Survey of Rhizosphere Competent Entomopathogenic Fungi on Small Fruits and Christmas Trees in the Willamette Valley, OR

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Presentation Outline

- Background and objectives
- Fungal survey material and methods
- *Metarhizium* survey
- *Beauveria* survey
- Larval bioassay
- Future research

System: Nursery and Small Fruit

- #1 industries in Oregon and important throughout the Pacific Northwest
  - Billion dollar industry
- Key soil pest: black vine weevil
  - Causes over $20 million dollars loss

Black Vine Weevil

- Invasive pest
- Wide host range
  - Over 150 species
- Parthenogenic

Damage to Nursery Crops

- Key crops impacted
  - Conifers
  - Ericaceous plants
- Zero tolerance in shipped nursery stock

Damage to Small Fruits

- Strawberry fields replanted every 2yrs
- Can cause severe loss in young blueberry and caneberry fields
- Contaminant in harvested fruits

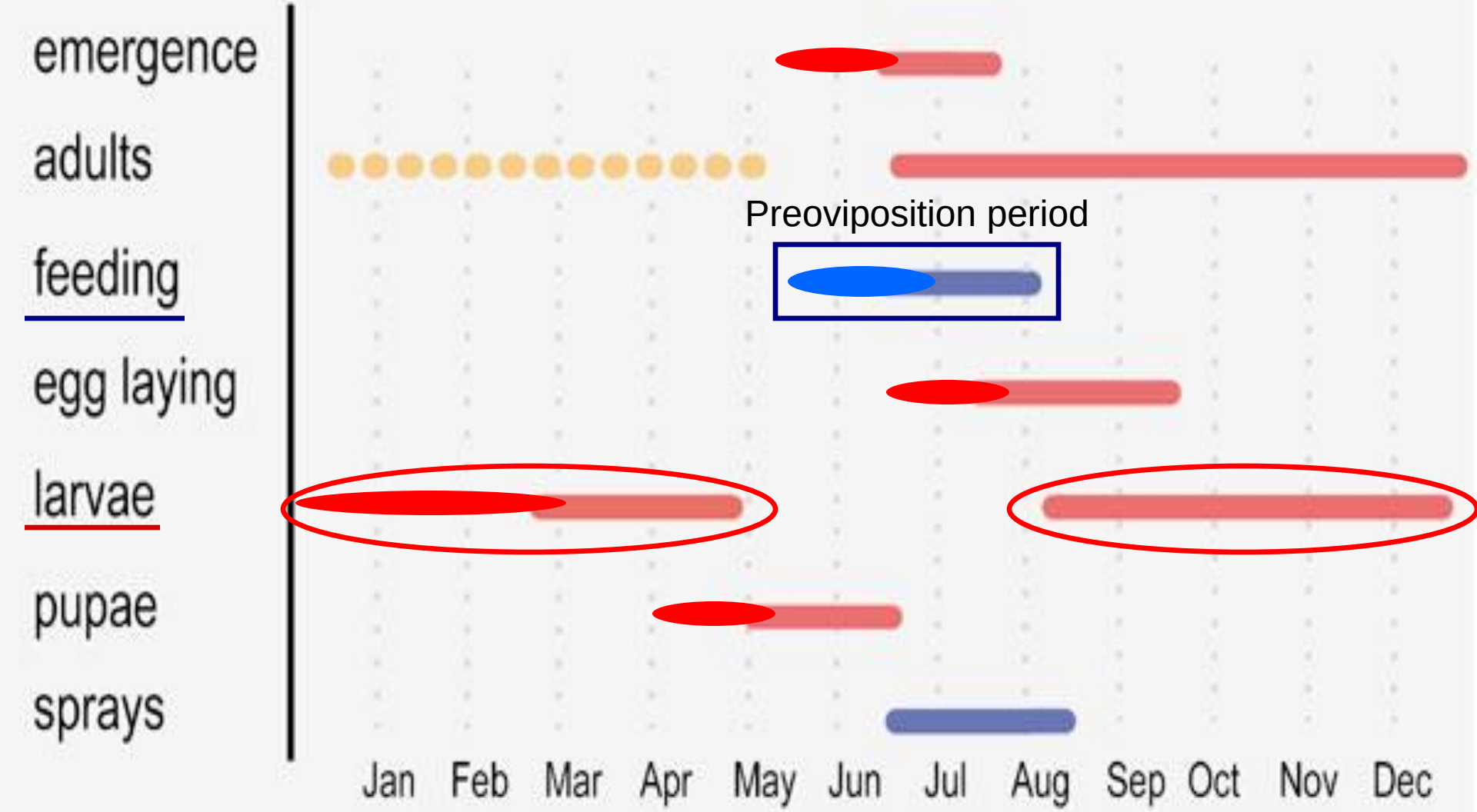
Black Vine Weevil infested strawberry field

Black Vine Weevil Control

- Pesticides-hard to contact insects
  - Larvae underground, adults feed at night
- Cultural Controls-limited efficacy
- Nematodes-don't persist
  - Need moist environment
- Entomopathogenic Fungi

Life Cycle & Management of BVW

DeAngelis, 10/96



Entomopathogenic Fungi

- Commercially available
- Two genera commonly found
  - *Metarhizium:* potted nursery crops
  - *Beauveria*: greenhouse pests

Metarhizium

Beauveria

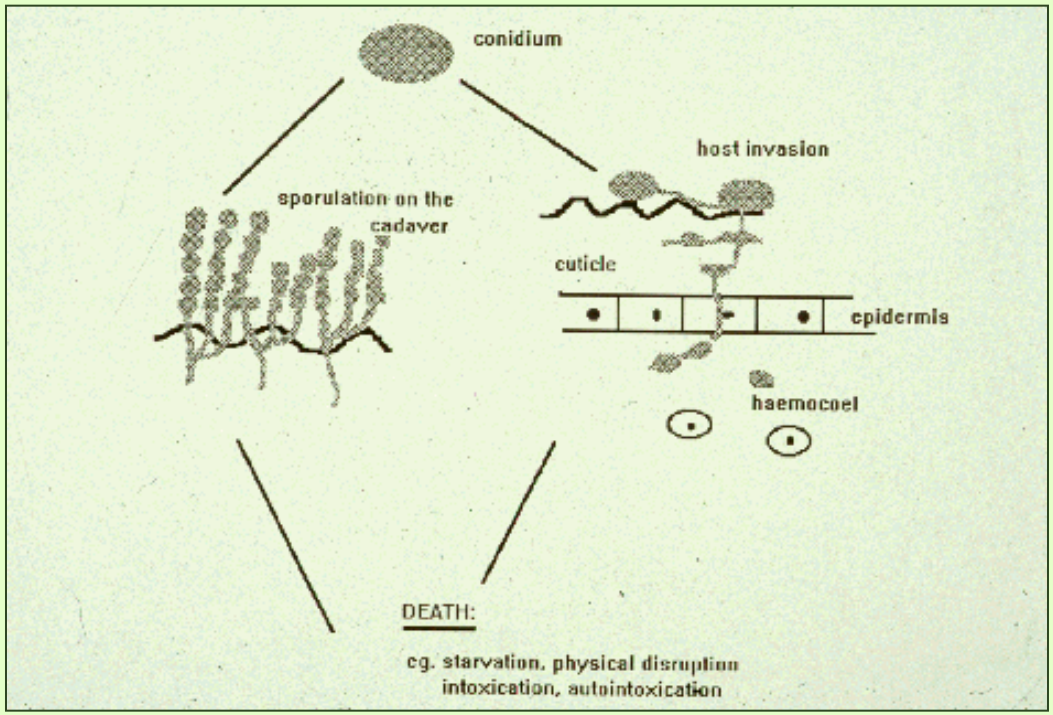
Mode of Infection

Penetration through body openings

Penetration using enzymatic and physical force

Fungal Infection

- Penetration of the cuticle
- Host invasion
- Multiplication in the host hemocoel
- Death of host
- Sporulation of fungi



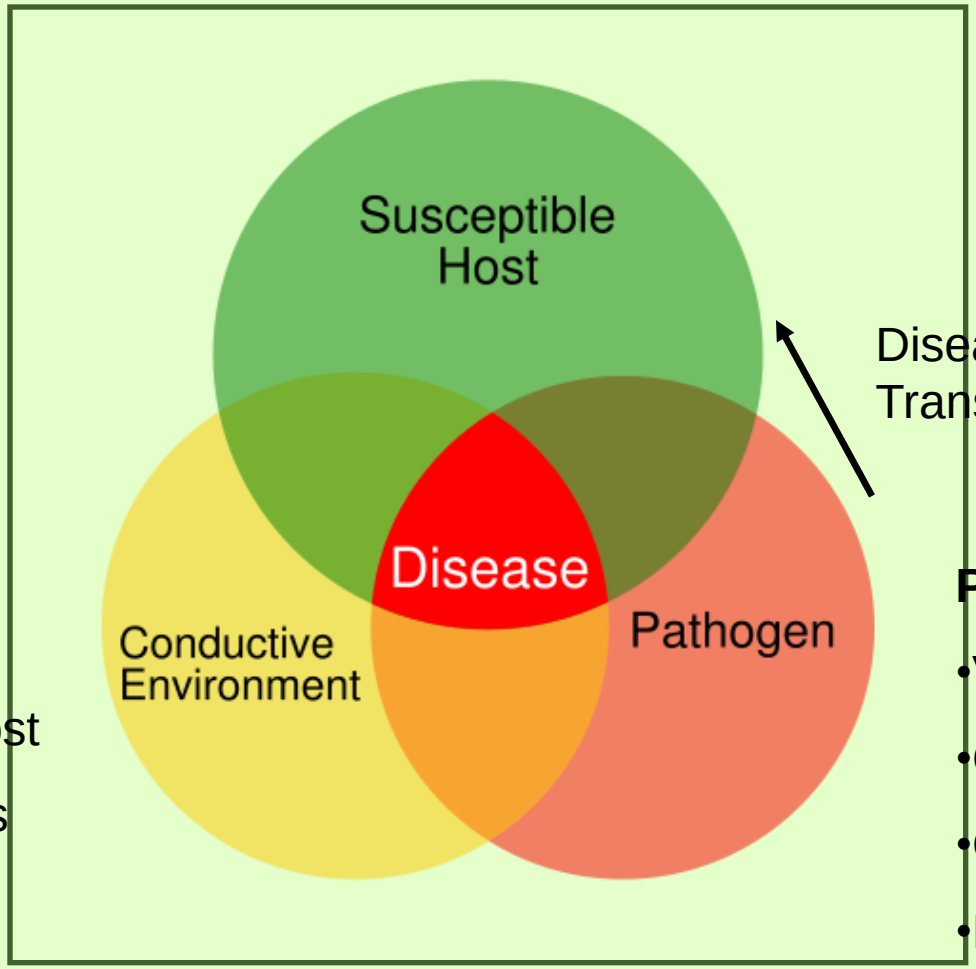
Key Factors of Epizoology

Host

- Susceptibility
- Behavior
- Density

Environment

- Conducive to pathogen & host
- Abiotic factors
- Biotic factors



Pathogen

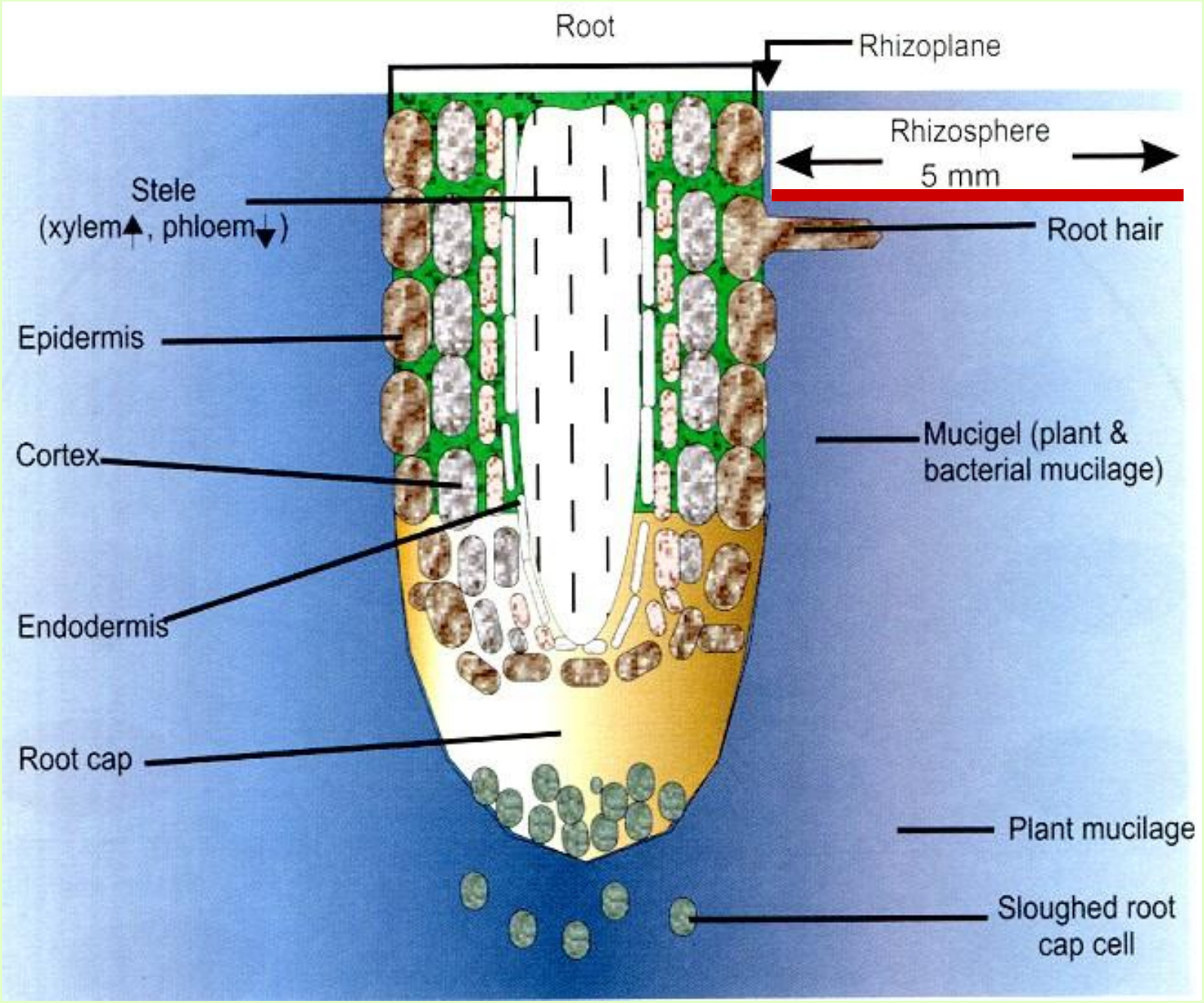
- Virulence
- Capacity to disperse
- Capacity to survive
- Density

Factors Limiting Adoption

- Adoption limited by
  - –Grower perceptions
  - –Inconsistent performance
  - –Lack of knowledge of ecology
  - –Cost

Rhizosphere Competency

Schimdt, 1979 - “Microorganisms that show enhanced growth in response to developing roots”



Maier et al. 2000. Environmental Microbiology

M. anisopliae Rhizosphere Competence

- In 2002 Hu and St. Leger demonstrated in field studies that *Metarhizium anisopliae* is rhizosphere competent.
  - Discovered in cabbage
  - Demonstrated that rhizospheric soils are a potential reservoir for *M. anisopliae*
  - Rhizospheric effect more profound from “inner” than “outer” rhizosphere soil
  - Suggests that root exudates may be responsible for rhizospheric effect

Efficacy of Rhizosphere Colonized Roots

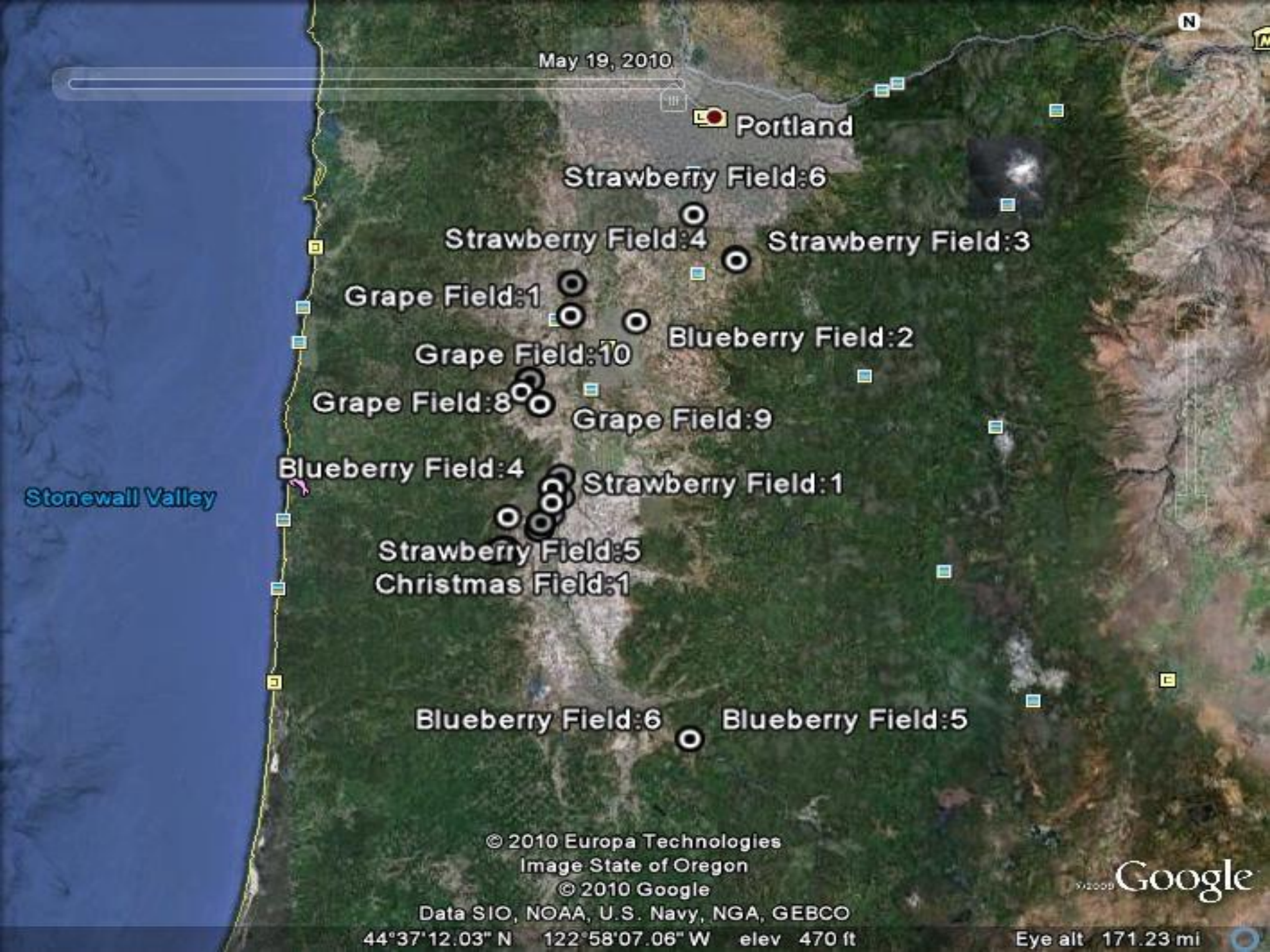
- 76% of black vine weevil larvae feeding on fungal treated roots were dead after 2 weeks

New Developments

- Rhizosphere competent
  - Close association with plant roots
- Can identify to species using genetic analysis-this could lead to understanding...
  - Habitat preference outside host
  - Inconsistent performance in the field

Objectives

	Determine relative abundance and diversity of *Metarhizium* spp. colonizing the rhizosphere of susceptible crops and identify using genetic analysis.
	Determine relative abundance and diversity of *Beauveria* spp. colonizing the rhizosphere of susceptible crops and identify using genetic analysis.
	Determine virulence of four *Metarhizium* spp. through larval bioassays.


May 19, 2010
Portland
Strawberry Field:6
Strawberry Field:4
Strawberry Field:3
Grape Field:1
Blueberry Field:2
Grape Field:10
Grape Field:8
Grape Field:9
Blueberry Field:4
Strawberry Field:1
Stonewall Valley
Strawberry Field:5
Christmas Field:1
Blueberry Field:6
Blueberry Field:5
© 2010 Europa Technologies
Image State of Oregon
© 2010 Google
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
44°37'12.03" N 122°58'07.06" W elev 470 ft
Eye alt 171.23 mi
Google

Materials and Methods

- Sample strawberry, blueberry, grape and Christmas trees roots
- Fungi was baited using a modified "*Galleria* bait method"

Material and Methods

- Fungal Isolation
  - Fungi from sporulating cadavers isolated using procedure outlined in Bruck 2004
  - Lyophilized and Cyro-preserved at -80°C
- Isolate Identification to species
  - Dr. Stephen Rehner USDA ARS Systematic Mycology and Microbiology Lab
    - using PCR amplification and nucleotide sequencing of EF-1α intron region

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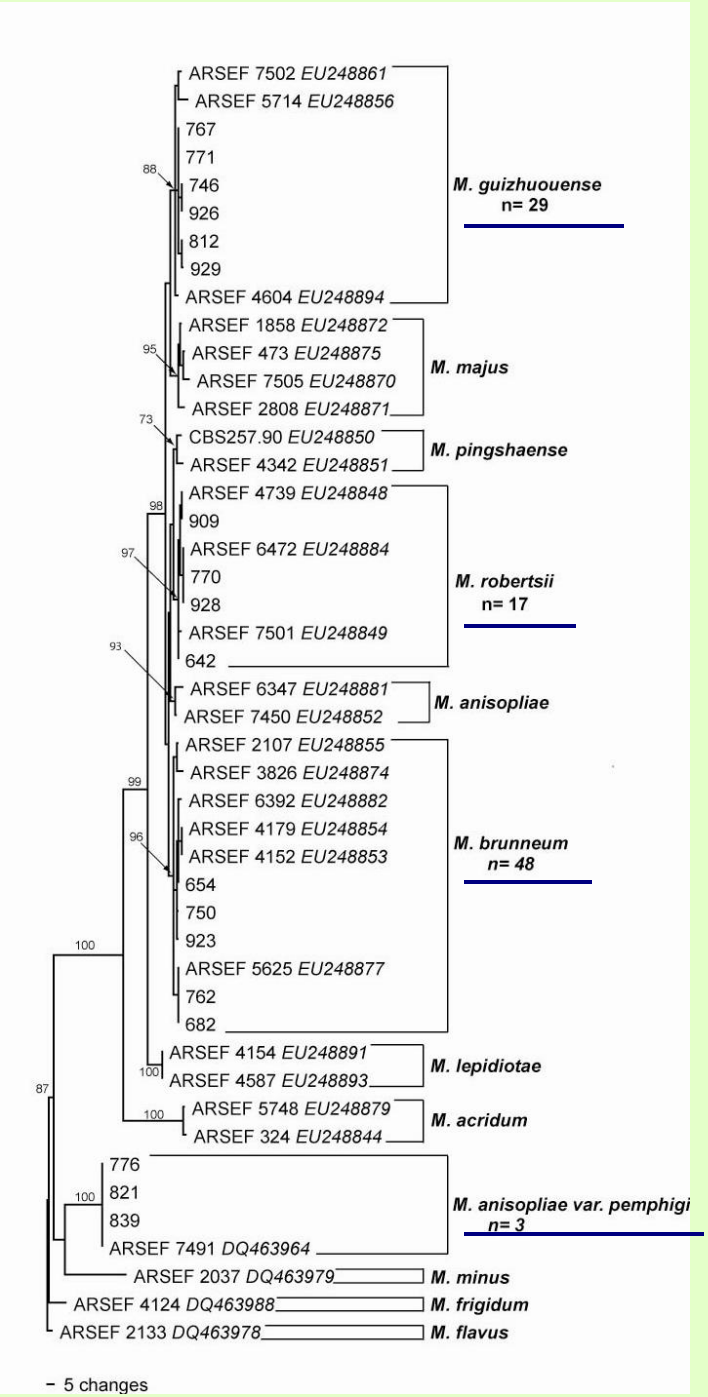
ARSEF 7502 EU248861
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M. guizhuouense
n= 29
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ARSEF 7505 EU248870
ARSEF 2808 EU248871
M. majus
CBS257.90 EU248850
ARSEF 4342 EU248851
M. pingshaense
ARSEF 4739 EU248848
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ARSEF 7501 EU248849
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M. robertsii
n= 17
ARSEF 6347 EU248881
ARSEF 7450 EU248852
M. anisopliae
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ARSEF 6392 EU248882
ARSEF 4179 EU248854
ARSEF 4152 EU248853
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M. brunneum
n= 48
ARSEF 4154 EU248891
ARSEF 4587 EU248893
M. lepidiotae
ARSEF 5748 EU248879
ARSEF 324 EU248844
M. acridum
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ARSEF 7491 DQ463964
M. anisopliae var. pemphigi
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M. minus
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M. frigidum
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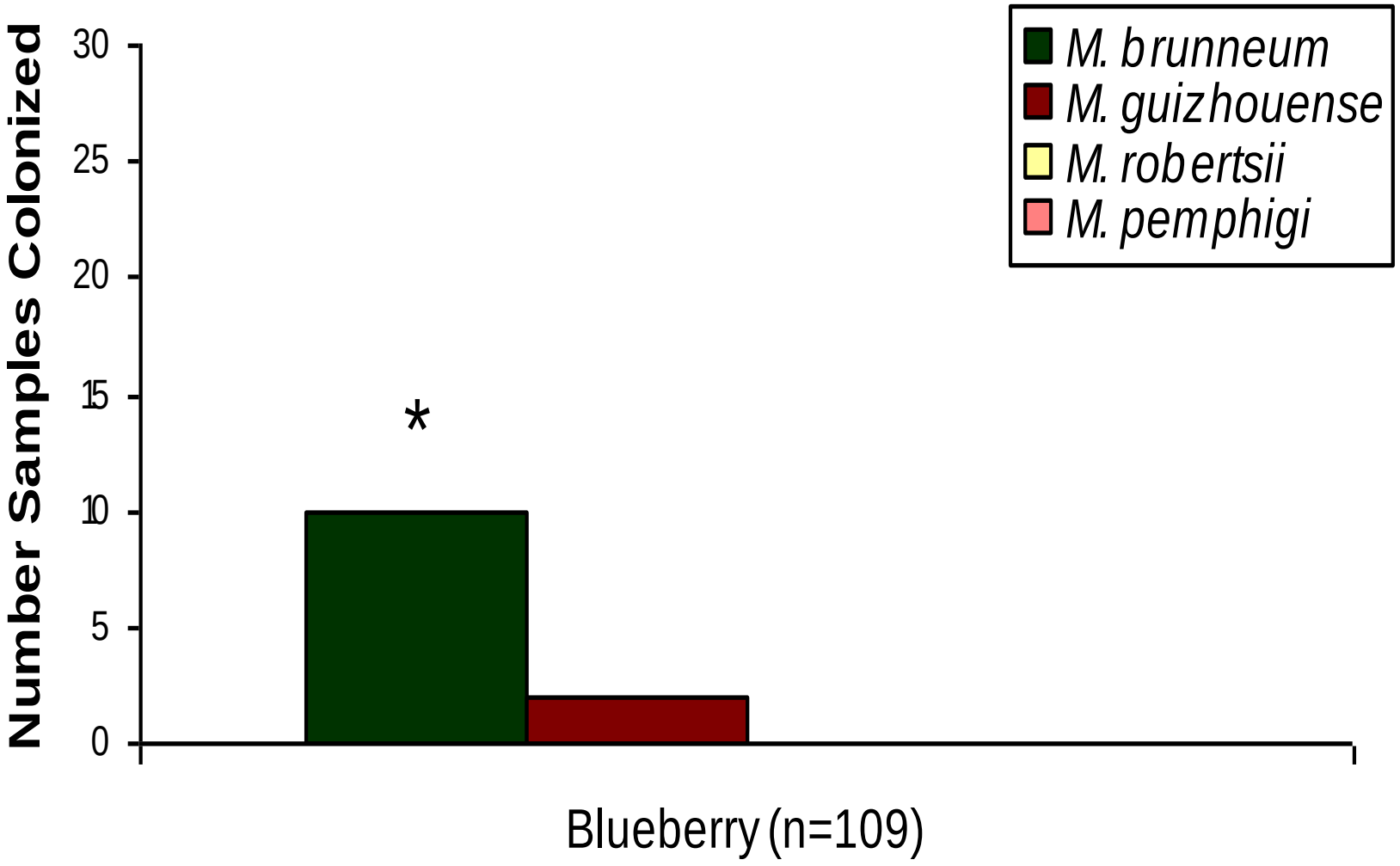
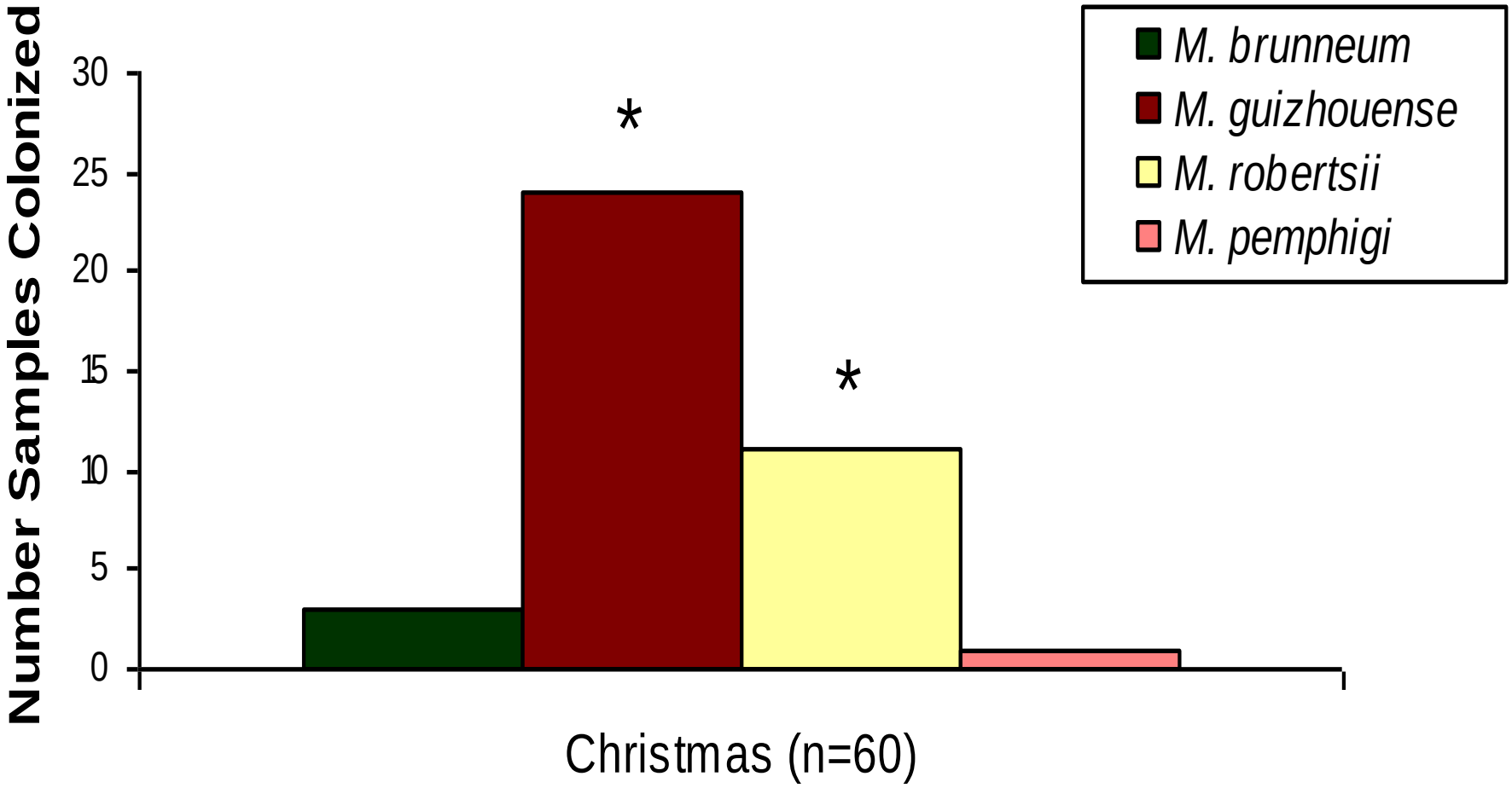
Metarhizium spp. Colonizing Blueberry



Fisher's exact test $p<0.05$, * indicates significance

Metarhizium spp. Colonizing Christmas Trees

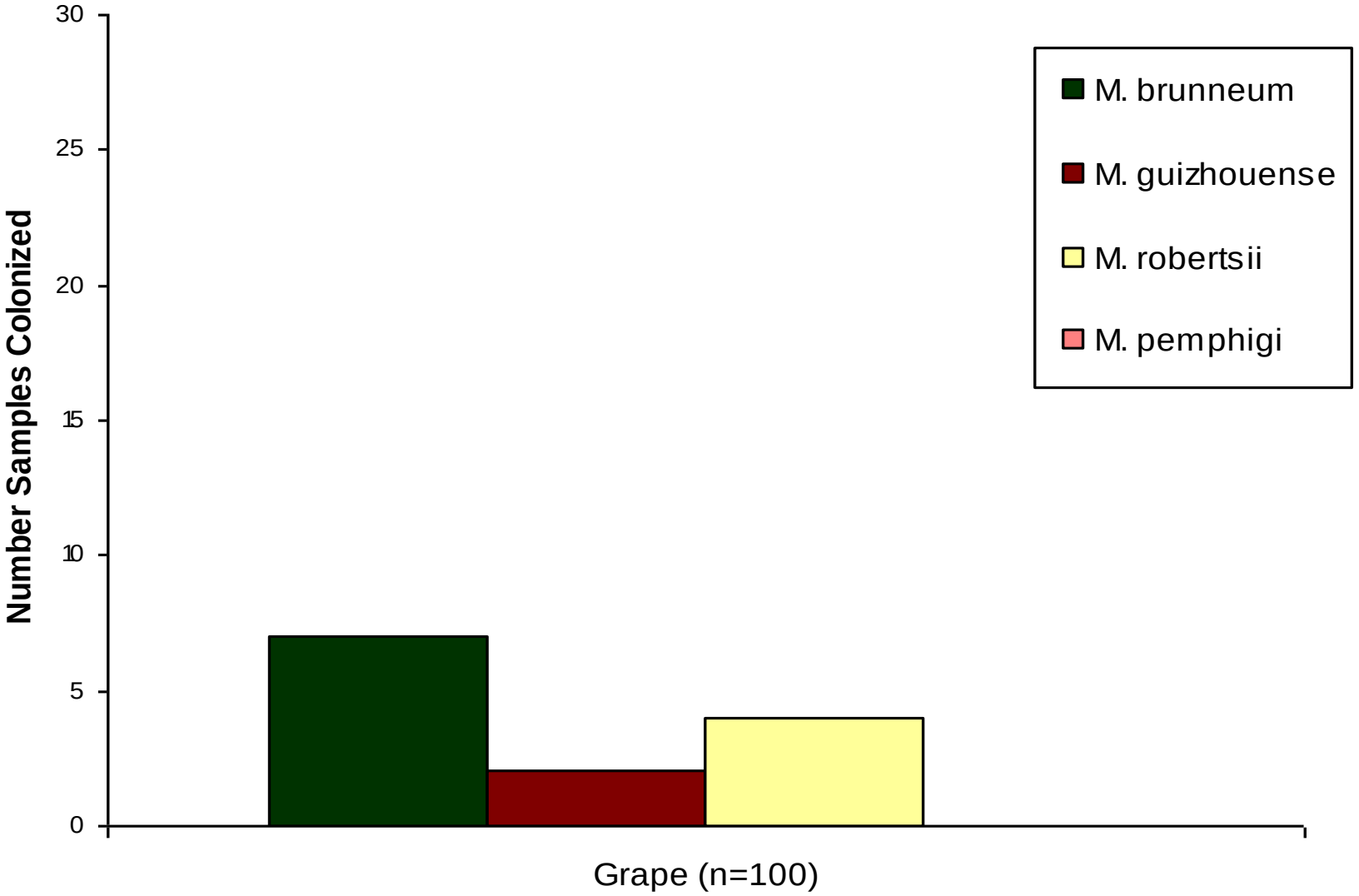


Fisher's exact test $p<0.05$, * indicates significance

Metarhizium spp. Colonizing Strawberry

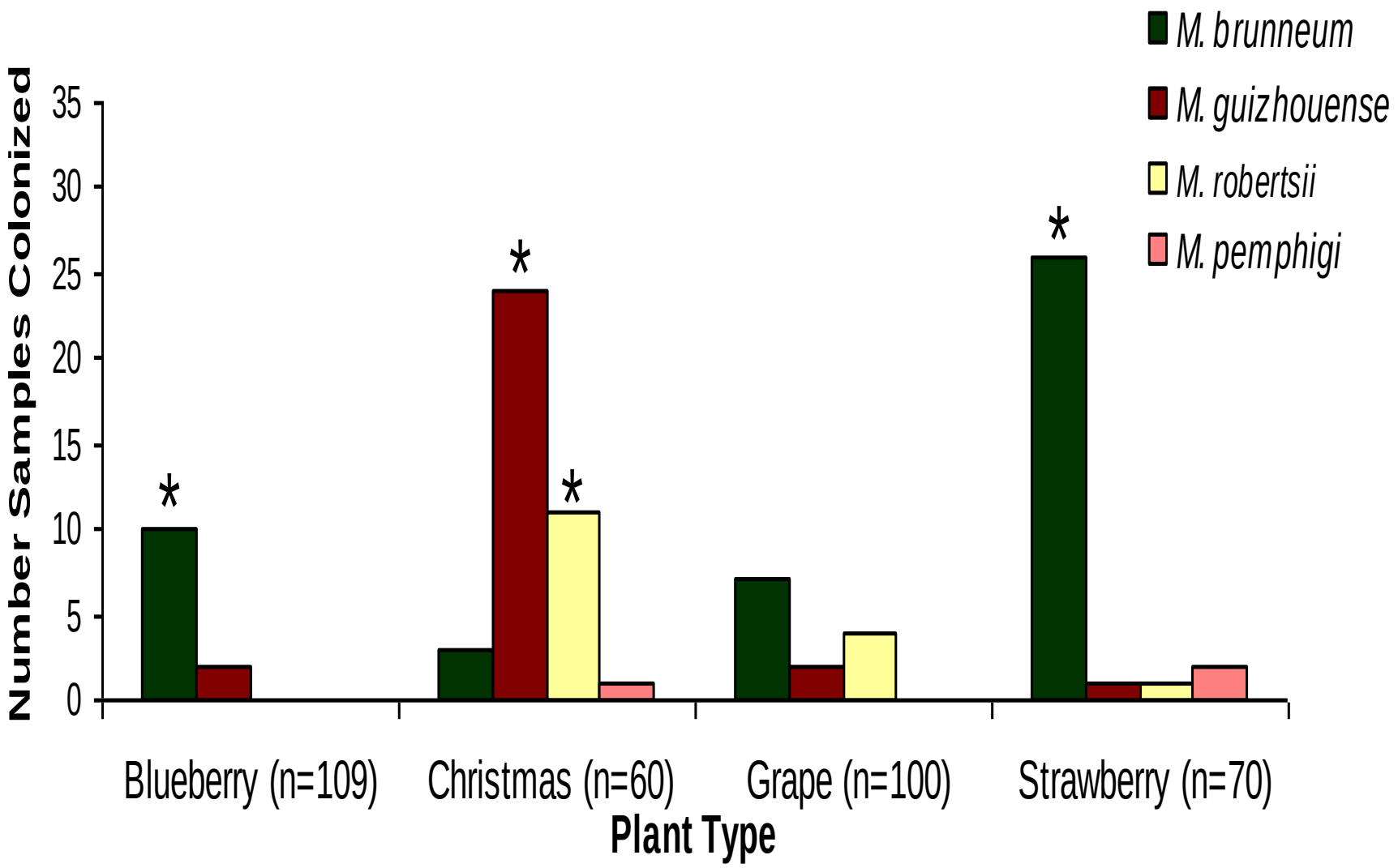
Fisher's exact test $p<0.05$, * indicates significance

Metarhizium spp. Colonizing Grape



Fisher's exact test $p<0.05$, * indicates significance

Nursery crops associated with *Metarhizium*



Fisher's exact test $p<0.05$, * indicates significance

Conclusion

- Strawberries and Christmas trees greatest number samples colonized and greatest diversity
- Strawberries and blueberries were significantly associated with *M. brunneum.*
- Christmas trees were significantly associated with *M. guizhouense* and *M. robertsii.*

Objectives

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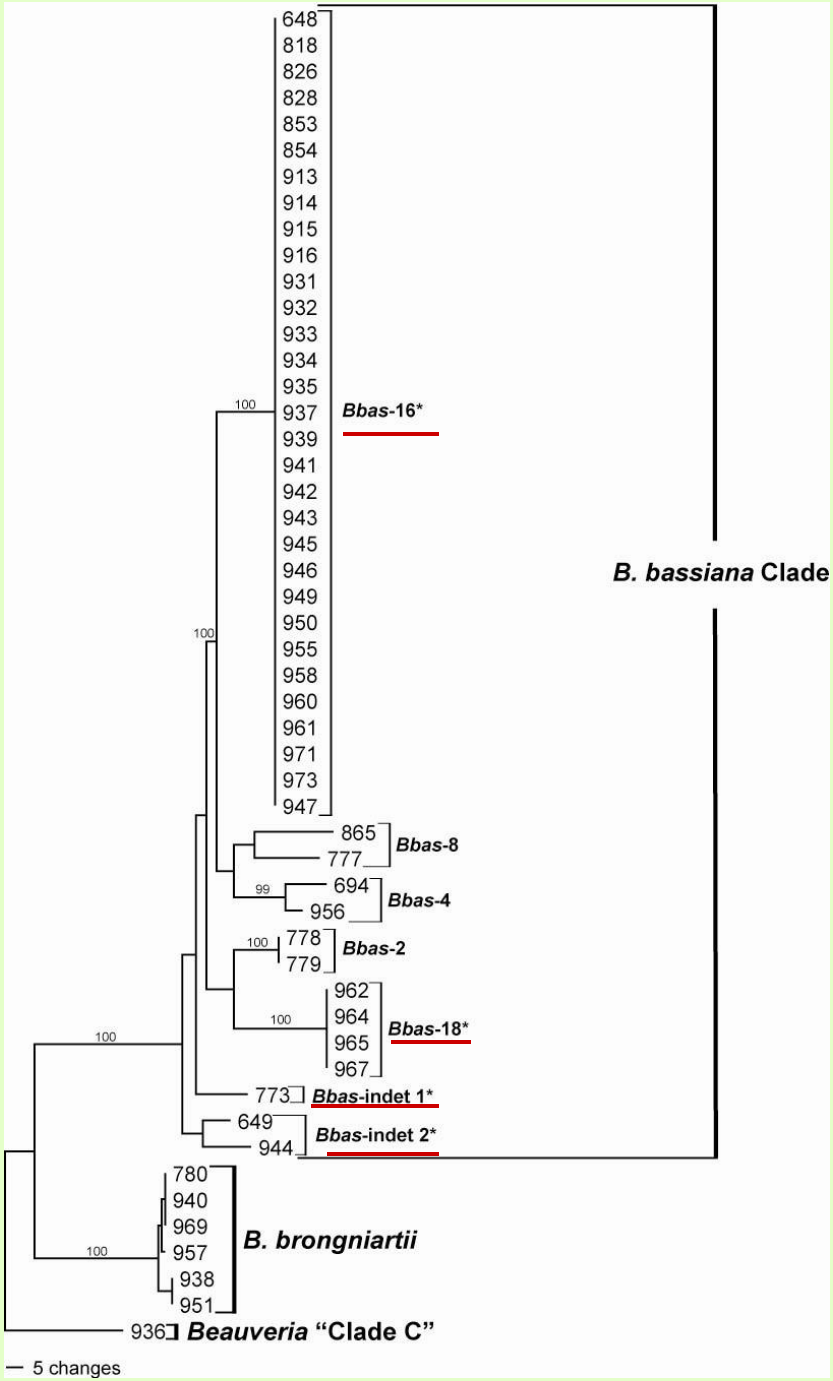
Determine relative abundance and diversity of *Beauveria* spp. colonizing rhizosphere of susceptible crops and identify using genetic analysis.

Determine virulence of four *Metarhizium* spp. through larval bioassays.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Bbas-16*
B. bassiana Clade
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Bbas-8
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Bbas-4
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Bbas-2
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Bbas-18*
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Bbas-indet 1*
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Bbas-indet 2*
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B. brongniartii
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Beauveria "Clade C"
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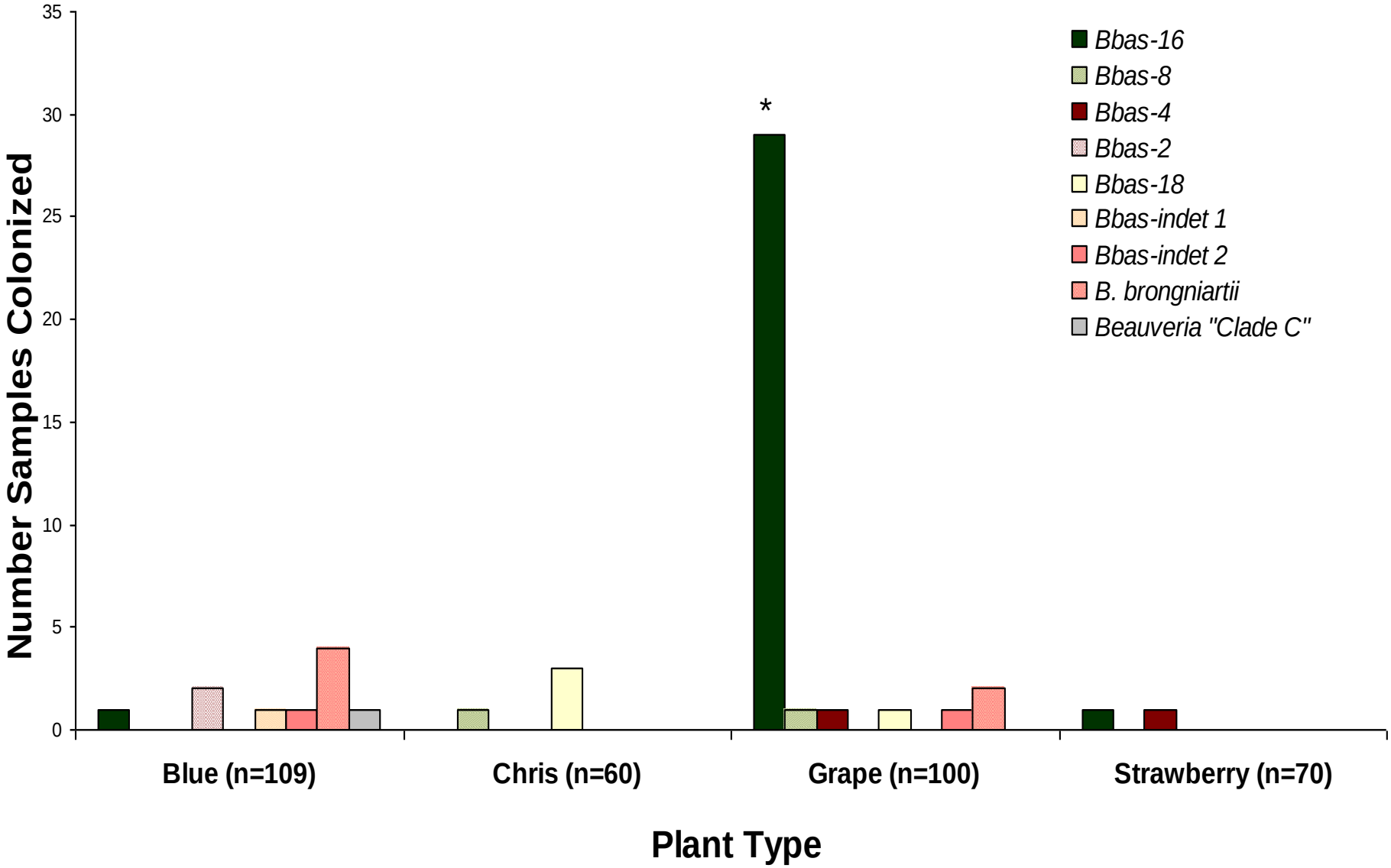
Susceptible Crops Associated with *Beauveria*



Fisher's exact test $p<0.05$, * indicates significance

Conclusion

- *Bbas-16* was significantly associated with grapes.
- Grapes were the only plant type significantly associated with *Beauveria*

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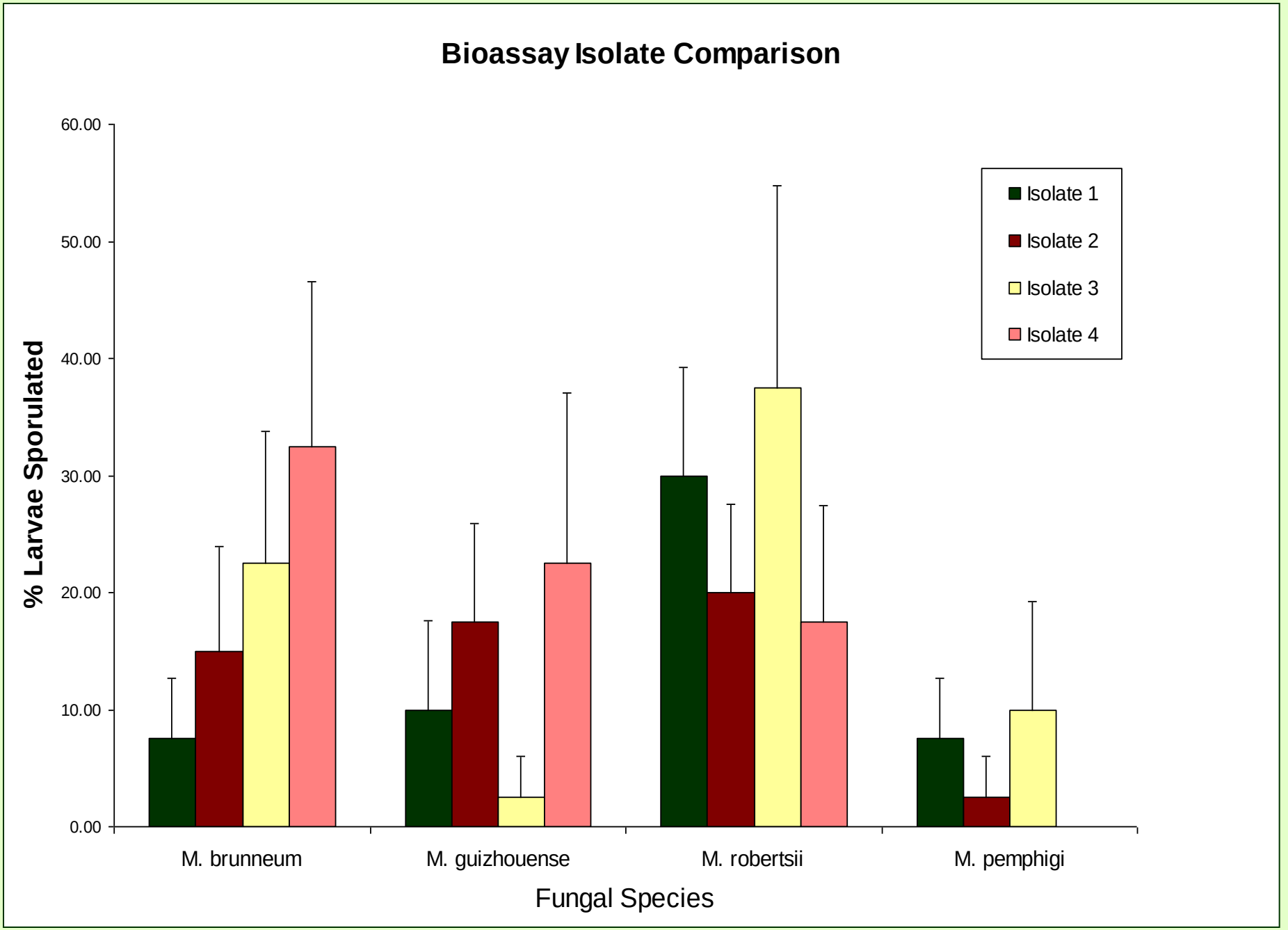
	Determine virulence of four *Metarhizium* spp. through larval bioassays.

Larval Bioassay Test-*Metarhizium spp.*

- 4 isolates of each spp. except *M. pemphigi* only 3 isolates available
- 4 reps/5 larvae
- Spore Concentration: $5x10^4$ spores/ml
- Experiment done 2X



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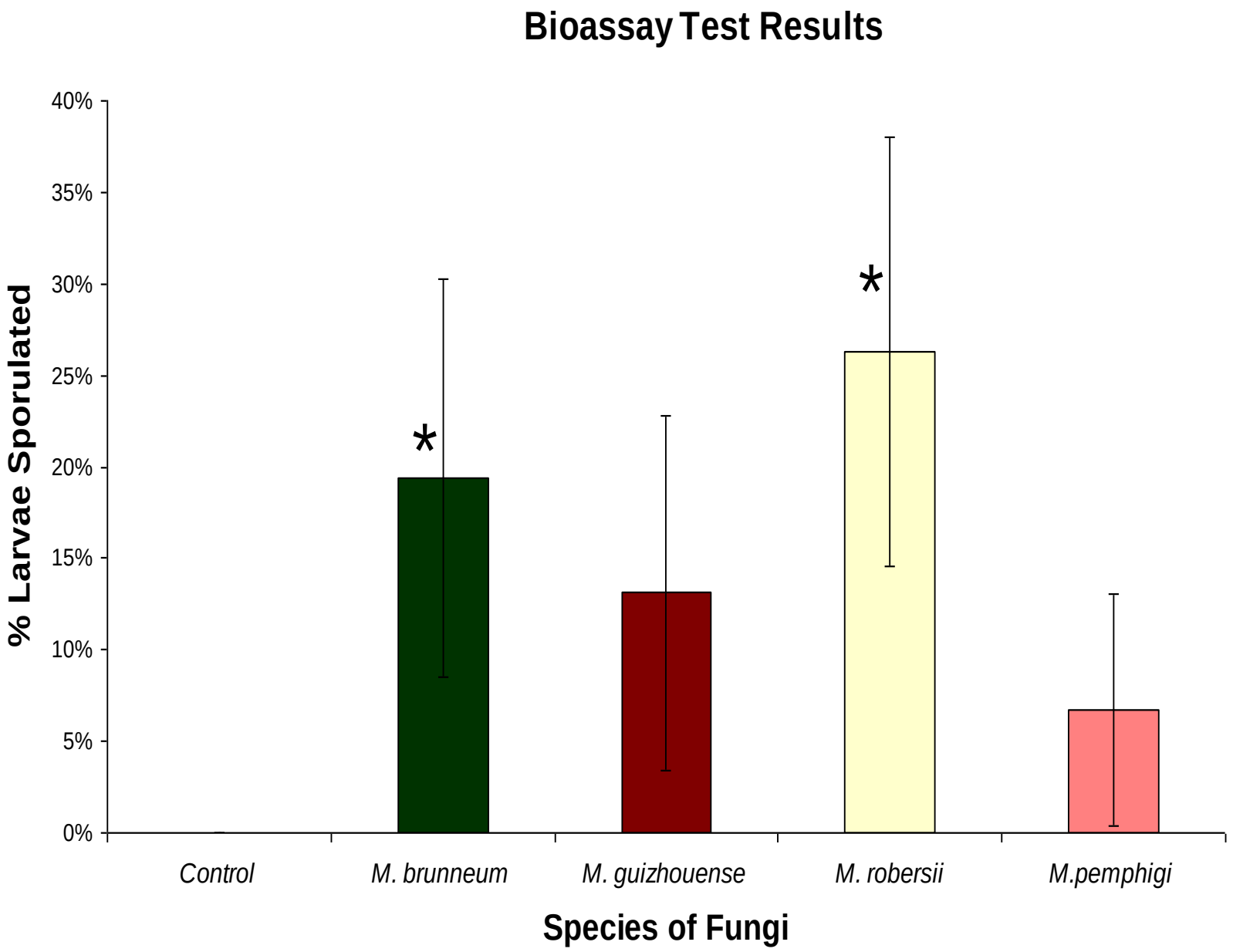


P<0.05, * indicates significance

Results Bioassay

- All four species were pathogenic to black vine weevil larvae
- Significantly more larvae sporulated when treated with *M. brunneum* and *M. robertsii* as compared to the control

Conclusions

- This is the first study to survey fungal rhizosphere interactions in agricultural field conditions.

- This study suggests that certain species of *Metarhizium* and *Beauveria* are associated with the strawberry, blueberry, grape and Christmas tree rhizospheres.

Implications and Limitations

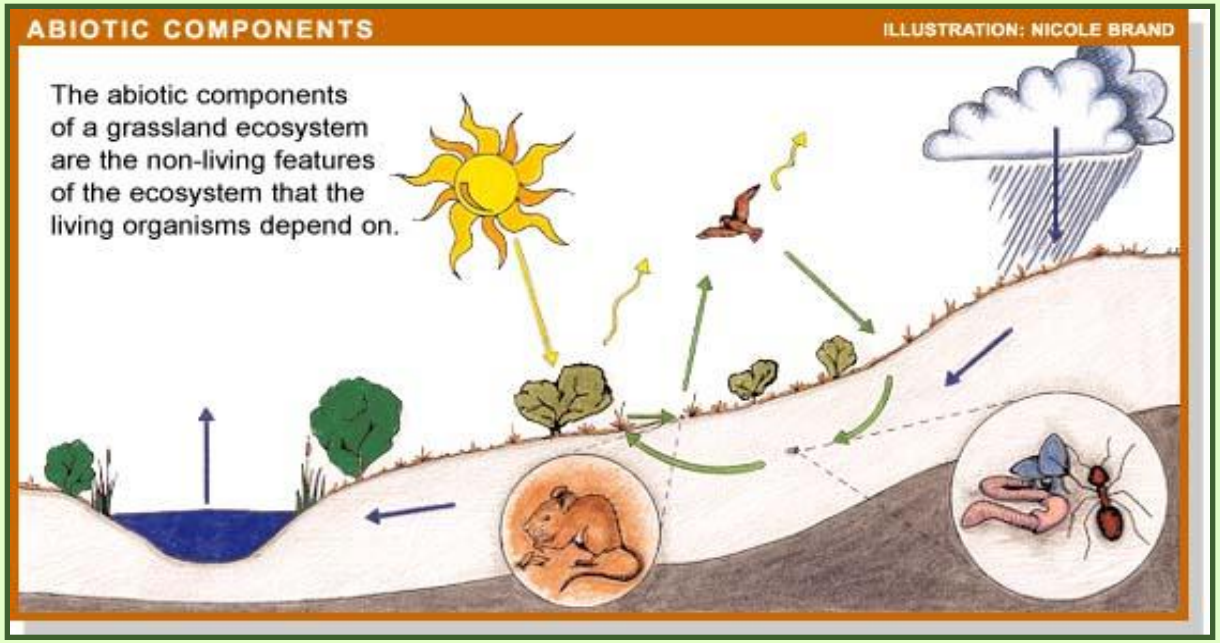
- It seems likely that matching of the prominent fungal species for use on each crop could enhance control of root-feeding insects.
- Plant-fungus relationships may be also influenced by other biotic and abiotic factors.

Why Develop Rhizosphere Competent Entomopathogenic Fungi?

- Economical
- Easy to apply
- Maintains itself on plant roots
- No harmful pesticides or residues

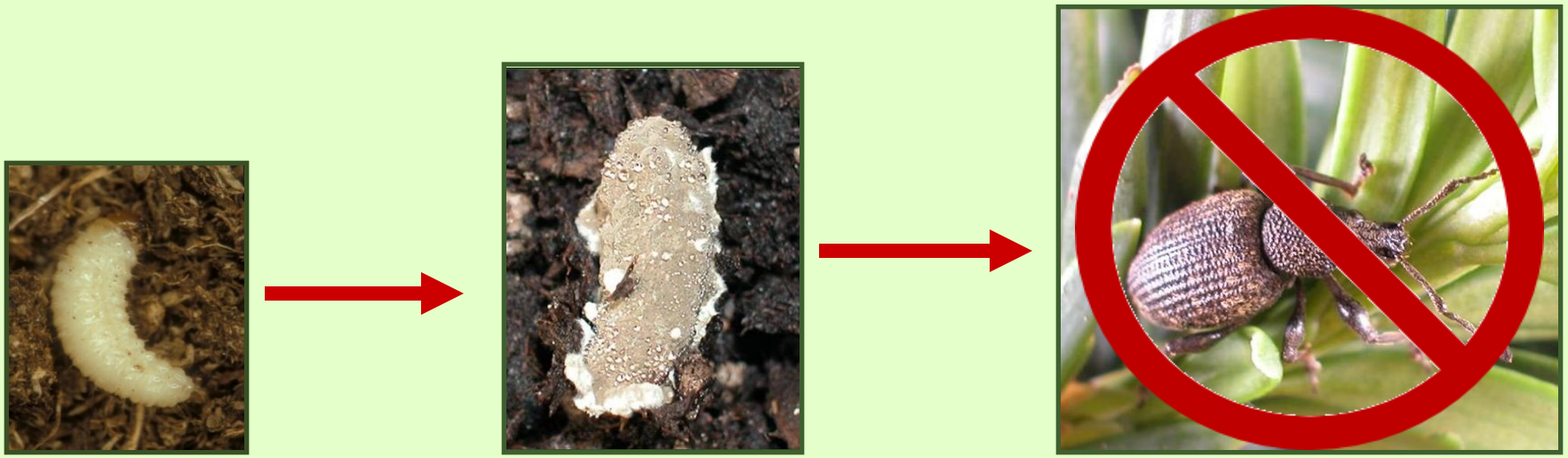
Future Research

- Determine the effects of soil moisture, type and temperatures on fungal rhizosphere colonization.
- Determine the effects of biotic influences on fungal rhizosphere colonization.



Future Research

- Develop method for inoculating roots prior to planting/potting
- Complete characterization of *Beauveria* taxonomy.



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- Funding was provided by the USDA Horticultural Crops Research Laboratory

Questions?