

### 1. Cross-phylogeny bioassays

#### Objective

Determine the extent and degree of antagonism within and between four agricultural systems

#### Methods

Symbiont isolations from 14 ant colonies spanning most of the phylogenetic diversity of the associations with attine ants

Petri plate bioassay experiments  
14\*14 pairings  
Three replicates

Cultivar inhibition of *Pseudonocardia*

*Pseudonocardia* inhibition of cultivar

Degree of antagonism measured as zone of inhibition

### 2. Within-*Acromyrmex* bioassays

#### Objective

Determine the extent and degree of antagonism at the population level

#### Methods

Symbiont isolations from 12 ant colonies distributed on five *Acromyrmex* species

Petri plate bioassay experiments  
12\*12 pairings  
Three replicates

Cultivar inhibition of *Pseudonocardia*

*Pseudonocardia* inhibition of cultivar

Without cultivar headstart

With cultivar headstart

Degree of antagonism measured as zone of inhibition and presence of discoloration in interaction zones

### 3. *In vivo* evaluation of effects of *in vitro* interactions

#### Objective

To determine whether antagonism *in vitro* correlates with reduced success of sub-colonies *in vivo*

#### Methods

Set up of twenty-one ant-fungus-*Pseudonocardia* combinations

Combinations chosen to include:

Pairings with antagonism from neither symbiont

Pairings with antagonism from cultivar

Pairings with antagonism from *Pseudonocardia*

Pairings with antagonism from both symbionts

Three replicates performed for each combination

Effect of cultivar inhibition of *Pseudonocardia* evaluated by examining *Pseudonocardia* cover on the ants

Effect of *Pseudonocardia* inhibition of cultivar evaluated by examining sub-colony fungus garden weight