Vestaron SPEAR™ Products Mechanisms of Action

March 9th, 2016

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Outline

- SPEAR™ Product Line
- Mode of action of GS-Omega/Kappa-HXTX-Hv1a
  - Phenotypic Symptoms
  - Neuromuscular Effects
  - Electrophysiology Measurements
- Omega & Kappa Peptides
- Synergy considerations

SPEAR™ Product Line

- SPEAR™-T : Comprised of GS-Omega/Kappa-HXTX-Hv1a active alone for control of thrips & whiteflies in glasshouses. Mode of exposure is primarily by contact.
- SPEAR™-C: Comprised of GS-Omega/Kappa-HXTX-Hv1a active co-formulated with a Bt_k for control of caterpillars. Mode of exposure is primarily oral ingestion which is facilitated* by perforation of the gut by the Bt_k.
- SPEAR™-P: Comprised of GS-Omega/Kappa-HXTX-Hv1a active co-formulated with a Bt_t for control of Colorado potato beetles. Mode of exposure is a mixture of contact and oral ingestion.

Note: appendix slide on Bt resistant insects showing synergy of Bt and SPEAR™ peptide

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GS-Omega/Kappa-HXTX-Hv1a: Insecticidal Activity

- Spectrum: GS-Omega/Kappa-HXTX-Hv1a has insecticidal activities against wide range of arthropod pests by injection, including those in the orders of diptera, lepidoptera, coleoptera, orthoptera, blattaria, Acari.
- Symptoms: Insects injected with GS-omega/kappa-HXTX-Hv1a typically showed neurotoxic symptoms. High dose injection results in quick paralysis of insects and death. At low dose injection, excitatory toxic phenotypes were observed initially in the injected insects, for example uncontrollable fasciculation of limbs, lost coordinate movements etc, followed by paralysis and death. These symptoms indicate GS-Omega/Kappa-HXTX-Hv1a is a neurotoxin (Sollod, 2006).

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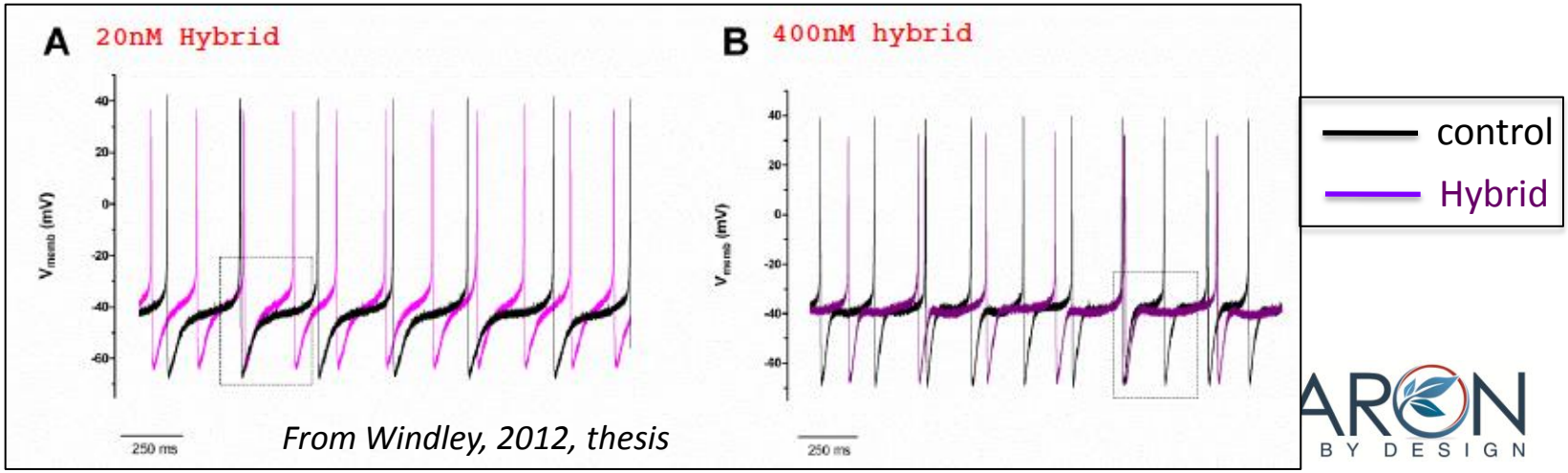
GS-Omega/Kappa-HXTX-Hv1a: Effects on Spontaneous AP frequency

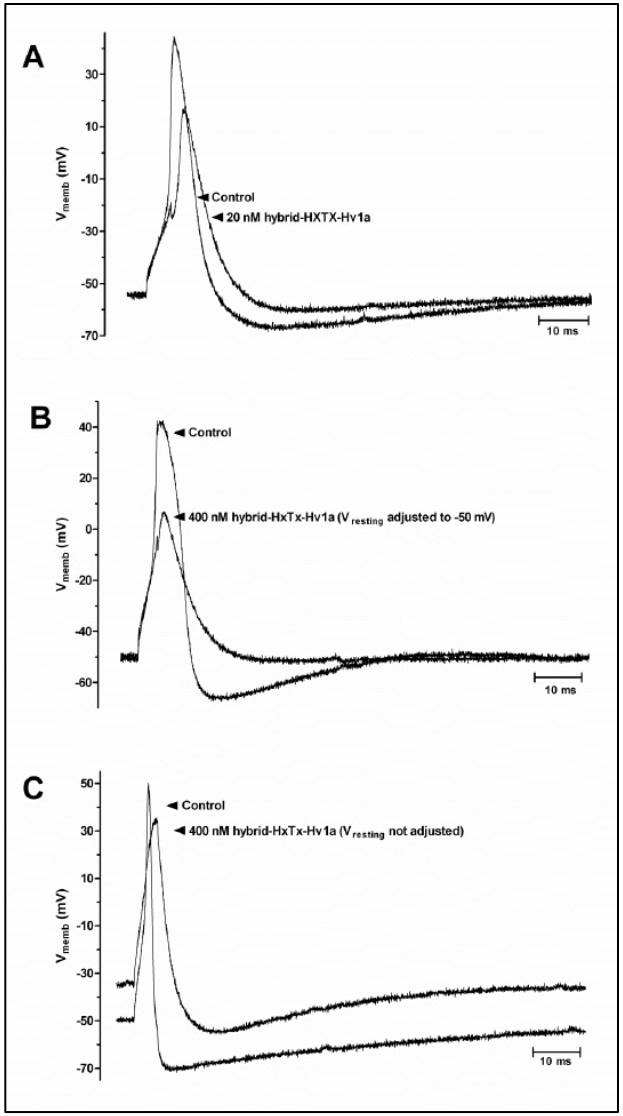
- The peptide effects on the action potential (AP) of Dorsal Unpaired Median neuron (DUM) isolated from the terminal abdominal ganglion (TAG) of American cockroaches (*Periplaneta americana*) were studied (Monique Windley, 2012), showing complicated modifications on the AP.
- DUM neurons spontaneously fire action potentials to maintain critical autonomous activities of cockroaches which are essential for cockroach survival. The peptide at low doses increased the spontaneous firing frequency from 4 Hz to 6.3 Hz, but decreased the firing frequency down to 1Hz at high doses.
- Higher firing frequency results in over-excitatory reaction as seen as uncontrollable fasciculation of limbs; Lower firing frequency results in fewer autonomous actions or even paralysis.



From Windley, 2012, thesis

GS-Omega/Kappa-HXTX-Hv1a: Modification of the AP Properties

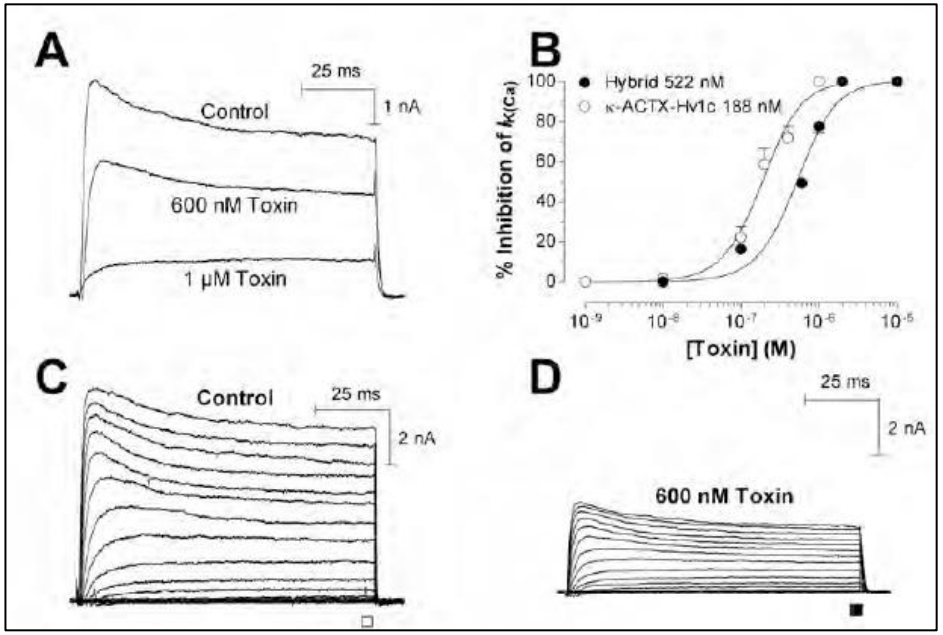
- The peptide affected the action potential properties as well, including decreased and even diminished after-hyperpolarization potential (AHP) and prolonged AP repolarization. At high dose, the peptide also depolarized the neuron membranes and decreased the AP amplitude.
- Neuron communication is through neuron synapses. Pre-synaptic membrane AP determines the neurotransmitter release. Diminished AHP and prolonged AP repolarization both promote the neurotransmitter release from the pre-synaptic neurons and generate excitatory reactions observed in the insect injection bioassay. High doses of the peptide decrease the AP amplitude which explains why high dose injections cause quick insect paralysis.



From Windley, 2012, thesis

GS-Omega/Kappa-HXTX-Hv1a: Inhibition of BK_{Ca} channels

- The peptide induced prolongation of AP repolarization can be explained by blockage of voltage-gated K channels since their open state is responsible for the repolarization phase of an AP.
- One of the K_v channels, the large conductance (B), Ca^{2+} activated potassium channel (BK_{Ca}) is responsible for the after hyperpolariztion potential (AHP) of an AP in cockroach neurons (Wicher, et al, 2001; Grolleau et al, 1995). This peptide's effects on AHP suggest that it may block the BK_{Ca} channel.
- Cockroach BK_{Ca} gene (pSlo) was cloned and heterologously expressed in HEK293 cell line, whose membrane has "clean" ion channel background. GS-Omega/Kappa-HXTX-Hv1a showed inhibition of pSlo channel currents with an IC_{50} of 520 nM (Sollod, 2006). This IC_{50} value is relatively high. However, the experiment was performed on heterologously expressed channels, the natural formation of which, may include extra auxiliary subunits. The absence of these subunits may alter the binding affinity of the peptide.



From Sollod, 2006, thesis

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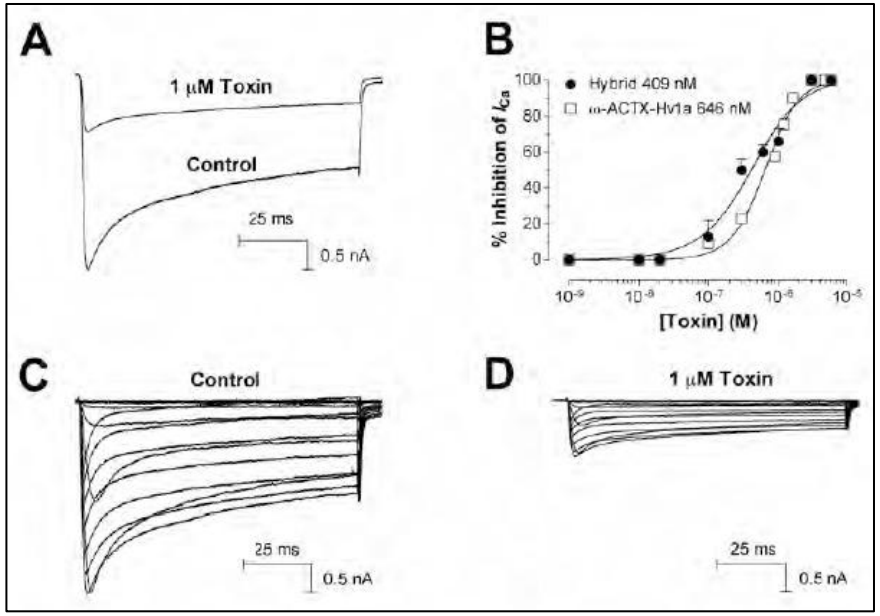
GS-Omega/Kappa-HXTX-Hv1a: Inhibition of Ca_V channels

- GS-Omega/Kappa-HXTX-Hv1a has multiple effects on the firing frequency of the spontaneous AP on DUM neurons. At low dose, the peptide increases the frequency, but at high dose the peptide decreases the frequency.
- That the peptide increases the firing frequency, can be explained by blockage of BK_{Ca} channels because this blockage decreases the amplitude of AHP, leading faster membrane depolarization to AP threshold.
- Voltage-gated Ca channel (Ca_V) blockade can explain the frequency reduction at high dose of the peptide. In cockroach DUM neurons, the spontaneous AP is generated when a "pace-maker" like membrane depolarization, which is induced by Ca_V channel opening, reaches the threshold. The blockade of Ca_V reduces AP firing frequency by slowing down the depolarization process.



From Sollod, 2006, thesis

- Patch clamp recording were performed to study this peptide effects on the isolated Ca^{2+} currents on the cockroach DUM neurons with blockade of the Na^+ currents by TTX and K^+ currents by 4-AP, TEA, CTX etc. (Sollod 2006), indicating that GS-Omega/Kappa-HXTX-Hv1a inhibited Ca_V currents with IC_{50} of 410 nM.

GS-Omega/Kappa-HXTX-Hv1a; Structural basis of its dual modes of action

- Alanine scanning mutagenesis has been performed to probe the pharmacophore of GS-Omega/kappa-HXTX-Hv1a (Sollod, 2006). Its pharmacophore is composed of Q10, P11, N30, V36 and Y37.
- The pharmacophore 3D structure of GS-omega/kappa-HXTX-Hv1a overlaps with both the pharmacophores of omega-HXTX-Hv1a, a Ca_V antagonist peptide, and kappa-HXTX-Hv1c, a BK_{Ca} channel antagonist peptide.

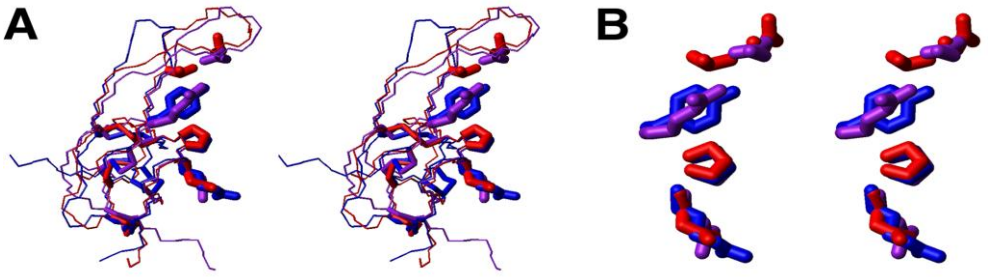


Figure 12: Overlay of toxin pharmacophores. **(A)** Stereoview of an overlay of the backbones (shown in thin neon tubes) of ω-ACTX-Hv1a (purple), κ-ACTX-Hv1c (blue), and the hybrid toxin (red). Also shown are the disulfide bonds (medium-sized neon tubes), and the sidechains of the pharmacophore residues (thick neon tubes). **(B)** Same view as (A), but just showing the sidechains of the pharmacophore residues.

From Glenn King, 2006, unpublished data.

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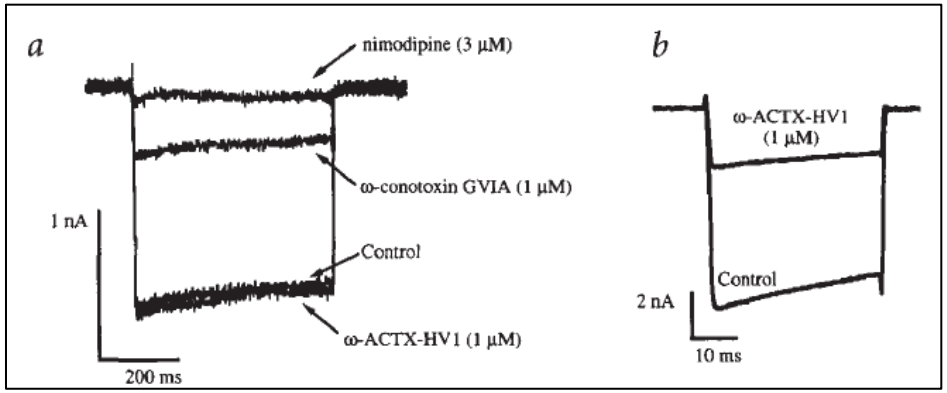
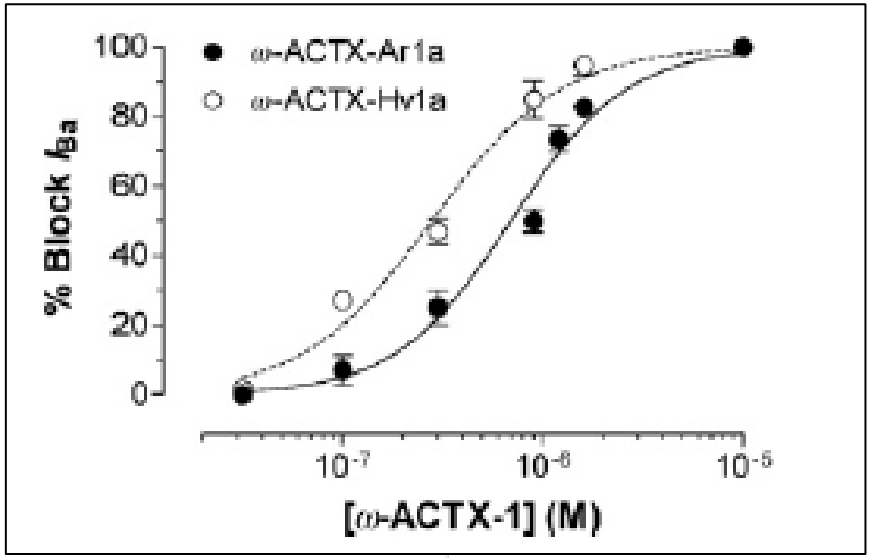
Isolated Ca_V activity of Omega-HXTX-Hv1a

- Omega-HXTX-Hv1a has broad insecticidal spectrum. Its mode of action was determined to be specifically blockade of insect Ca_V channels. It had no effect on the mammalian L, N, P/Q types of Ca^{2+} channels at the concentrations up to 10 µM (Tedford, et al., 2004).
- However, omega-HXTX-Hv1a had potent inhibition on the cockroach DUM neuron Ca_V channels. Sollod (2006) reported omega-HXTX-Hv1a had an IC_{50} of 650 nM against the isolated overall Ca^{2+} current from cockroach DUM neuron. Chong et al (2007) further identified that omega-HXTX-Hv1a was more specific to block medium to low voltage gated Ca^{2+} channel (M-LVA Ca_V) with IC_{50} of 280 nM. It also inhibit high voltage-activated Ca^{2+} channels (HVA Ca_V) with an IC_{50} of 1100 nM.



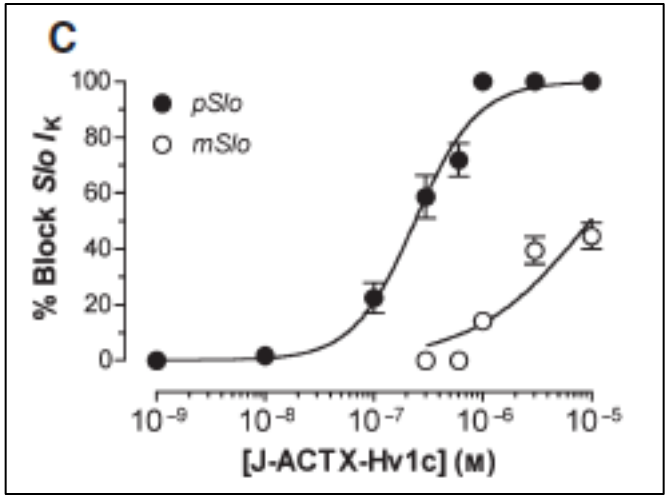
Data from Fletcher et al., 1997

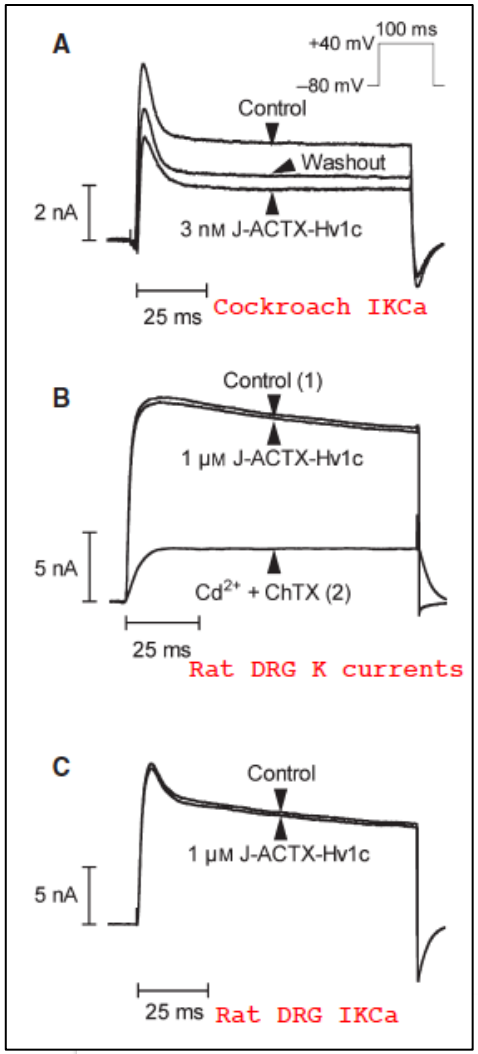


Data from Chong et al., 2007

Isolated BK_{Ca} Activity of Kappa-HXTX-Hv1c

- Kappa-HXTX-Hv1c has a broad insecticidal spectrum. Gunning et al (2008) studied its mode of action by electrophysiology performed on cockroach DUM neurons. It was reported that this peptide had no effects on the Na^+ currents, Ca^{2+} currents, delayed-rectified K^+ current or A-like K^+ currents in the cockroach DUM neuron membranes. It specifically inhibited BK_{Ca} currents in the DUM neurons with an IC_{50} of 3 nM.
- Kappa effects on BK_{Ca} channels were specific to insects and not mammals (Gunning et al, 2008), as it failed to inhibit rat DRG neuron BK_{Ca} currents.
- In the heterologously expressed BK_{Ca} channels, this peptide had an IC_{50} of 240 nM against cockroach BK_{Ca} channels, at least 50 times lower than that against mouse BK_{Ca} channels (IC_{50} > 9.7 µM).





Data from Gunning et al., 2008

GS-Omega/Kappa-HXTX-Hv1a: Potentiation due to dual modes of action

- GS-Omega/Kappa-HXTX-Hv1a had an IC_{50} of 520 nM on insect BK_{Ca} channels which is slightly less potent than Kappa-HXTX-Hv1c (IC_{50} 240 nM) (Gunning, 2008).
- GS-Omega/Kappa-HXTX-Hv1a had an IC_{50} of 410 nM on insect Ca_V channels which is roughly equivalent to that of Omega-HXTX-Hv1a (IC_{50} 650 nM by Sollod, 2006; IC_{50} 280 nM by Chong et al, 2007).
- In a fly injection bioassay, GS-Omega/Kappa-HXTX-Hv1a had LD_{50} of 38 pmol/g (Sollod, 2006), more potent than either Omega (87 pmol/g, Tedford et al, 2004) or kappa peptide (320 pmol/g, Maggio et al, 2002) in the same assay. These data indicate synergy of the dual functions of GS-Omega/kappa-HXTX-Hv1a.
- It is known that Ca_V and BK_{Ca} co-localize on the neuronal membrane (Berkefeld et al, 2006), and that Ca^{2+} influx through the Ca_V channel can activate BK_{Ca} channels. Therefore the blockage of Ca_V channels could *potentiate* the blockage of the BK_{Ca} channels.

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Summary: GS-Omega/Kappa-HXTX-Hv1a

- Clear CNS effects in insect motion
- Effects isolated to BK_{Ca} and Ca_v
- Dual effects can be isolated from each other – not the reflection of an upstream event
- Omega and Kappa peptides are development candidates and should have separate codes to avoid later confusion in the market.
- Hence, SPEAR™ has dual modes of action (Ca_v & BK_{Ca})
- SPEAR™ used in combination with Bt products should be considered to have three independent* modes of action (Ca_v, BK_{Ca} & Bt)

Note: appendix slide on Bt resistant insects showing synergy of Bt and SPEAR™ peptide

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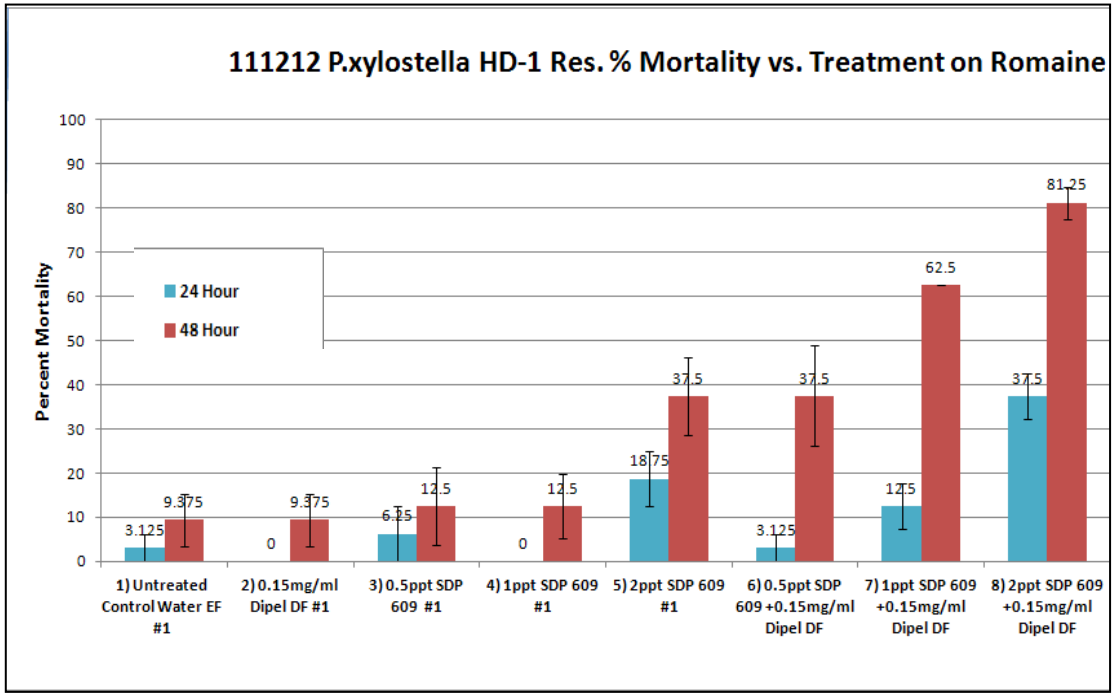
120 hr old Bt-Resistant DBM on Lettuce



- CONCLUSION: amount of Bt activity required for peptide mediated control (oral activity) is a small fraction of that required for Bt alone control (sepsis).
- Bt_k synergy seen with HD-1 DBM (800 fold resistant to Bt_k)
- HD-1 exhibit mode 1 resistance – down regulation of Bt gut receptor
- 0.15 mg/mL Dipel – laboratory rate for cut leaf disks
- Laboratory rates do not map directly to field rates
- SDP 609 - SPEAR™ peptide
- Ppt = parts per thousand of SPEAR™ peptide active

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