980);
  - C3-41, China, *Cx. quinq.* (1989).
- Toxins: Syn. bin toxins (52 KDa and 43 Kda), also Mtx;
- MoA: Gut toxins;
- IRAC – Group 11;
- Targets: Most mosq. genera, except some *Ae.* spp.;
- Non-targets: Safe at label rates;
- Resistance concern: Yes (Lab and field – Su, 2014; 2015, 2016).



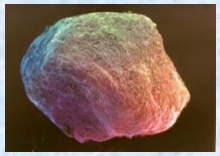






Lsph products

Product	Formulation	Potency (ITU/mg)	Rate	Application	Residual Efficacy (wks)
VectoLex CG	Corn grit G	50	5-20 lb/ac	Aerial, hand or ground equipment	2-3
VectoLex WSP	WSP	50	1 pouch/50 sq. ft.	Hand	Up to 5
VectoLex WDG	WDG	650	0.5-1.5 lb/ac	Mix w/ water, spray (aerial/ground)	2-3
Spheratax SPH	Corn grit G	50	5-20 lb/ac	Aerial, hand or ground equipment	2-3
Spheratax SPH WSP	WSP	50	1 pouch/50 sq. ft.	Hand	Up to 5



Resistance to *Lsph* in *Cx. pipiens* complex

Laboratory selections

Gen's	*Lsph* Strain	RR	Ref.
80	2362	27-37	Rodcharoen & Mulla 1994
8		23,000	Nielsen-LeRoux et al. 1997, 2001
12		7,000	Wirth et al. 2000
18		5,000	Nielsen-LeRoux et al. 2001
70		45	Zahiri, Su & Mulla 2002
30		20	Zahiri & Mulla 2003
35		139	
46		162,000	Pei et al. 2002, Oliveira et al. 2004
6	B101	52,000	Adak et al. 1995
7	1593	6,223; 31,325	Rao et al. 1995, Poopathi et al. 1999, Nielsen-LeRoux et al. 2001,
13	C3-41	144,000	Pei et al. 2002, Yuan et al. 2003
18	IAB59	46	Pei et al. 2002, Yuan et al. 2003, Oliveira et al. 2004,
12		6	Pei et al. 2002
72		40,000	Amorim et al. 2007

Resistance to *Lsph* in *Cx. pipiens* complex (Cont'd)

Resistance in field populations

Country	RR	Strains	Treat. History	Ref.
France	70	2362	n/a	Sinègre et al. 1994;
Brazil	10	2362	2 yrs	Silva-Filha et al. 1995
India	150	B-101	25 treats., 1 yrs	Adak et al. 1995;
India	146-180	1593	35 treats., 2 yrs	Rao et al. 1995
France	5,958	2362	2 yrs	Chevillon et al. 2001
China	20,000	C3-41	8 yrs	Yuan et al. 2002
Tunisia	750	2362	n/a	Nielsen-Leroux et al. 2002
Thailand	Control failure	2362	19 treats.	Mulla et al. 2003
Thailand	187,000	2362	5 treats.	Su & Mulla 2004
U.S.A.	537-9,049	2362	1.5 – 10 yrs	Su et al. 2017, 2019

Cross Resistance to *Lsph* in *Cx. pipiens*

Laboratory studies on cross resistance (xRR)

R	High xRR to other *Lsph*	Low xRR to other *Lsph*	No xRR	Ref.
2362	1593, 2297, C3-41, IAB881, IAB872	ISPC5, LP1-G, 47-6B, IAB59	*B.t.i.*	Rodcharoen & Mulla 1996 Wirth et al. 2000, Wirth 2010 Nielsen-LeRoux et al. 2001 Pei et al. 2002, Mulla et al. 2003 Yuan et al. 2003, Zahiri et al. 2003 Oliveira et al. 2004, Su & Mulla 2004
1593	2362, IAB881, IAB872	2297 IAB59	*B.t.i.*	Rao et al. 1995 Poopathi et al. 1999 Wirth 2010
C3-41	2362, 2297, 1593	47-6B, IAB59	*B.t.i.*	Pei et al. 2002 Yuan et al. 2003 Wirth 2010
IAB59	2362, 2297, 1593, C3-41	47-6B, LP1-G	*B.t.i.*	Pei et al. 2002, Yuan et al. 2003 Oliveira et al. 2004 Amorim et al. 2007, Wirth 2010

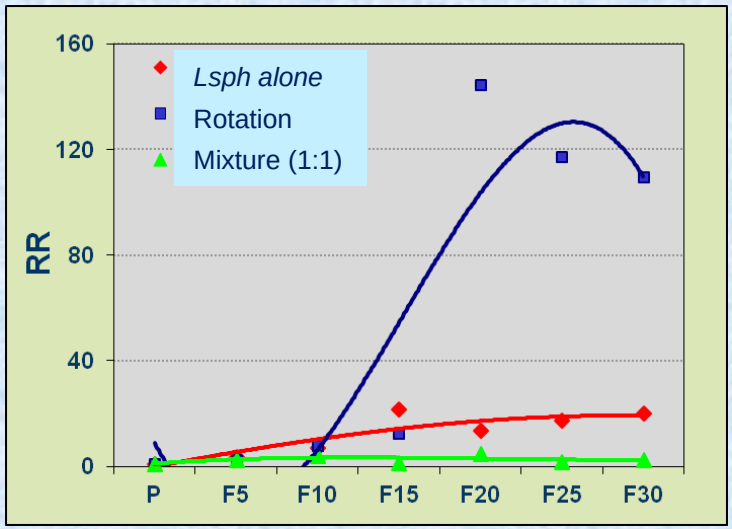
Resistance Management

- **Prevention of *Lsph*-R development**
  - Mixture (*B.t.i.* : *Lsph* = 1 : 1 to 1 : 3) (Zahiri & Mulla 2003);
  - NOT *B.t.i.* and *Lsph* rotation (Zahiri & Mulla 2003);
  - Recombinant: Bt Cyt1A + Cry11B + Lsph Bin; Bt Cyt1A + Lsph BinA (Federici et al. 2003, 2010).

- **Restoration of *Lsph* susceptibility**
  - Switch to *B.t.i.* (Zahiri, Su & Mulla 2002; Su & Mulla 2004);
  - Mixture (*B.t.i.* + *Lsph*) or Rotation (Zahiri, Su & Mulla 2002);
  - Recombinants - Bt Cyt1A + Lsph BinA, Bt Cyt1A + Cry11B + Lsph Bin (Wirth et al. 2000a,b; 2001).

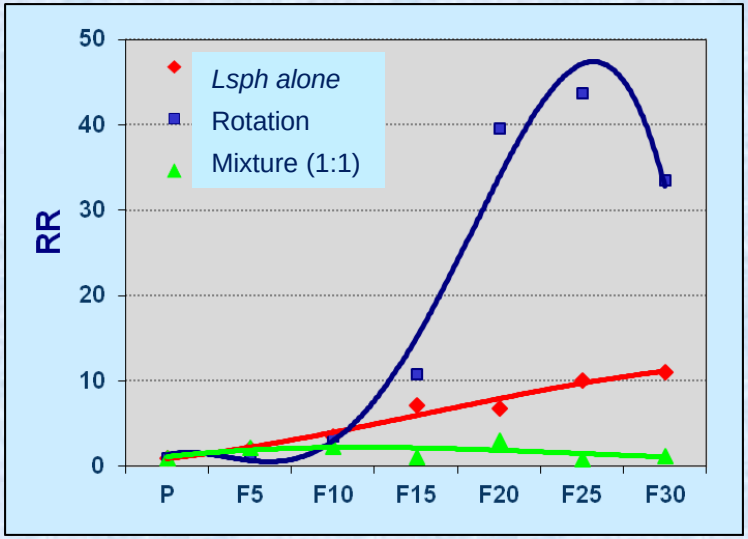
Prevention of *Lsph* Resistance in *Cx. quinquefasciatus*

(Zahiri & Mulla 2003)



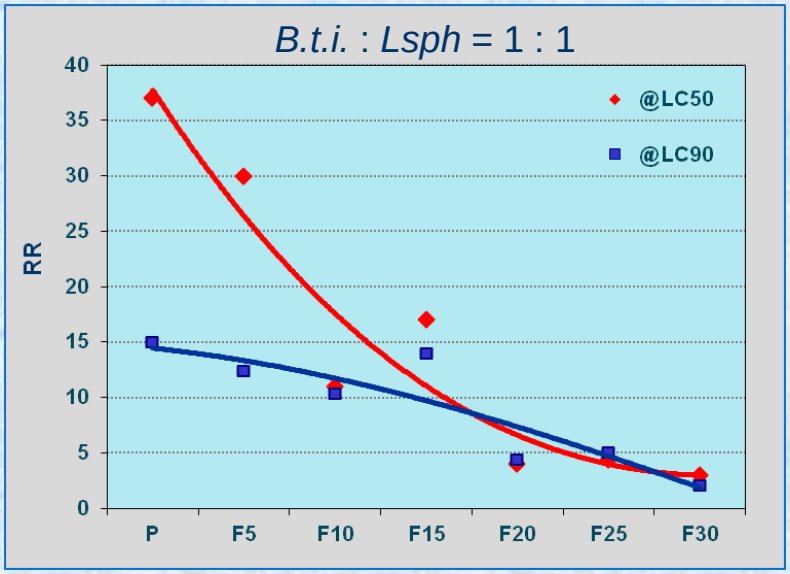
@ LC_{50}

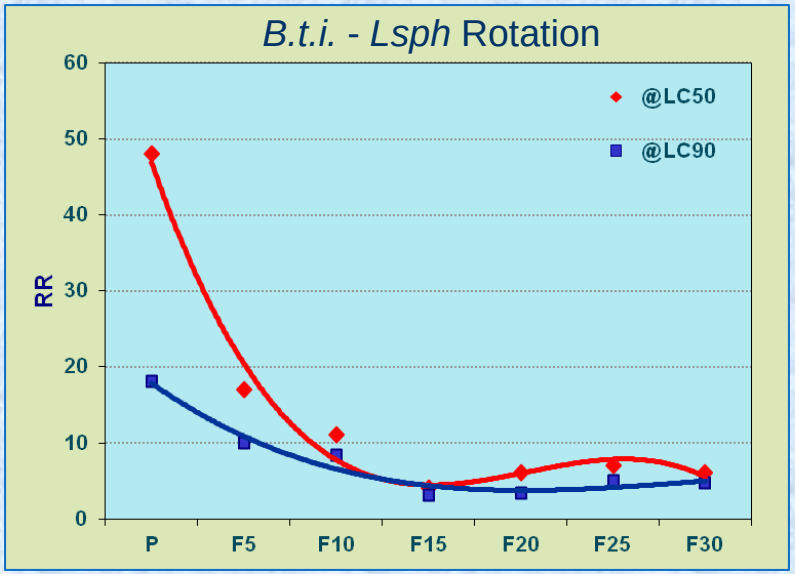
@ LC_{90}

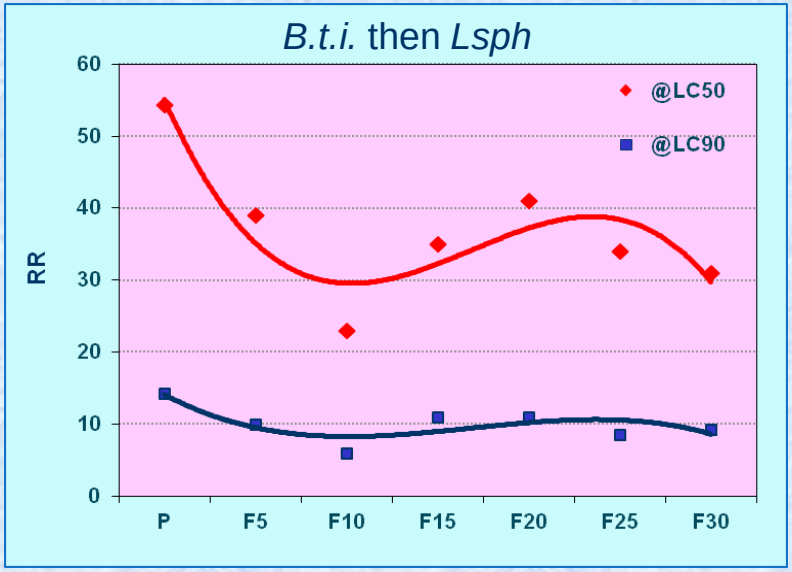


Lsph Susceptibility Restoration in *Cx. quinq.*

(Zahiri, Su & Mulla 2002)





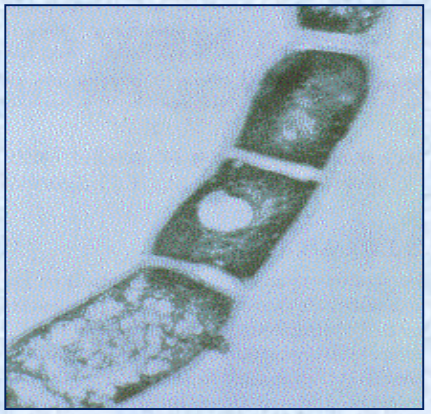


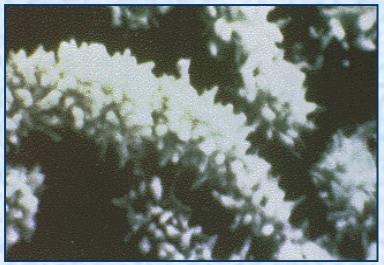
B.t.i. + *Lsph* products

Product	Formulation	Potency (ITU/mg)	Rate	Application	Residual Efficacy (wks)
VectoMax G/CG	Corn grit G	*B.t.i.* 50 *Lsph*	5-20 lb/ac	Aerial, hand or ground equipment	Up to 4
VectoMax WSP	WSP	*B.t.i.* 50 *Lsph*	1 pouch/50 sq. ft.	Hand	Up to 5
FourStar Briquet	Briquet (45, 90, 180d)	70 *B.t.i.* 60 *Lsph*	1 ea/100 sq. ft.	Hand	6, 13, 26

Spinosad – *Saccharopolyspora spinosa*

- Tetracyclic-macrolide compounds with an amino sugar and a neutral sugar
- 20 natural spinosyns, 200 sythetic spinosoids
- Only spinosyns A & D (spinosad), J & L (spinetoram) developed for commercial insecticide use
- Environmentally friendly -
  - Reduced risk pesticide (USEPA)
  - IRAC: Group 5
- Targets: Agricultural and public health pests.
- Non-targets: Good safety margin at label rates.
- Resistance concern: Yes
  - Documented in several Ag. pests
  - Mosquitoes: Lab colony of *Cx. quinq.* (Su & Cheng 2012, 2014)

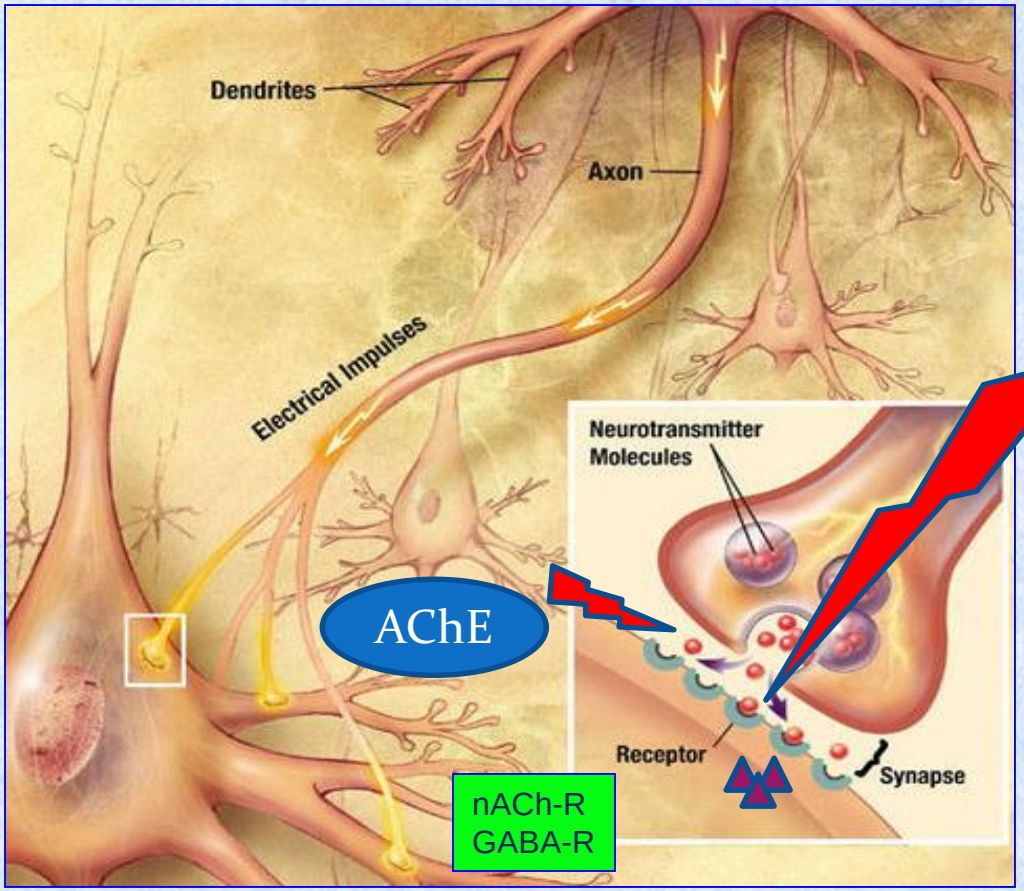




Neurotoxin

- Mainly via ingestion, lesser by contact
- Activation of post-synaptic nACh receptor and GABA receptor
- Causes over excitation of insect nervous system, leading paralysis

Spinosyns: Simulating neurotransmitters (Activating nAChR-Dα6)

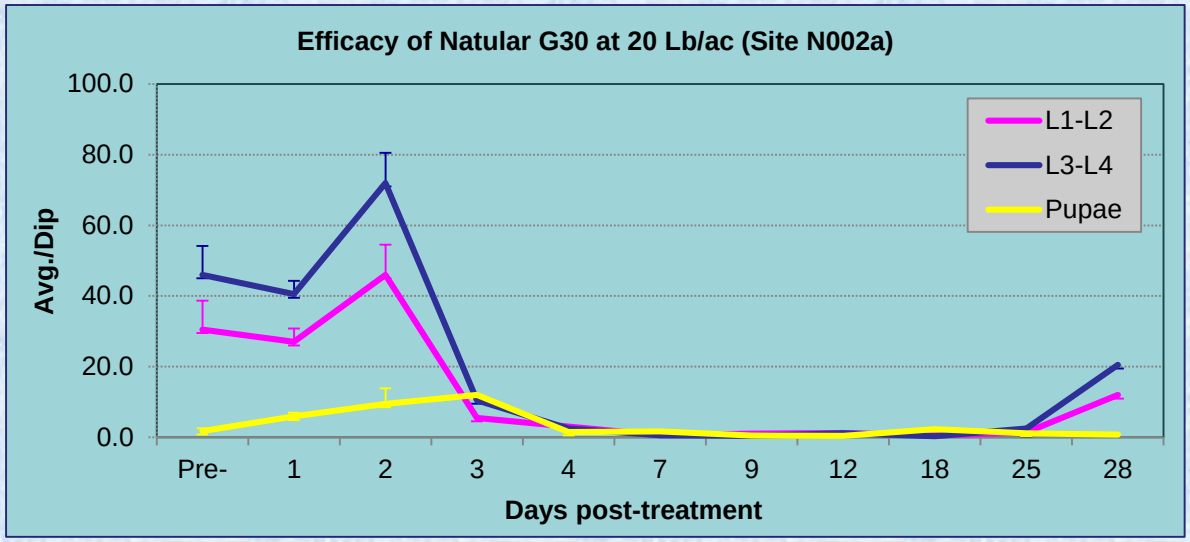


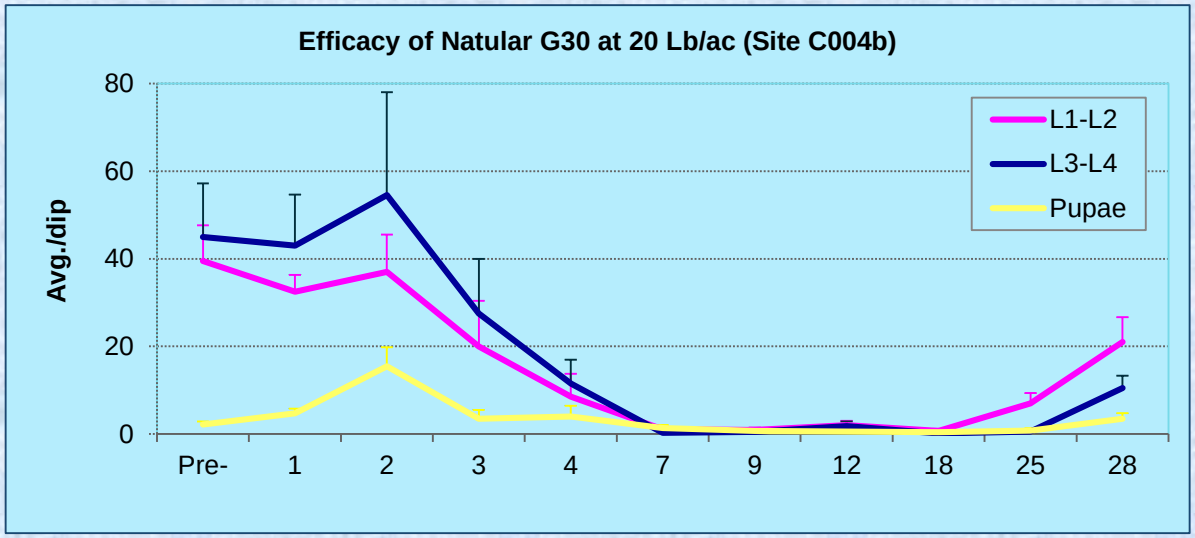
Dead mosquito larvae killed by spinosad

Spinosad products

Product	Formulation	AI%	Rate	Application	Residual Efficacy (d)
Natular EC	EC	20.6	1.1-2.8 fl. Oz/ac	Aerial or ground equipment	7
Natular G	Corn grit G	0.5	3.5-9 lb/ac	Aerial or ground equipment	7
Natular G30	SG	2.5	5-20 lb/ac	Aerial or ground equipment	30
Natular DT	Tablet	7.4	1 ea/container	Hand	30
Natular T30	Tablet	8.33	1 ea/100 sq. ft.	Hand	30
Natular XRT	Ingot	6.25	1 ea/100 sq. ft.	Hand	150

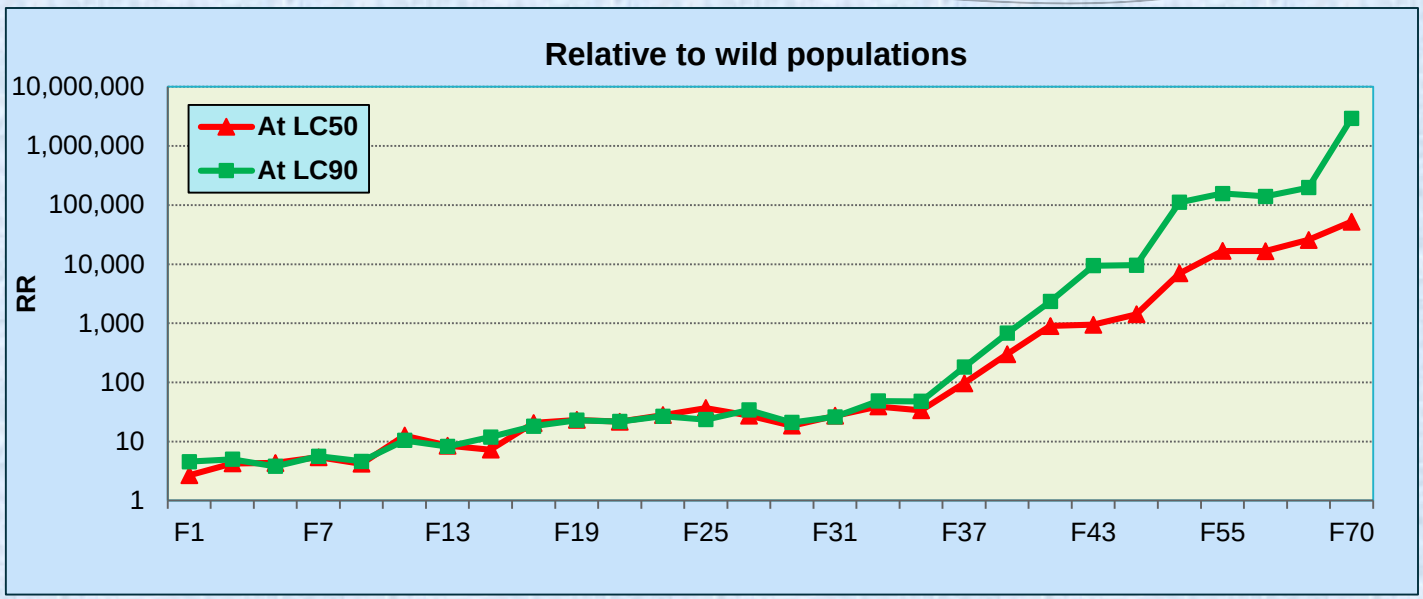
Filed Test Results (Exp. 073010 – Dairy pond WV)

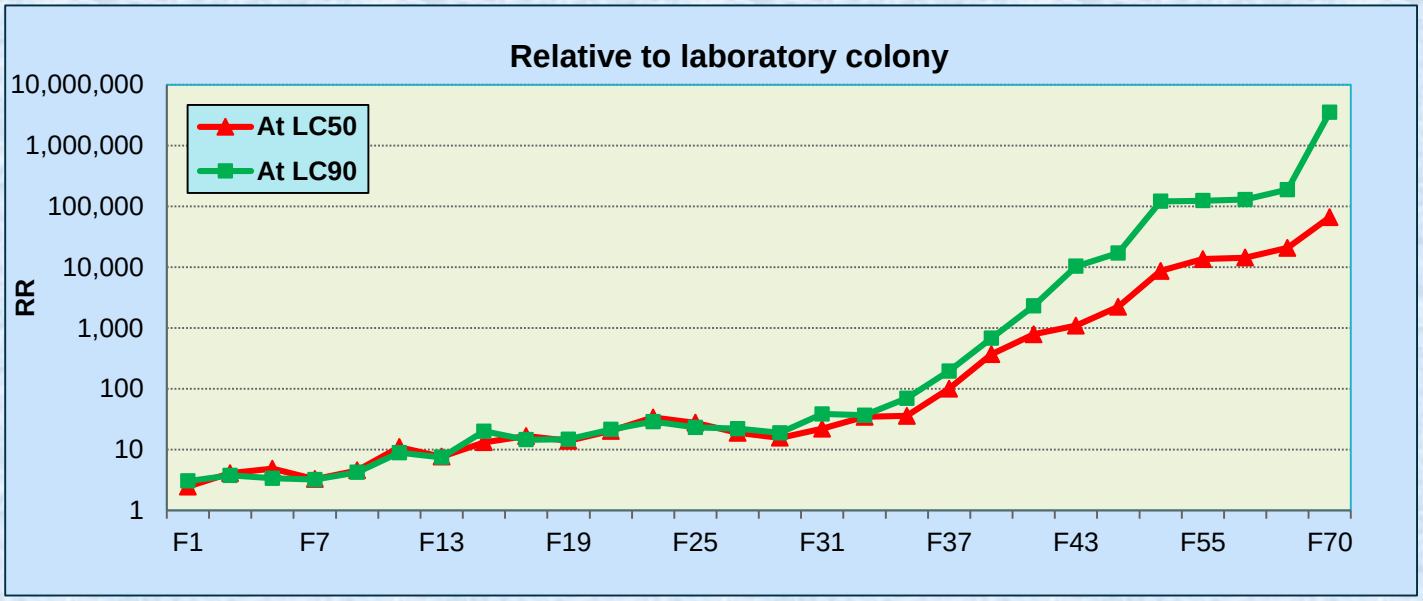




Resistance Development to Spinosad in *Cx. quinq.*

(Su et al. 2021)

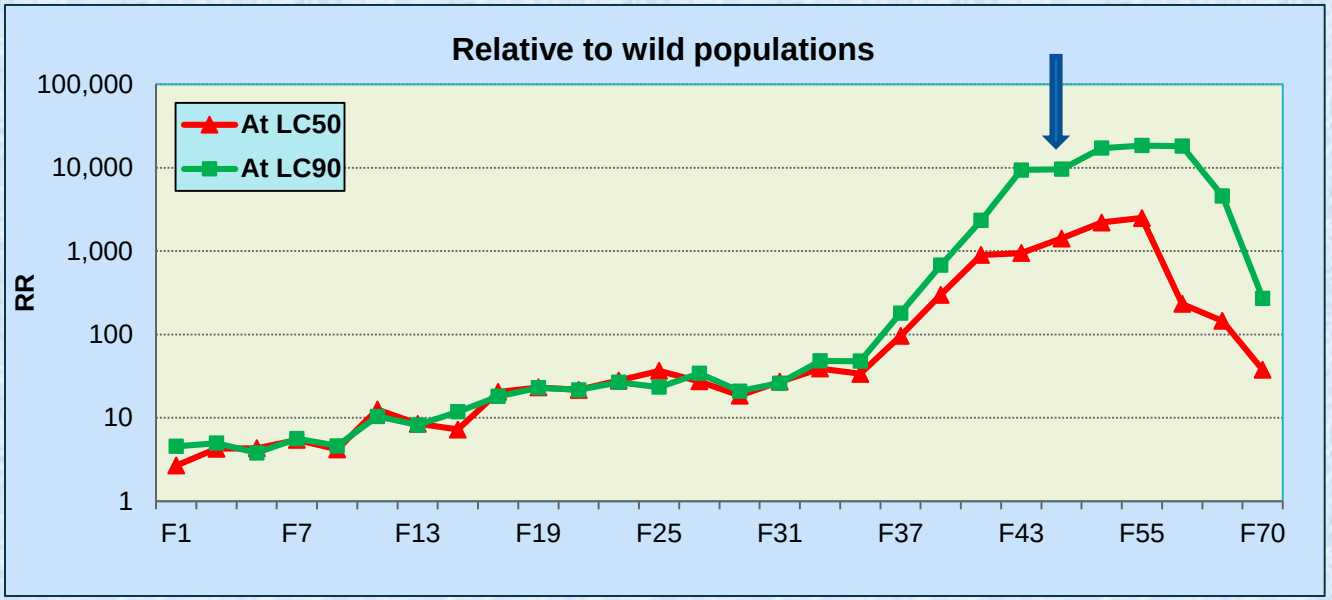


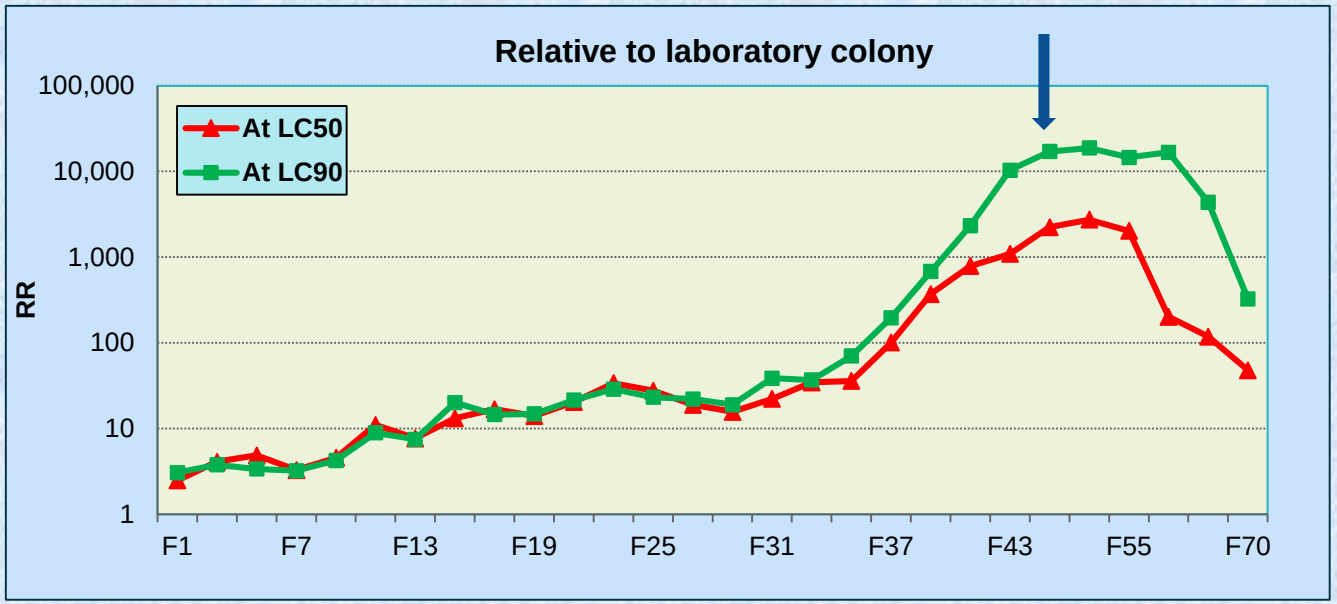


Stability of Resistance in *Cx. quinq.*

Removal of Selection

(Su et al. 2021)

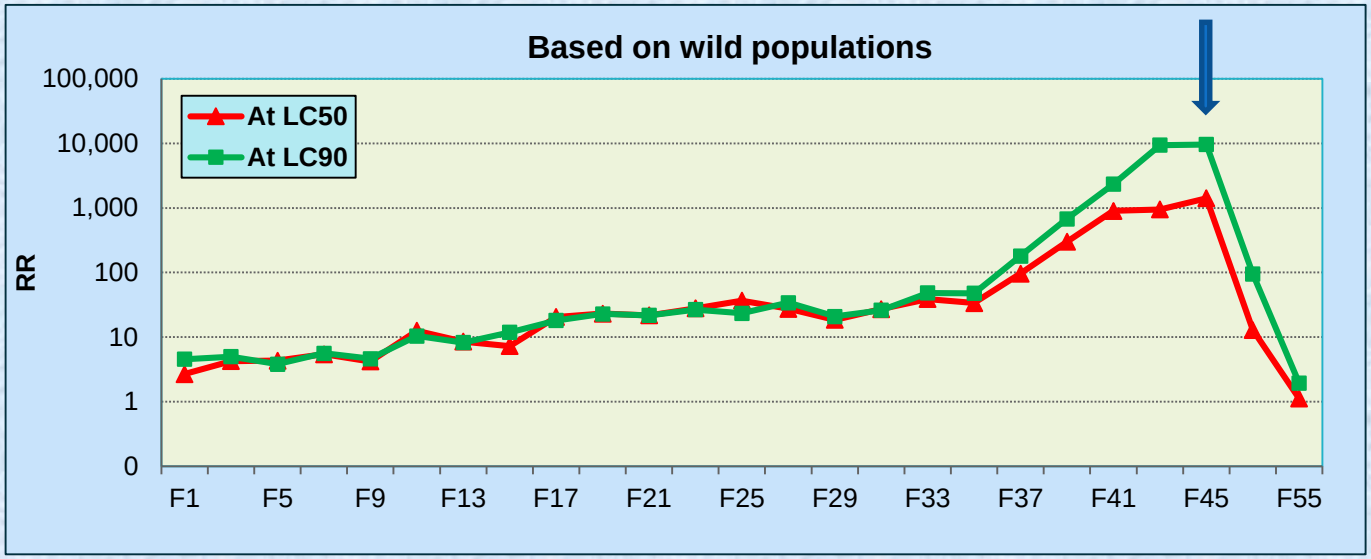


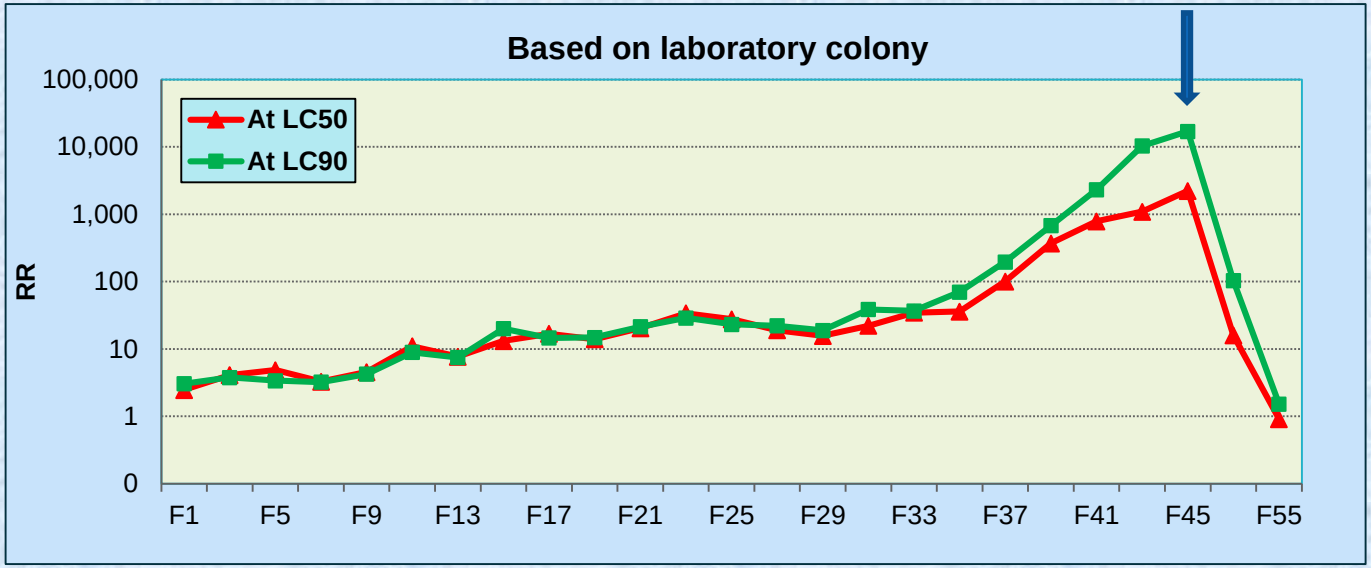


Stability of Resistance in *Cx. quinq.*

Removal of Selection + Addition of Susceptible Individuals at 10%

(Su et al. 2021)





Absence of cross Resistance to Other Larvicides In Spinosad – R *Cx. quinq.* (Su & Cheng 2014)

Larvicides Tested	IRAC group/ MoA	Gen of selections	RR to Natular G30		cRR*	
			@ LC_{50}	@ LC_{90}	@ LC_{50}	@ LC_{90}
B.t.i. (VectoBac WDG, 3,000 ITU/mg)	11/ Midgut disruptor	F_{25}	**28-37**	**23**	**0.6**	**0.5**
B.t.i. + *L. sphaericus* (VectoMax CG, Unknown Bti+50 Bs ITU/mg)		F_{27}	**19-27**	**22-34**	**1.9**	**2.3**
S-methoprene (Altosid LL, 5% S-methoprene)	7/ IGR-JHA	F_{25}	**28-37**	**23**	**2.9**	**1.4**
Pyriproxyfen (Archer, 1.3% pyriproxyfen)		F_{33}	**35-39**	**37-48**	**1.5**	**0.5**
Novaluron (Mosquiron 0.12CRD, 0.12% novaluron)	15/ IGR-CSI	F_{47}	**2,845-2,907**	**11,948-22,928**	**1.3**	**0.8**
Diflubenzuron (Dimilin 25 WP, 25% diflubenzuron)		F_{31}	**22-27**	**26-39**	**1.5**	**1.0**
Temephos (Skeeter Abate, 5% temephos)	1B/ AChE inhibitor	F_{27}	**19-27**	**22-34**	**2.0**	**1.9**
Imidacloprid (ImidaPro 4SC, 40.7% imidacloprid)	4/ nAChR agonist	F_{41}	**787-900**	**2,318-2,334**	**2.1**	**1.4**
Indoxacarb (Advion RIFA bait, 0.045% indoxacarb)	22A/ Na^+ channel blocker	F_{50}	**6,992-8,649**	**110,962-120,920**	**1.4**	**2.2**

Presence of cross Resistance to Other Larvicides In Spinosad – R *Cx. quinq.* (Su & Cheng 2014)

Larvicdes Tested	IRAC group/ MoA	Gen of selections	RR to Natular G30		xRR*	
			@ LC_{50}	@ LC_{90}	@ LC_{50}	@ LC_{90}
L. sphaericus (Spheratax TP, 1,000 ITU/mg)	11/ Midgut disruptor	F_{29}	16-19	19-21	81	9,472
		F_{37}	93-101	181-196	125	20,743
L. sphaericus (VectoLex WDG, 650 ITU/mg)		F_{31}	22-27	26-39	110	9,870
		F_{37}	93-101	181-196	89	15,792
Spinetoram (Radiant, 11.7% spinetoram)	5/ nAChR allosteric modulators	F_{33}	35-39	37-48	59	83
		F_{37}	93-101	181-196	96	109
Abamectin (Advance 375A, 0.011% abamectin)	6/ Chloride channel activators	F_{45}	1,415-2,320	9,613-17,063	26	83
Fipronil (Taurus SC, 9.10% fipronil)	2B/ GABA-gated chloride channel antagonists	F_{41}	787-900	2,318-2,334	18	11

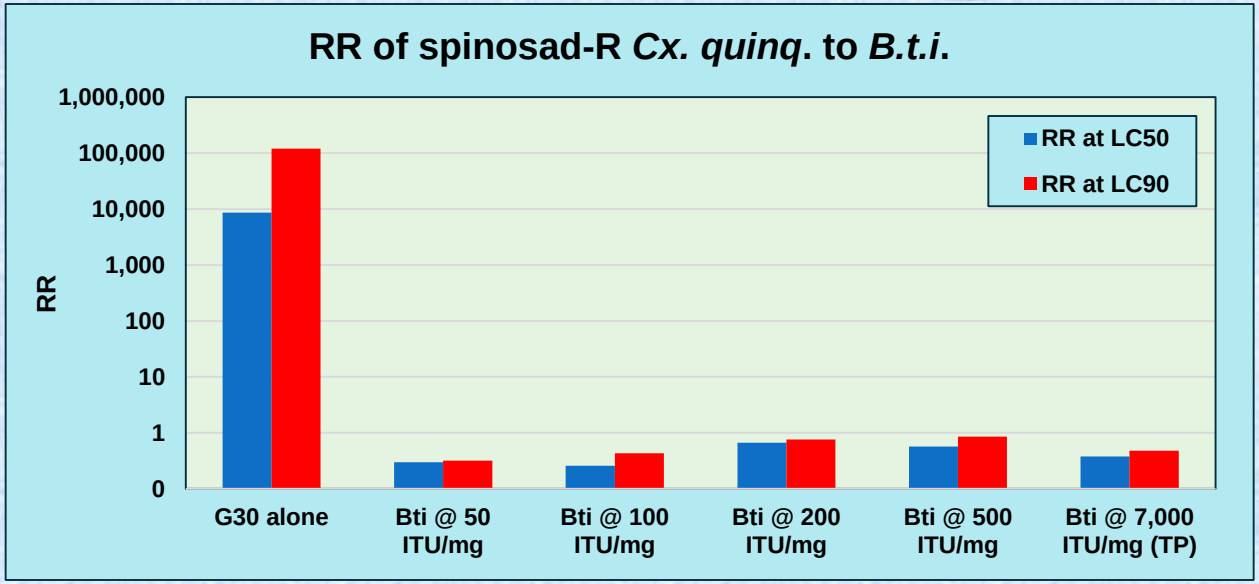
Absence of Cross Resistance to Spinosad and Spinetoram in *Lsph*–R *Cx. quinq.* (Su & Cheng 2014)

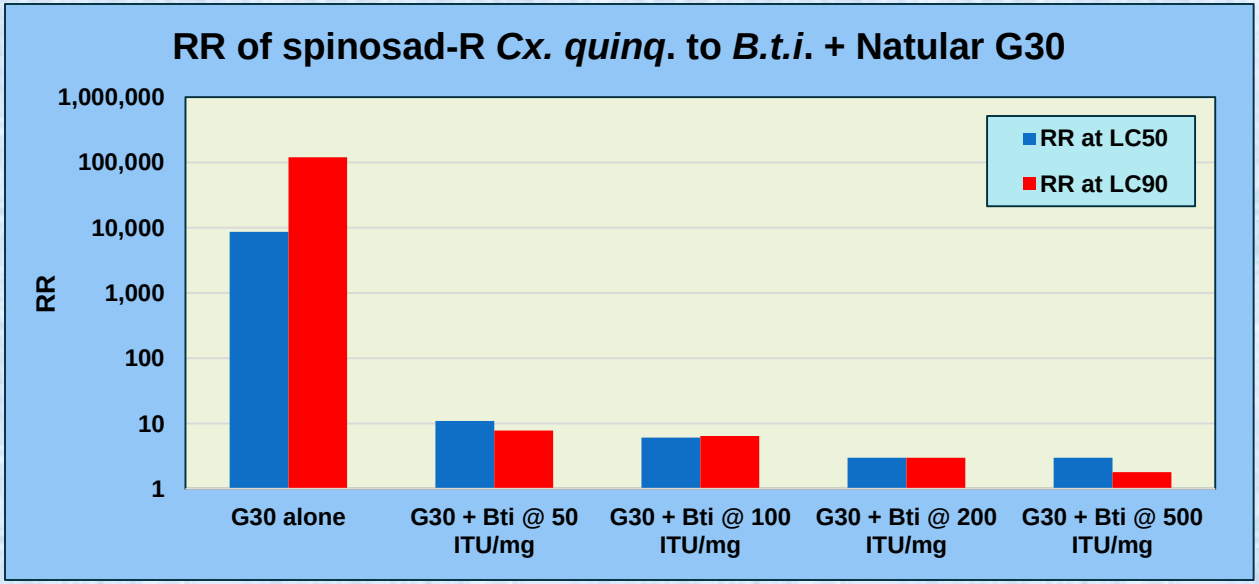
Bsph-R Generations of selections	RR to VectoLex WDG (650 ITU/mg)		RR to Spheratax TP (1,000 ITU/mg)		cRR to spinosad		cRR to spinetoram	
	@ LC_{50}	@ LC_{90}	@ LC_{50}	@ LC_{90}	@ LC_{50}	@ LC_{90}	@ LC_{50}	@ LC_{90}
Since 1990	58,512	510,852	24,840	74,669	**1.2**	**1.2**	**0.6**	**0.8**

Absence of Cross Resistance to Spinosad and Spinetoram in Methoprene–R *Cx. quinq.* (Su & Cheng 2014)

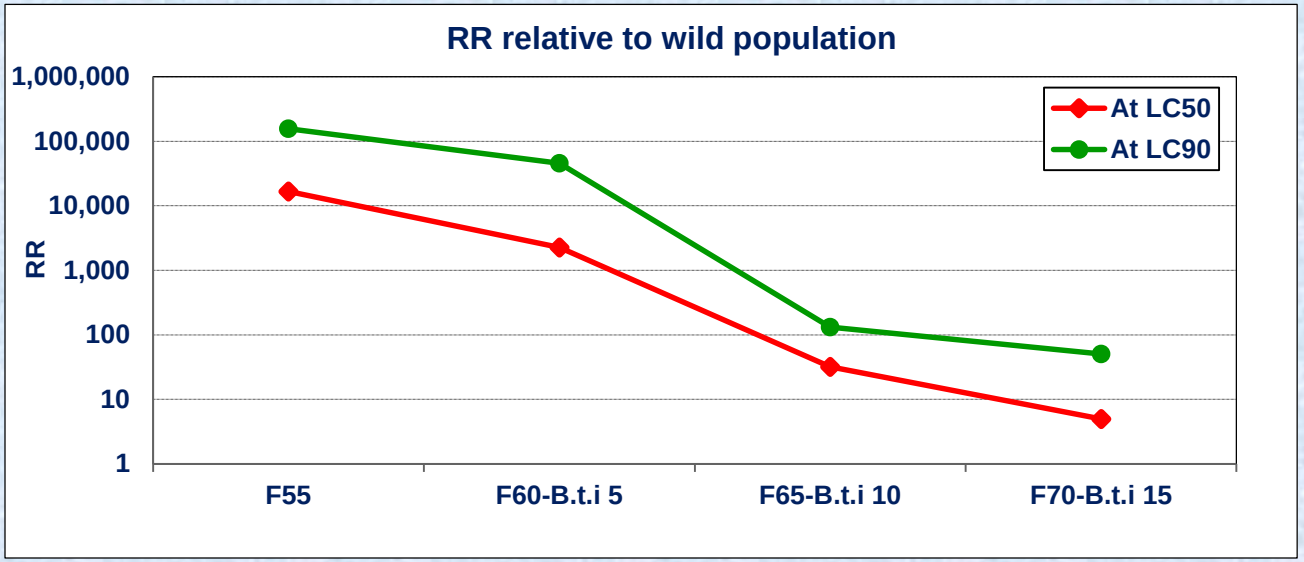
Bsph-R Generations of selections	RR to methoprene		cRR to spinosad		cRR to spinetoram	
	@ IE_{50}	@ IE_{90}	@ LC_{50}	@ LC_{90}	@ LC_{50}	@ LC_{90}
Since 1990	8-40	9-54	**0.8-0.9**	**1.0-1.2**	**1.0**	**1.9**

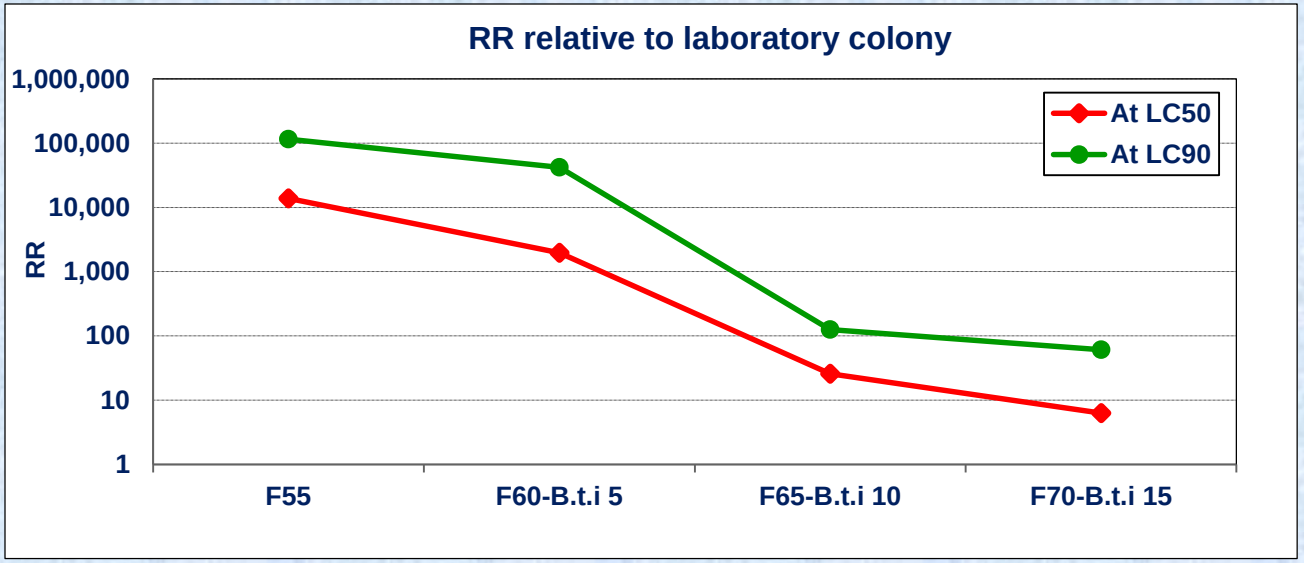
Potential of *B.t.i.* in Spinosad Resistance Management





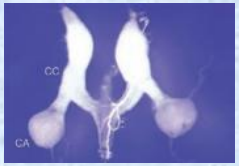
Spinosad susceptibility Restoration by *B.t.i.*

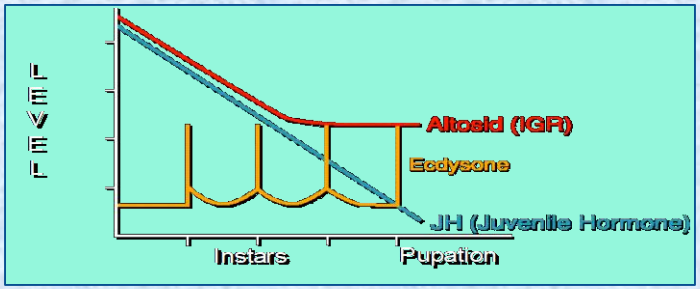


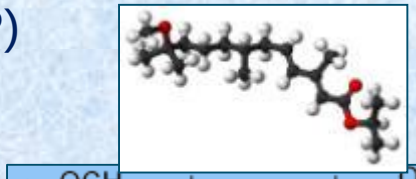


S-methoprene

- Growth regulation by interrupting JH balance
- Most susceptible window period: 12-24 h before pupation
- Mortality: Majority-Pupae or intermediate form (L-P)



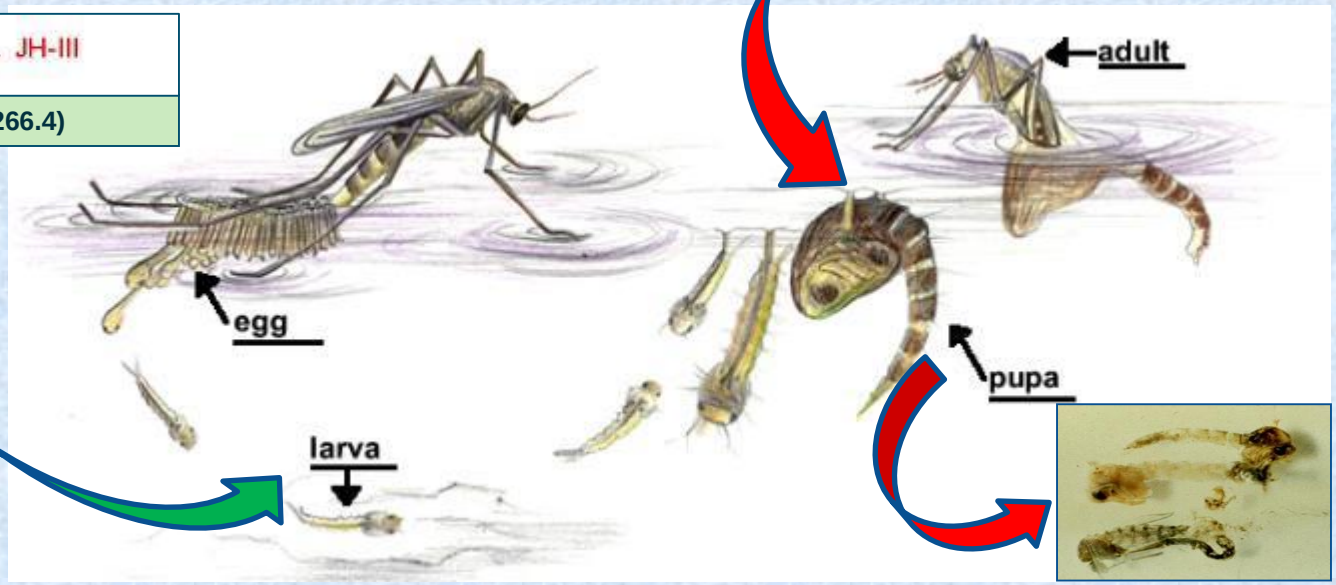




Methoprene

($C_{19}H_{34}O_3$ MW 310)

($C_{16}H_{26}O_3$ MW 266.4)



S-methoprene products

Formulations	AI %	Dose	Residual (d)	Pre-hatch treat.
Altosid SR-5	5	4 fl. OZ/ac	3-5	No
Altosid SR-20	20	¾ -1 OZ/ac	3-5	No
Altosid SBG	0.2	5-20 Lb/ac	7 to 10	Yes
Altosid Pellets	4.25	5-10 Lb/ac	30	Yes
Altosid 30-d Briquet	8.62	Ea/100 sq. ft	30	Yes
Altosid 150-d Briquet	2.1	Ea/100 sq. ft	150	Yes
Altosid 150-d Ingot	2.1	Ea/100 sq. ft	150	Yes

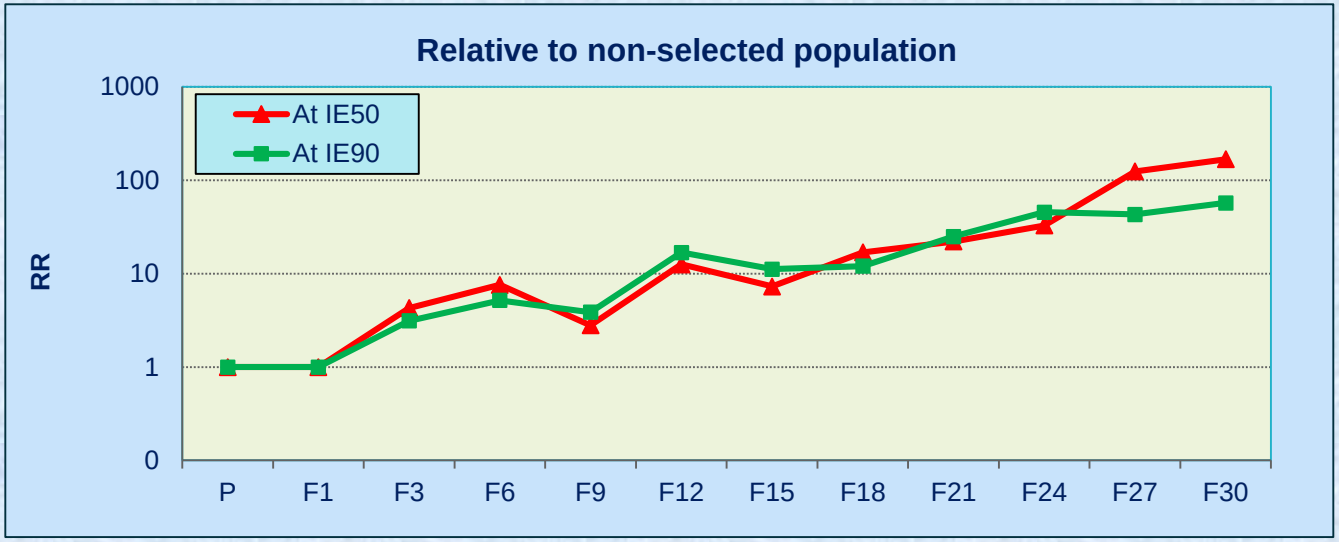
Resistance in Laboratory Populations

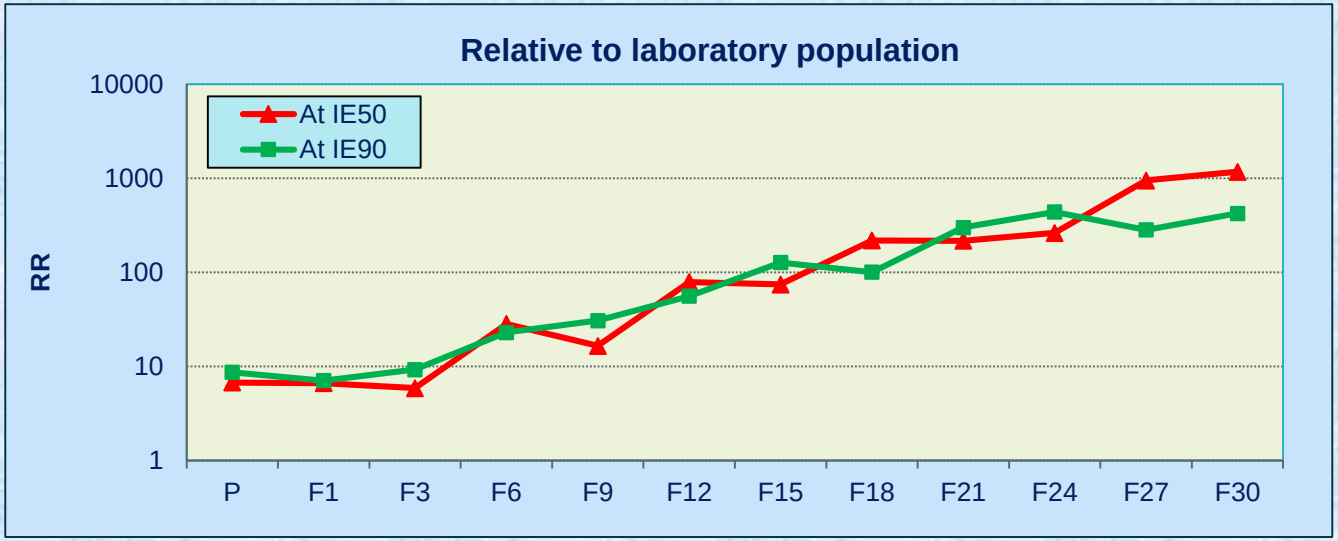
Species	Gen's of selection	Chemicals	RR	Cross resistance	References
Cx. quinq.	20	methoprene	1		Schaefer & Wilder (1973)
Cx. pipiens	8	methoprene	8	hydroprene	Brown & Brown (1974)
Cx. tarsalis	62	methoprene	86		Georghiou, et al. (1974)
Cx. pipiens	40	methoprene	218	hydroprene & triprene	Brown et al. (1978)
Cx. pipiens	27	triprene	10		Brown et al. (1978)
Cx. pipiens	8	hydroprene	1		Brown et al. (1978)
Cx. quinq.	10	methoprene	3.9-21.3		Amin & White (1984)
Cx. quinq.	30	methoprene	1,025		Su et al. (2020)

Resistance in Field Populations

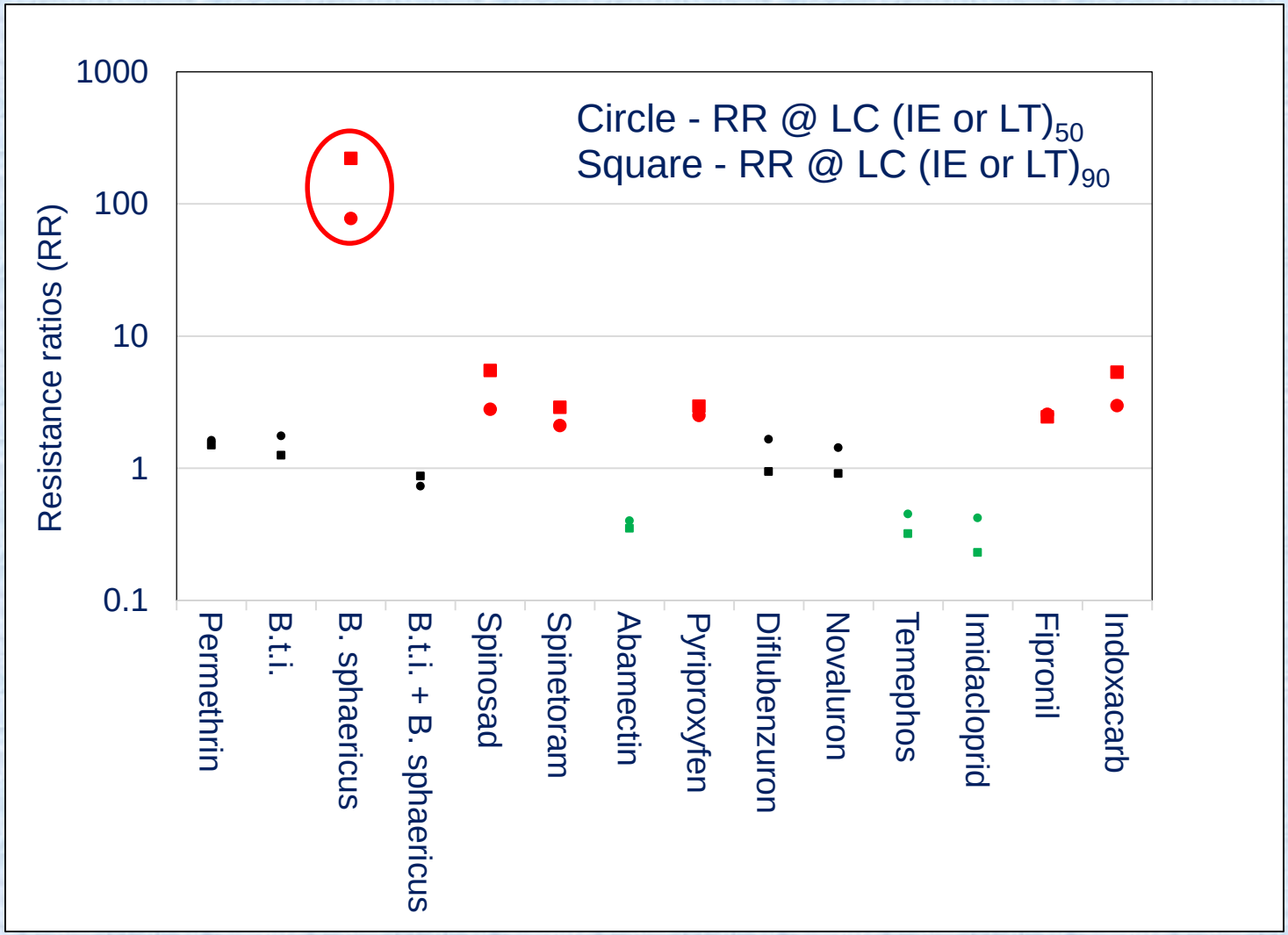
Species	Years of appl.	Chemicals	RR	References
Ae. taeniorhynchus	5	methoprene	15	Dame et al. (1998)
Ae. nigromaculis	20	methoprene	1000s	Cornel et al. (1999, 2000)
Ae. nigromaculis	?	methoprene	141	Su et al. (2018)
Cx. quinq.	?	Methoprene	8-28	Su and Cheng (2014)

Resistance in Lab Selection after Field Occurrence (Su et al. 2020)





Cross resistance to common pesticides in *Culex quinq.* resistant to methoprene (Su et al. 2020)



Summary

- **Flood water mosquitoes: *Aedes*, *Psorophora***
- **Larvicides: *B.t.i.*, *Lsph*, spinosad, s-methoprene**
- **Larviciding: Liquid or granular formulations**
- **Resistance management: monitoring, rotation or combination of products with different mode of actions**

Feel free to talk even you are not sure that it is better than being silent…