Evaluation of *Bacillus thuringiensis* for Management of Adult Emerald Ash Borer (*Agrilus planipennis*) on Ash Trees

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The Emerald Ash Borer (EAB)

From http://www.emeraldashborer.info

- Exotic pest of ash trees native to Asia
- Found in Michigan and Ontario in 2002
- Currently 23 additional US states as well as Quebec have EAB infestations

From http://www.emeraldashborer.info/map.cfm#

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The Emerald Ash Borer (EAB)

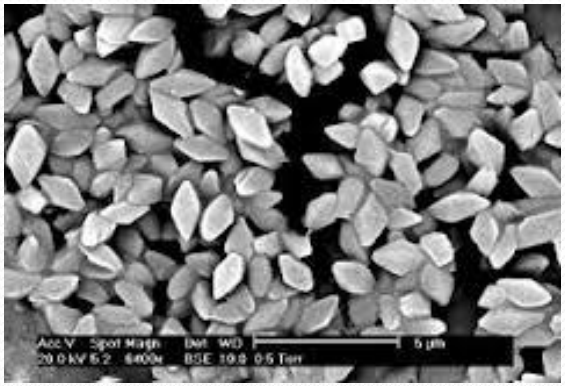
- 1-2 year life cycle
- EAB damage to ash trees
- Treatment options limited
  - Systemic treatments
  - Parasitoid wasps
  - Removal of infested trees
- Economic impacts
  - Estimated 2010-2020 cost of US $12.4B to remove/replace landscape ash trees
  - Decreased property values

Photos by Jerry Dowding, Canadian Food Inspection Agency, and David Cappaert, Michigan State University

Bacillus thuringiensis (*Bt*)

- First isolated in 1901 by Ishiwatari from diseased silkworms (*Bombyx mori*)
- Isolated again in 1911 by Berliner from a diseased Mediterranean flour moth larvae (*Ephestia keuhniella*)
  - Named after German province of Thuringia
- Over 82 serovars
- Produces crystal composed of δ-endotoxin (Cry protein) during sporulation
- Spore/crystal mixtures used to formulate biological insecticides

From http://bacillusthuringiensis.pbworks.com/w/page/9916080/FrontPage



Microscopy by Jim Buckman

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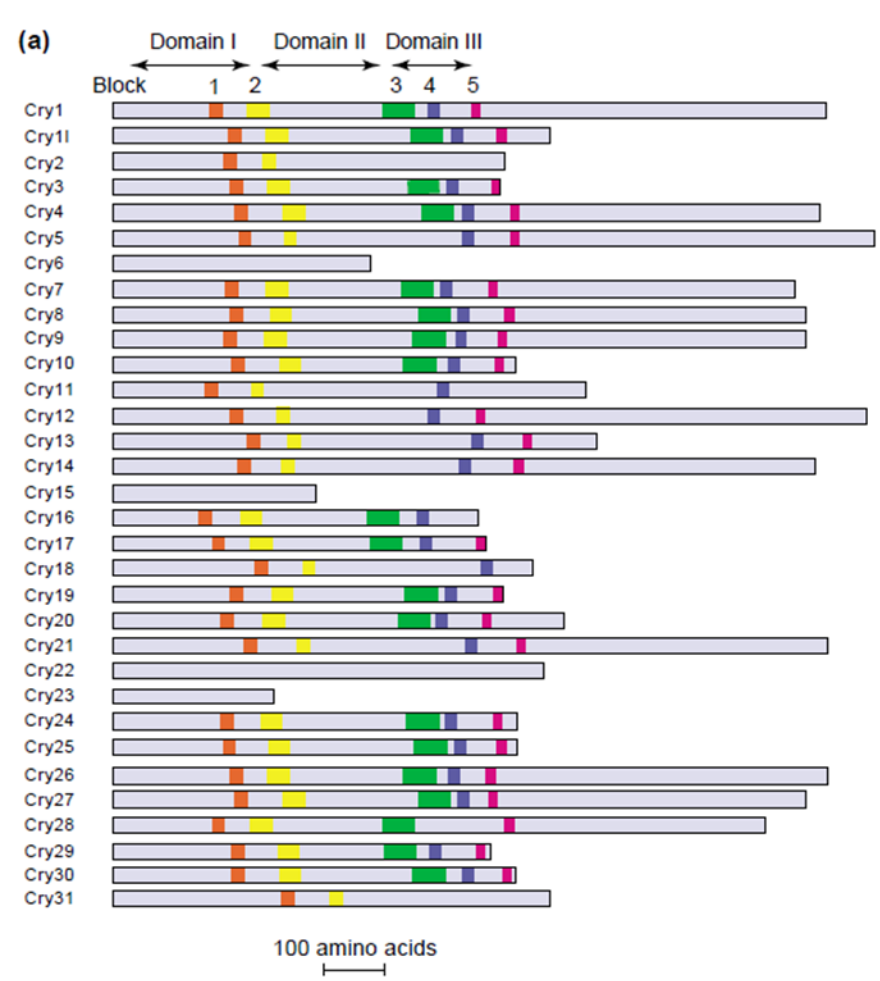
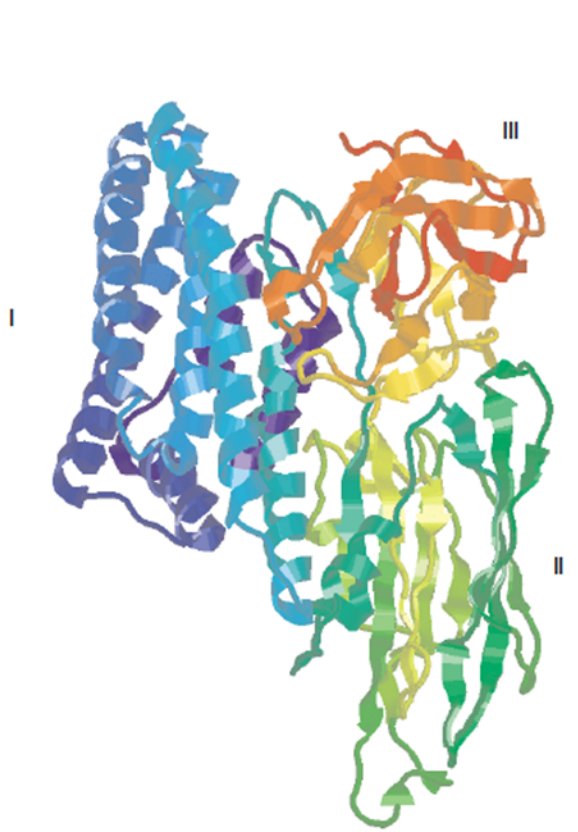
Cry Toxins





From de Maagd *et al.* 2001. *Trends Genet*. 17:193-199

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Bt Mode of Action

Ingestion of crystals by a susceptible insect

Crystal solubilization
Toxin activation

Receptor binding

Pore formation

Disruption of mid-gut lining

Cessation of feeding

Septicemia

DEATH

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Bt galleriae SDS-502

- Isolated in 2002 by Asano
- Produces Coleopteran-active Cry8Da
- Active against both larvae and adults
- Toxin binds to glucosidase in adult Japanese beetles
- Used by Phyllom BioProducts to produce:
  - bore*GONE!*®
  - beetle*GONE!* ag™ / beetle*GONE!* tlc™
  - grub*GONE!*®

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Cry8Da is Active Against Adult EAB

- LD_{50} of *Btg* SDS-502 crystal/spore mixture in EAB adults dosed using a droplet imbibement bioassay

Bioassay	LD_{50} (95% CI) (µg Cry8Da)	Slope	Pearson χ^2	n
Rep. 1	0.22 (0.15-0.34)	2.2	1.58	90
Rep. 2	0.25 (0.14-0.88)	1.2	0.03	90
Combined	**0.23 (0.16-0.35)**	1.6	0.53	180

Study performed by Leah Bauer, USDA Forest Service and Michigan State University

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Field Trial of bore*GONE!*® Sprayed on Potted Ash Trees

- Two rates: 100 and 200 grams/liter
- Small ash trees growing inside a hoop house under a shade cloth were hand sprayed onto until the foliage was completely wet
- Foliage collected on Days 0, 1, 2, 4, and 7 post-treatment
- Seven adult EAB male-female pairs/replicates used per treatment and the untreated control

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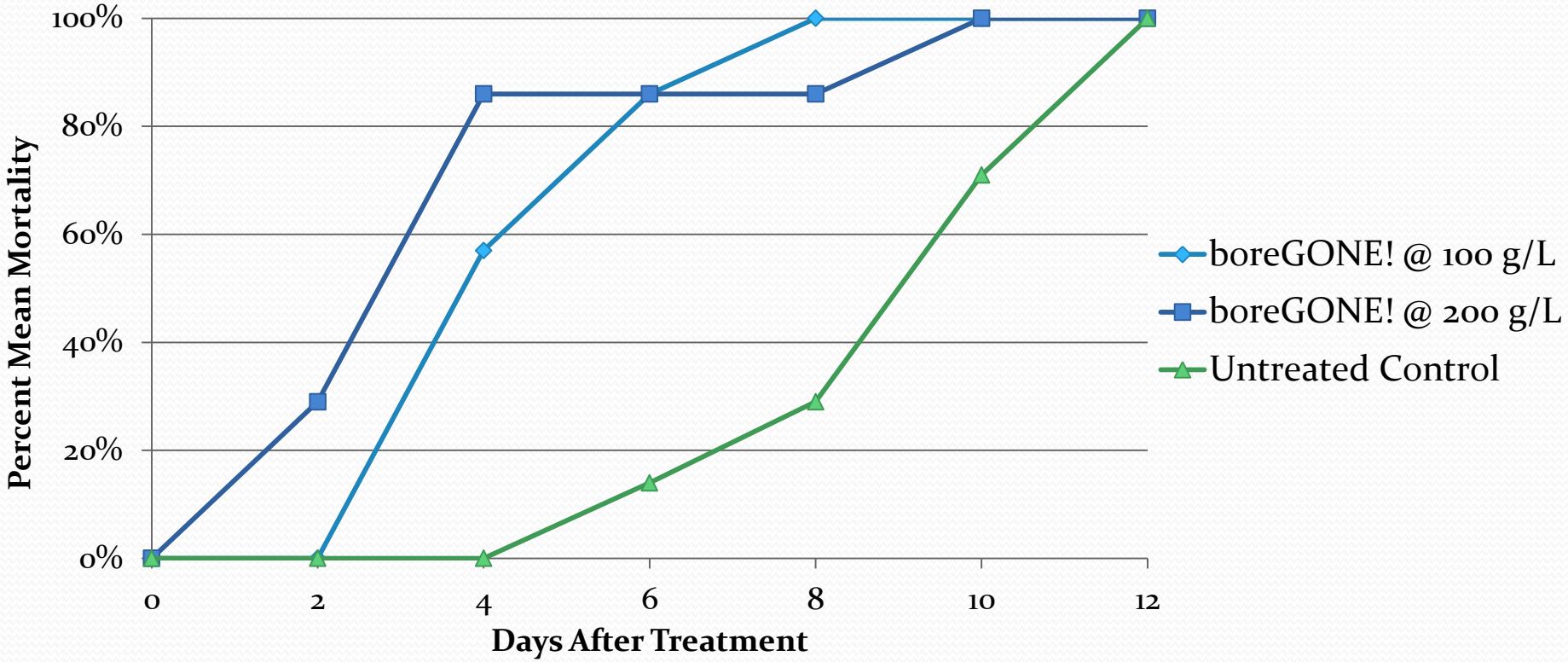
Adult EAB Mortality Over Time Post-treatment



Study performed by Fredric Miller, Morton Arboretum and Joliet Junior College

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boreGONE!® Lowers Adult EAB Longevity

Treatment	Mean Adult Longevity (Days)[1]
boreGONE! @ 100 g/L	5 a
boreGONE! @ 200 g/L	3 a
Untreated Control	9 b
Significance:	**F=26.69**
	P<0.001

[1]Means followed by the same letter are not significantly different. (P<0.05: Holm-Sidak method)

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bore*GONE!*® Lowers Time of Adult EAB Feeding Activity

Treatment	Mean Adult Feeding Activity (Days)[1]
bore*GONE!* @ 100 g/L	7 a
bore*GONE!* @ 200 g/L	6 a
Untreated Control	14 b
Significance:	**F=37.4**
	P<0.001

[1]Means followed by the same letter are not significantly different. (P<0.05: Holm-Sidak method)

Study performed by Fredric Miller, Morton Arboretum and Joliet Junior College

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boreGONE!® Lowers Adult EAB Damage to Ash Foliage

Treatment	Mean Leaf Tissue Consumed (%)[1, 2]	Mean Frass Produced (mg)[1]
boreGONE! @ 100 g/L	3.33 ab	44 ab
boreGONE! @ 200 g/L	2.61 a	33 a
Untreated Control	5.73 b	68 b
Significance:	**F=4.26**	**F=5.72**
	P=0.031	**P=0.012**

[1]Means followed by the same letter are not significantly different. (P<0.05: Holm-Sidak method)
[2]Seven measurements per leaf

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2014 bore*GONE!*® Aerial Trial

- Assess ability of bore*GONE!*® to knock down population of subsequent EAB generation
- Made two aerial applications of bore*GONE!*® on EAB-infested ash trees in Starved Rock State Park, Utica, IL
- Sprayed in late June/early July during peak flight as determined by day degree models
- Sprayed 16 acres at 7.5 lbs./acre
- Pre- and post-spray branch sampling in progress to assess effect

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Conclusions

- bore*GONE!*® is a new tool for controlling Emerald ash borer infestations
- The Cry8Da crystal protein in bore*GONE!*® is highly active against the adult Emerald ash borer
- Feeding bore*GONE!*®-sprayed ash foliage to adult Emerald ash borer resulted in:
  - Lower longevity
  - Lower feeding activity
  - Less foliar damage

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