The Feasibility and Benefits of Mixed Microbial Pesticides

Dr. Robert Behle

Crop Bioprotection Research Unit, USDA-ARS

Peoria, Illinois

Examples of Microbial used for Biopesticides

- Bacteria
- Fungi
- Baculoviruses
- Nematodes
- Fermentation Products

Background Information

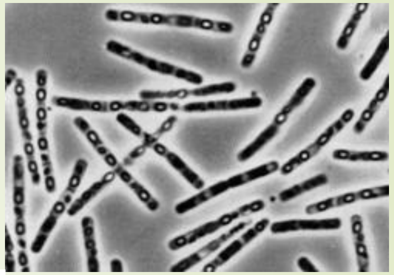
Bacillus thuringiensis kirstaki (Bt)

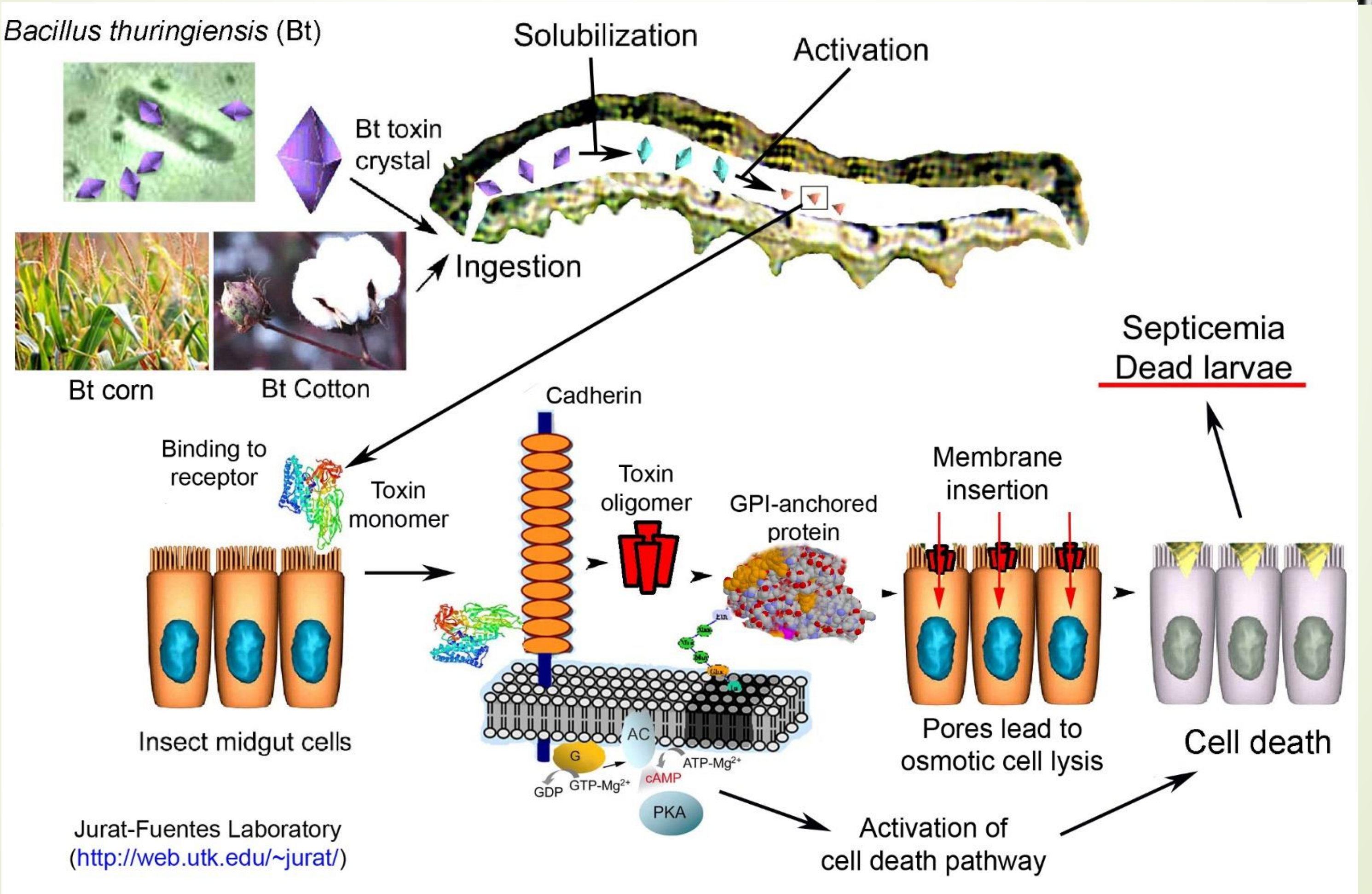
Beauveria bassiana GHA (Bb)

Literature Supporting Bacteria (*Bt*) and Fungus (*Bb*) Mixtures

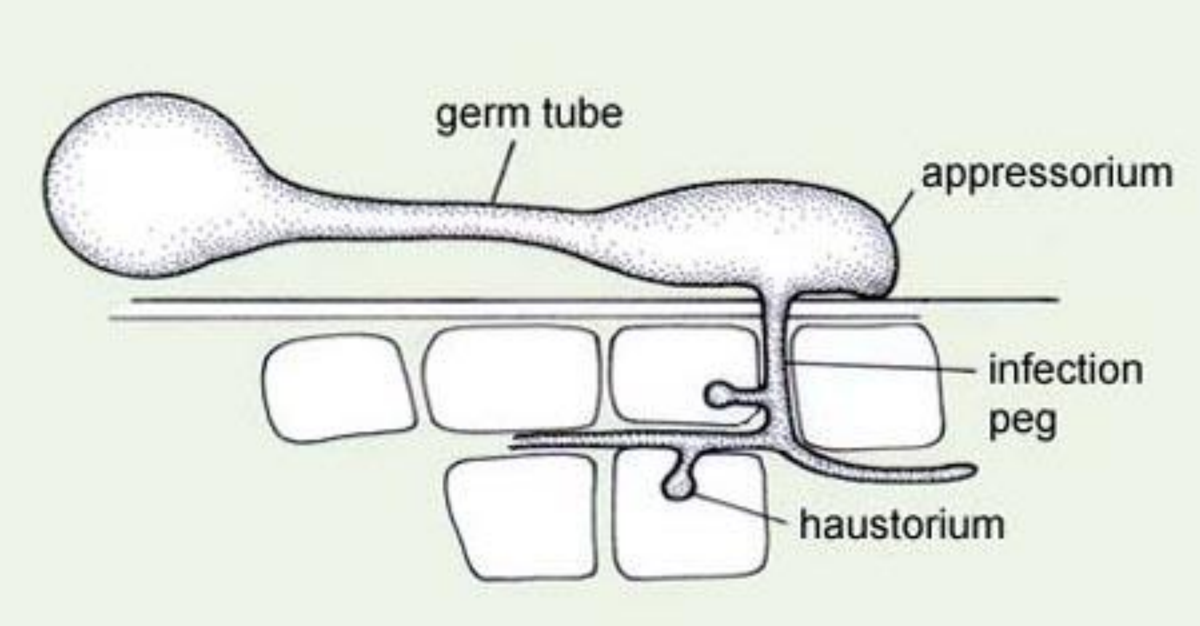
Authors	Journal	Target	Observation
Lewis & Bing, 1991	Cannadian Entomologist 123:387-393	European Corn Borer	Independent
Wraight & Ramos, 2005	Journal of Invertebrate Pathology 90:139-150	Colorado Potato Beetle	Synergistic
Mwamburi, Laing & Miller, 2009	Poultry Science	House Fly	Additive
Zhi-ying, Li-li & Chuan-wang, 2014	Journal of Beijing Forestry University	Lackey Moth (tent caterpillar)	Enhanced

Bt Infection Process





Fungal Infection Process - Conidia

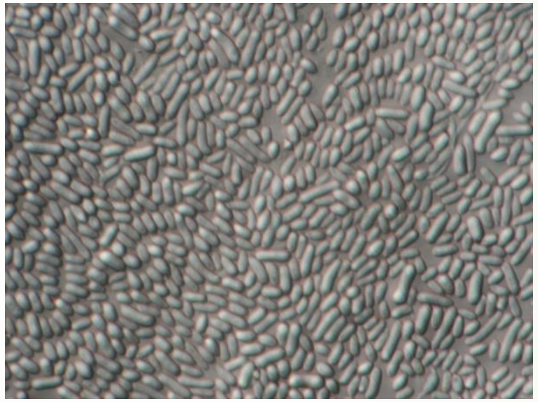


Mixed Microbe Product *Bacillus* and *Beauveria*

- Why?
  - Greater Efficacy
  - Wider Host Range for a Single Product
  - Reduced Development of Resistance
- Why Not?
  - Incompatibility (processing or storage)
  - Cost of microbe production

Fermentation for Fungal Biopesticides

Blastospores



Spore Comparison between Conidia and Blastospores

- Conidia
  - Commonly used for biopestisides
  - Relatively hardy structure
  - Contacts, germinates, infects
  - Produced using solid substrate production (dry)
  - Hyrophobic
  - Relatively slow germination

- Blastospore
  - Relatively new for biopesticides
  - Yeast-like, less hardy structure
  - Contacts, geminates, infects
  - Produced using liquid fermentation (aqueous)
  - Hydrophylic
  - Relatively fast germination

Production Considerations

- Fermentation conditions promote blastospore production
- Fermentation time 4-5 days
- Media cost estimated at $0.10 / L
- Spore yields about 2×10^{12} / L
- Equivalent application cost is $0.50 / A ($1 \times 10^{13}$ spore/A)

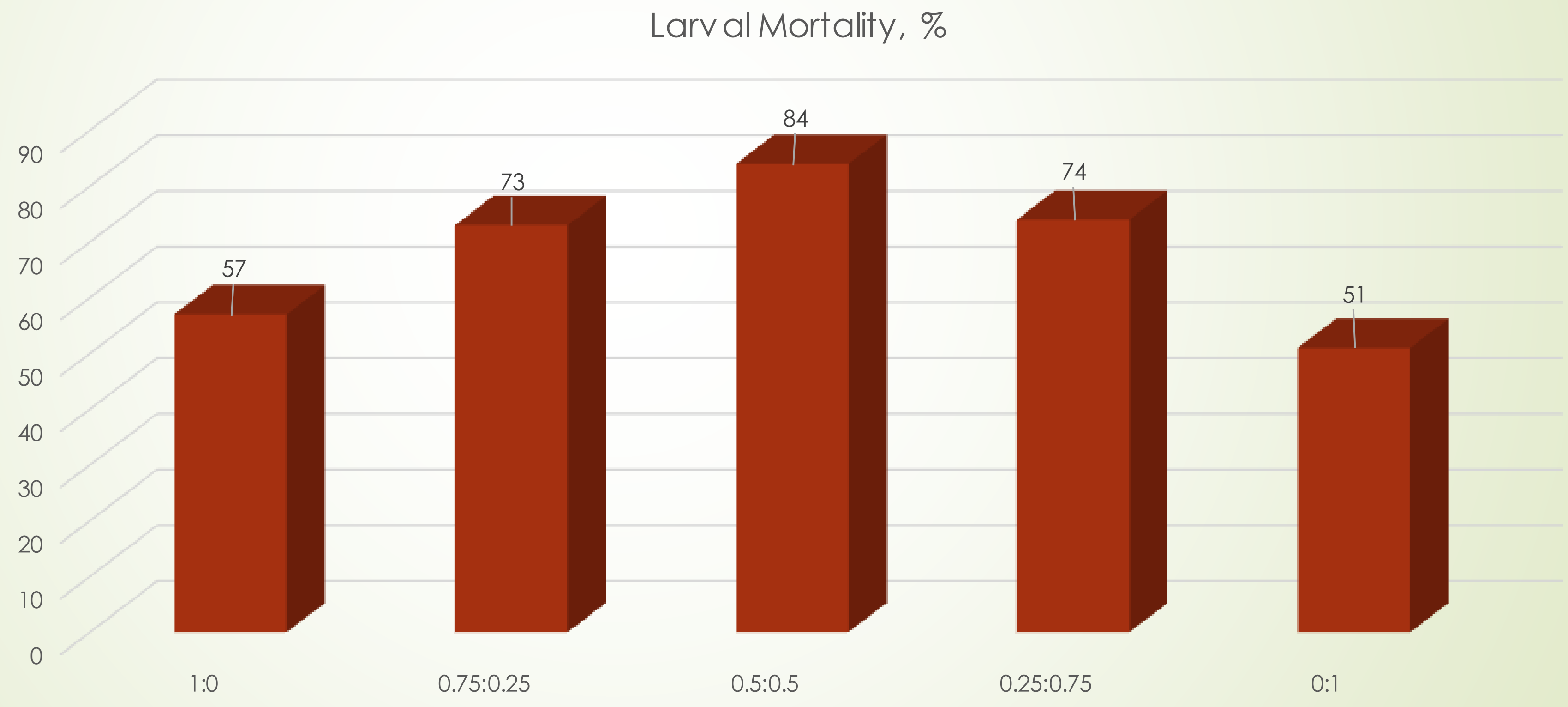
Experiments

Goal: To evaluate blastospores for insecticidal activity alone and in combination with Bt

I. Product Development

- Re-isolated pathogen from commercial products
  - Bt – Deliver Insecticide
  - Bb – BotaniGard EC
- Determined dosage response vs *Trichoplusia ni* (cabbage looper)
- Evaluated mixtures at LC50 ratios for individual microbes (Bt:Bb)
  - 1:0 0.75:0.25 0.5:0.5 0.25:0.75 0:1

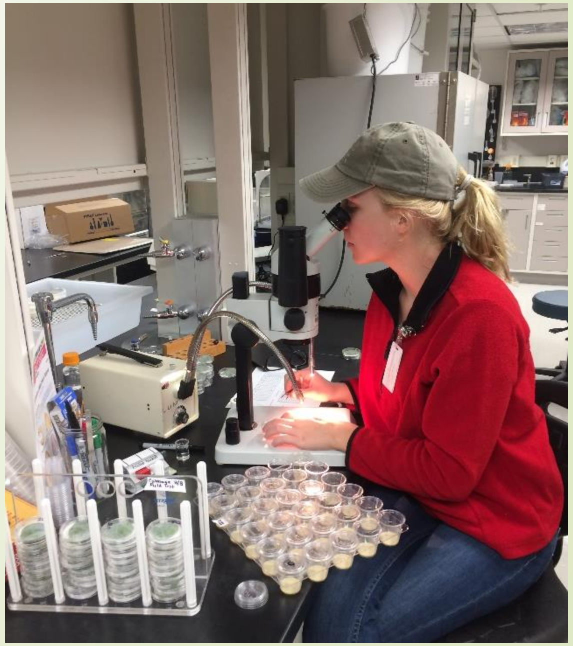
Mixed Microbe Treatments Bt:Bb



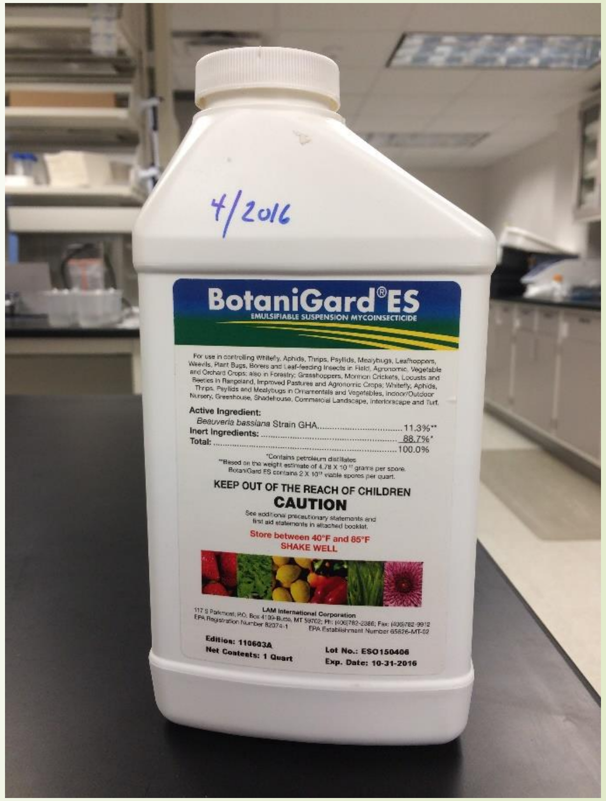
II. Field Application Experiment

- Evaluated the 0.5:0.5 ratio mixture to 5 control treatments:
  - Untreated, Deliver, BotaniGard, Bt (1:0 ratio), Bb (0:1 ratio)
  - Rates targeted low field rates on commercial product labels
- Applied to field grown cabbage plants (5 weekly applications)
- Samples treated leaf tissue and evaluated under laboratory conditions
- Two similar bioassay Evaluations
  - 3 day exposure to treated leaves, evaluate for mortality at 3 days (Bt), exactly 10 larvae per leaf
  - 1 day exposure to treated leaves, transfer to diet, evaluate at 7 days (Bb), excess larvae, transfer live larvae

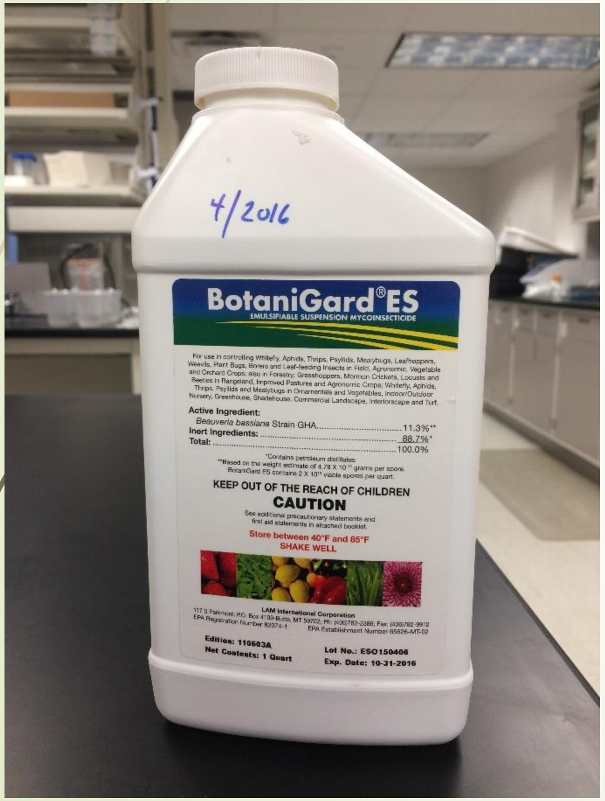


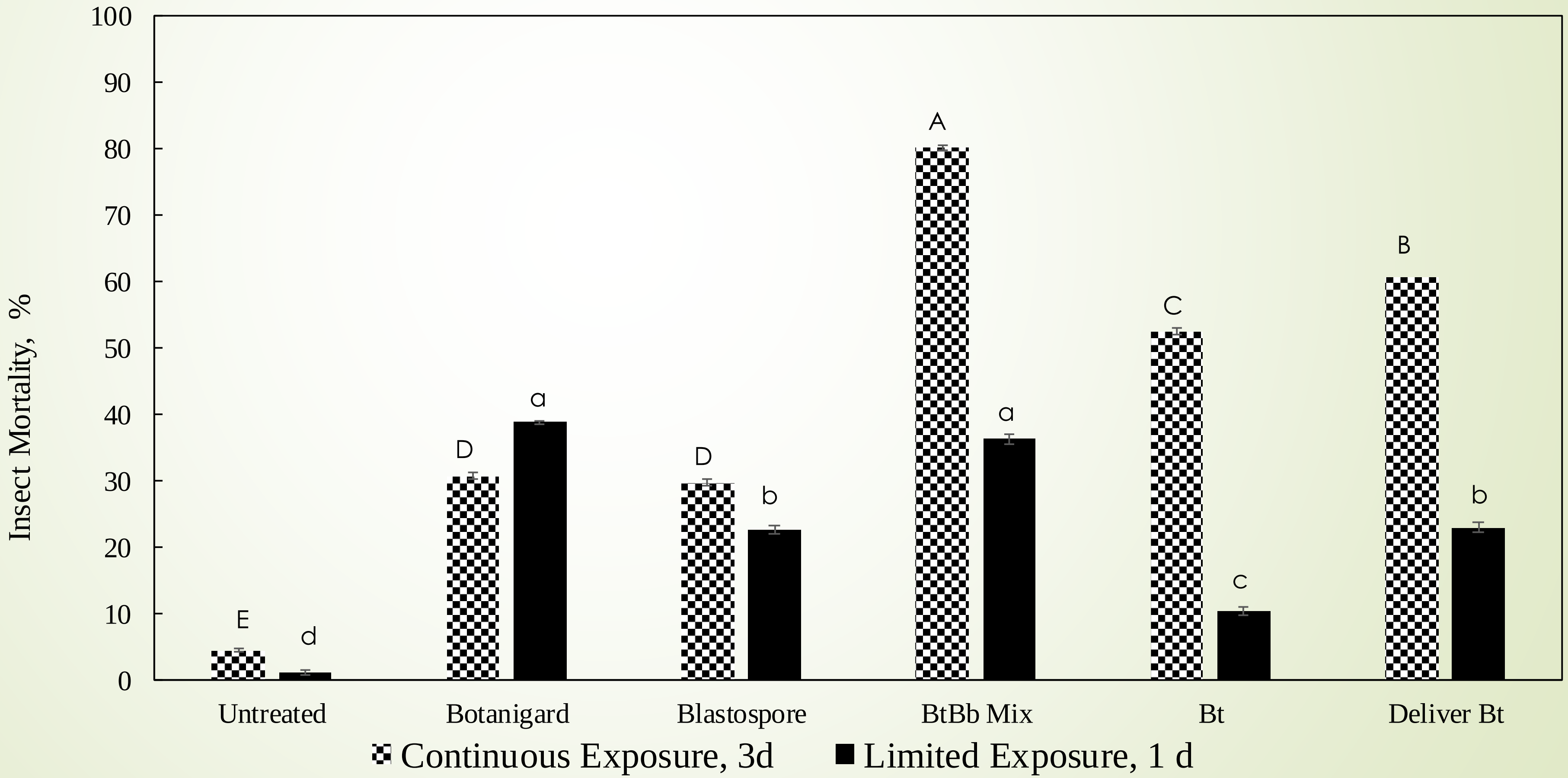
Treatments applied to field grown cabbage



Treatments applied to field grown cabbage



Average of five field applications to field grown cabbage



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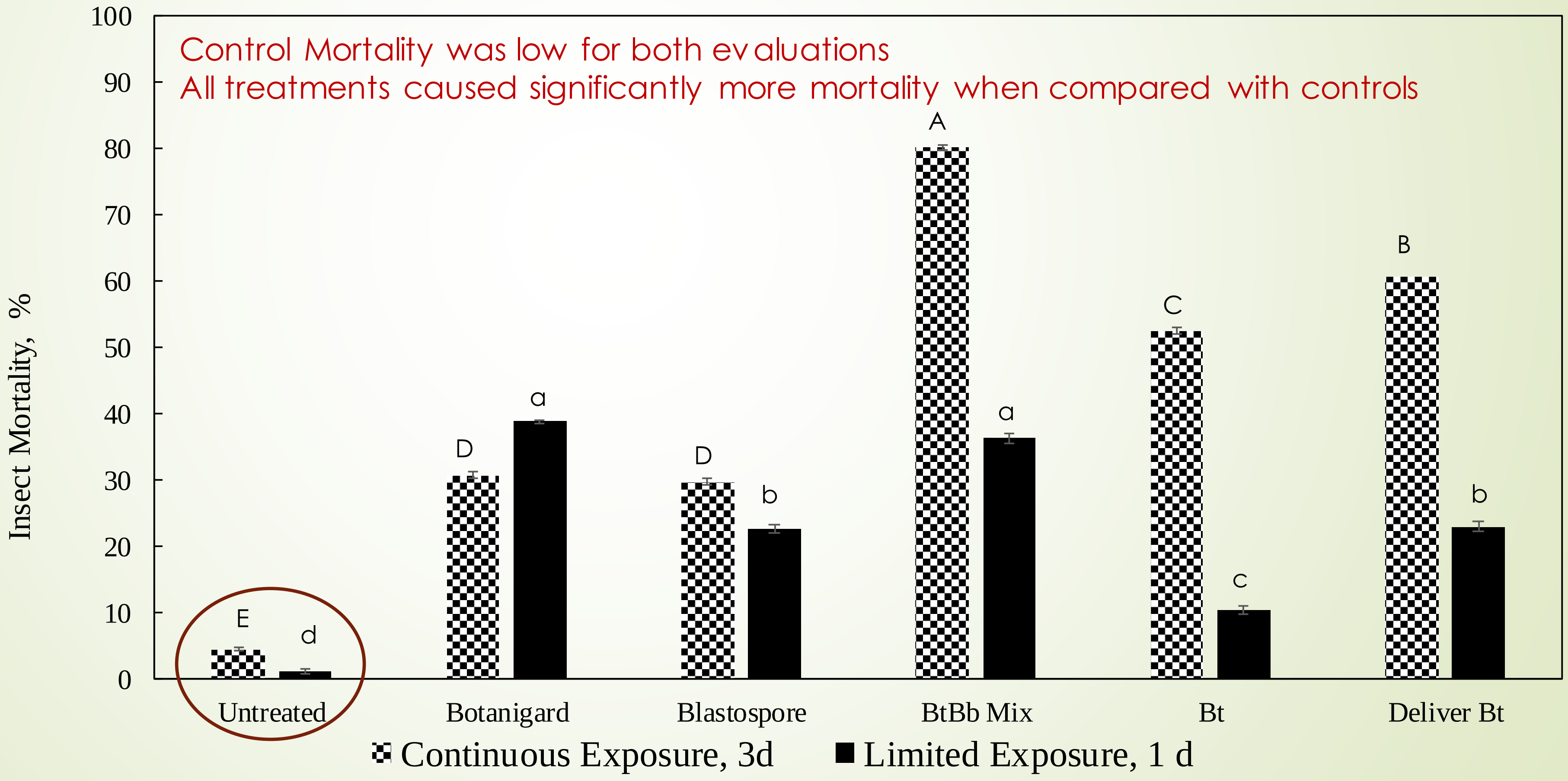

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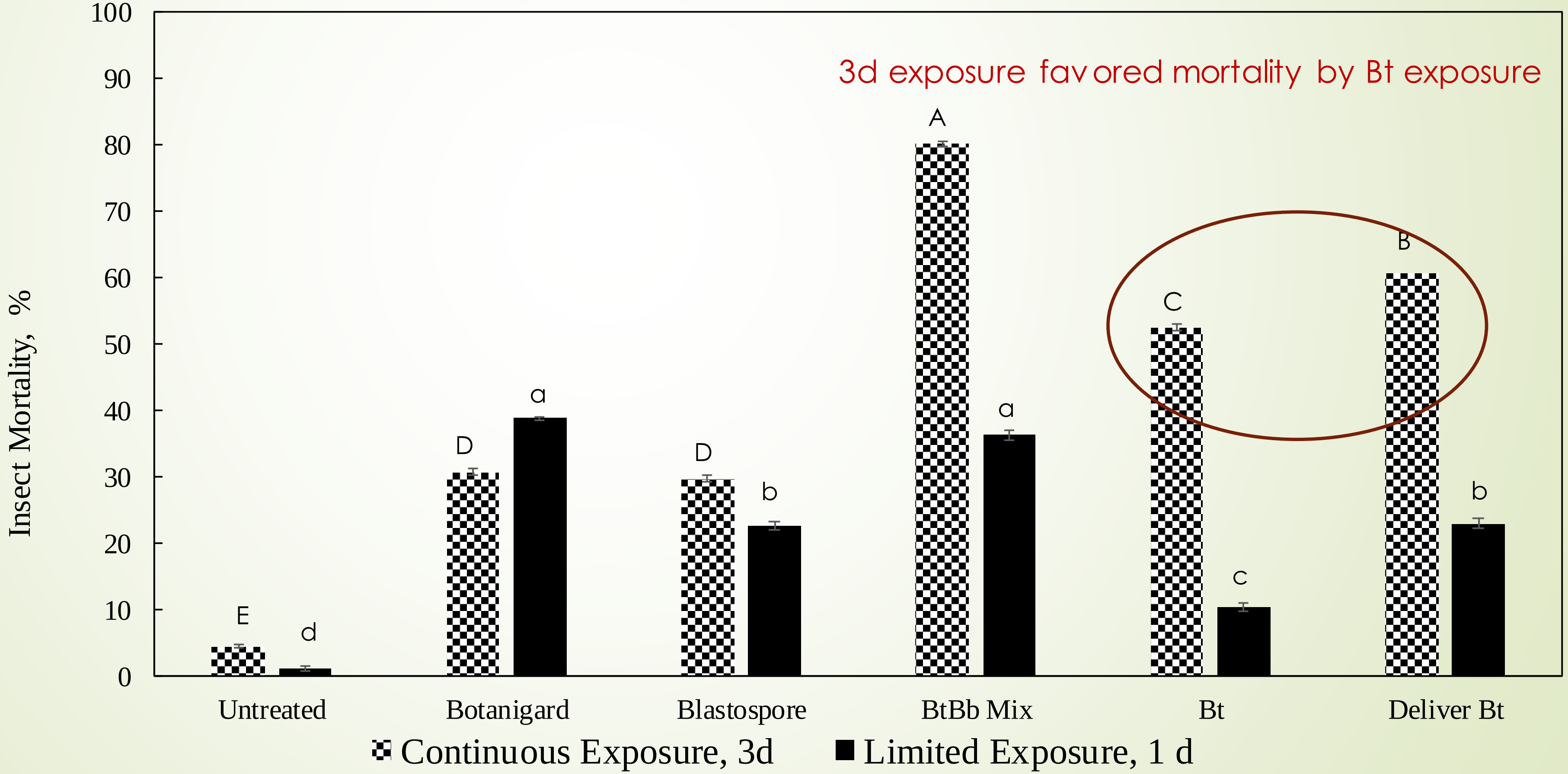

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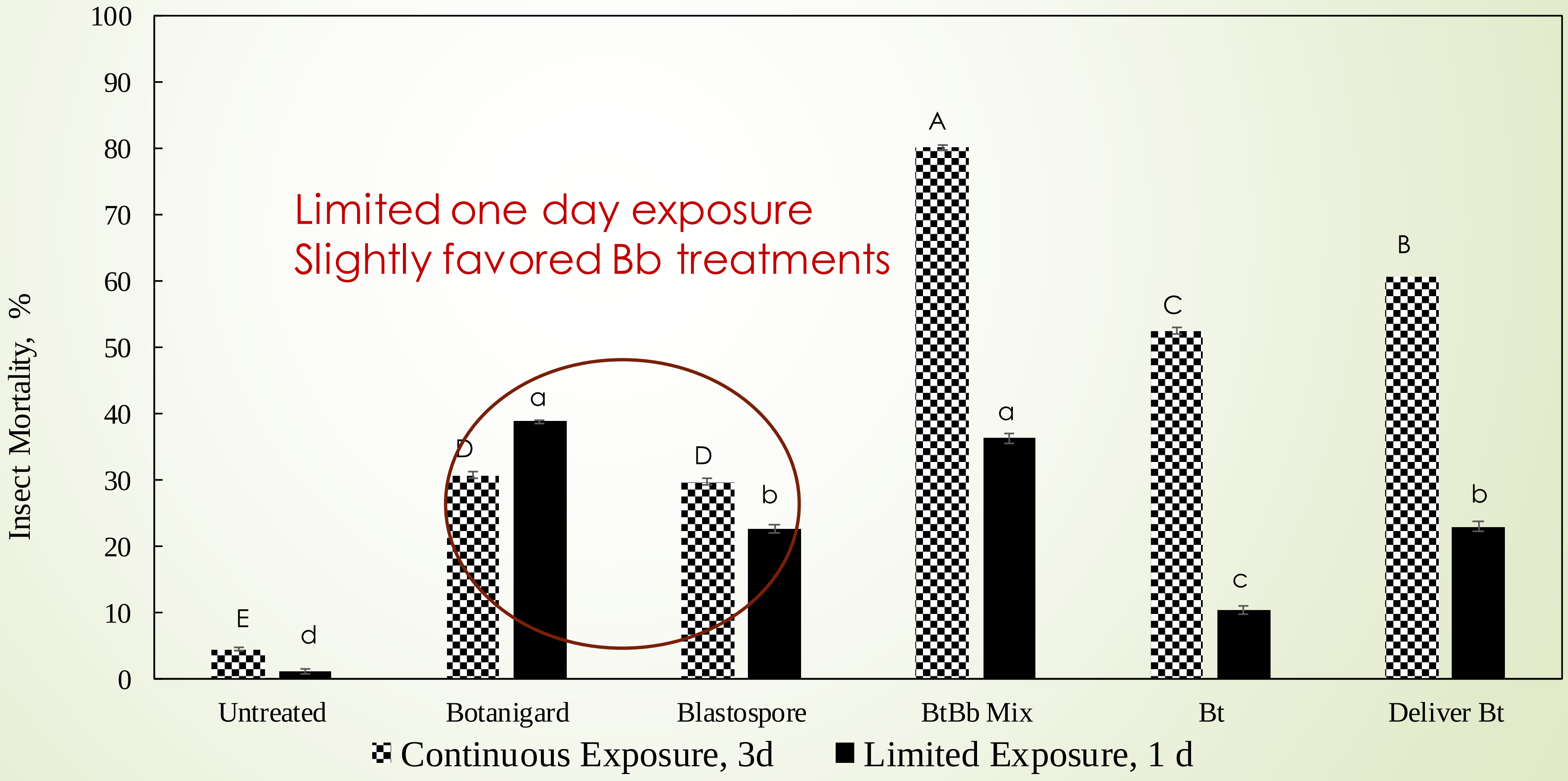

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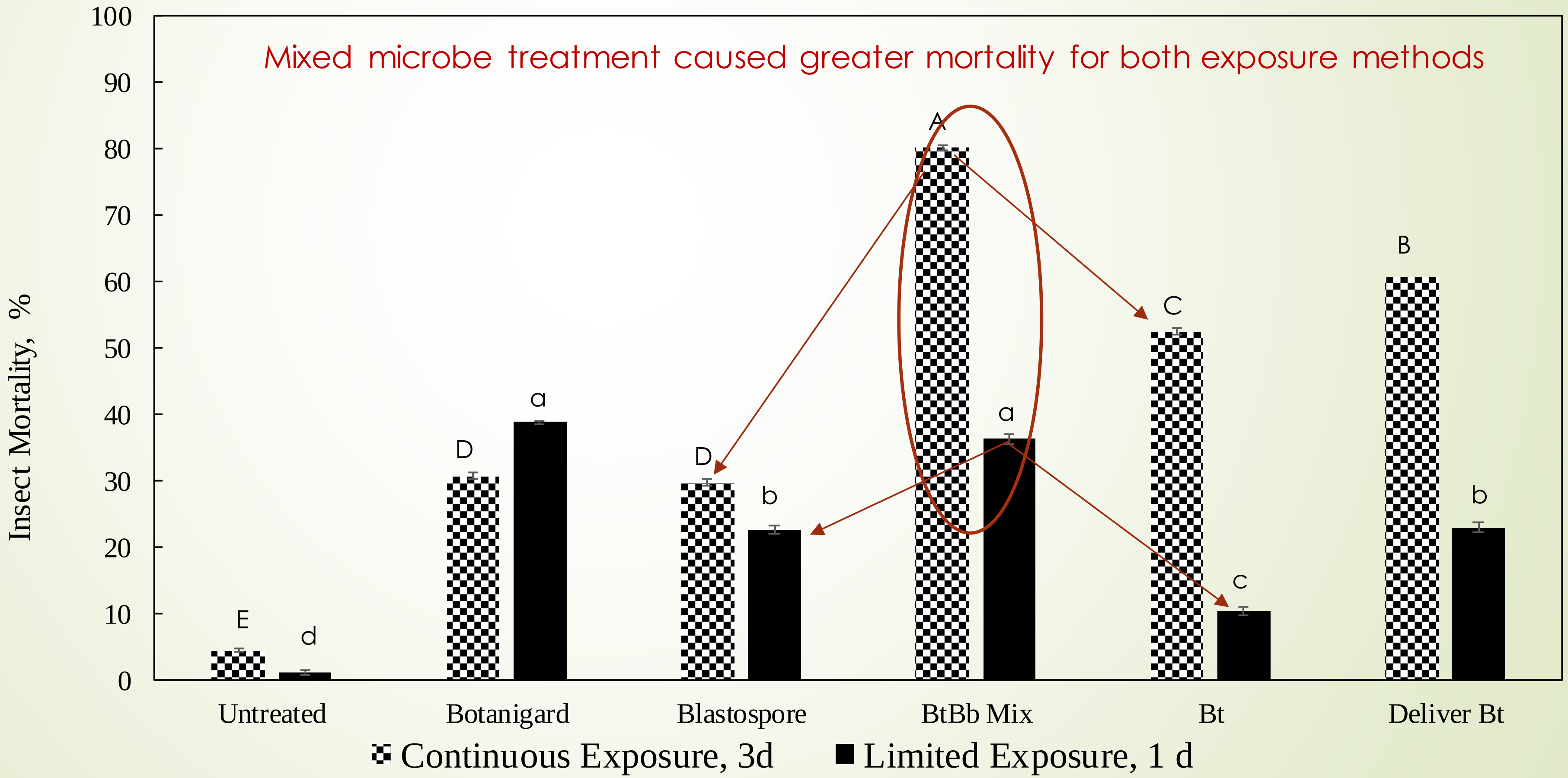

III. Preliminary and Future Research

- Evaluate activity against non-target pests – black cutworm
  - Turf pest evaluated by laboratory applications to bentgrass
  - Individual microbes required high rates to initiate infection
  - Preliminary evaluations do not indicate a benefit for mixed microbe application
- Evaluate combinations with baculovirus (*Agip*MNPV - Black Cutworm; *Af*MNPV – Cabbage Looper)
  - Baculovirus treatments alone result in significant mortality
  - No benefits have been observed for adding baculovirus

Conclusions to date

- Combining Bt and Bb enhanced insecticidal activity against a target pest known to be susceptible to both pathogens
- Blastospores were successfully used in the mixed microbe formulation, providing a lower cost option relative to conidia
- Generalizations about control of other pests is risky without specific evaluations of treatments for control of each target pest

Thank You

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Nakanishi, Brian
(301) 504-4879
brian.nakanishi@ars.usda.gov
Business Licensing Officer

Co-Toxicity Factor
Sun and Johnson, 1960

- Co-toxicity = 100 x (observed mortality – expected mortality)/expected mortality

  - $\geq$ +20 = Potentiation (Synergy)
  - $\leq$ -20 = Antagonism
  - >-20 to <+20 = Additive Effect

Disadvantages of Microbial Biopesticides

- Cost
- Maintaining microbe viability
- Limited application targets limits commercial interest
- Added requirements for effective control
- Short residual activity