Progress on Microbial Control of Sweetpotato Weevil in sub-Saharan Africa

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Problem: Sweetpotato Weevil

- Most important sweetpotato pest worldwide
- Eggs (200-250/♀) are laid singly in holes in stem or tuber and sealed with frass
- Larvae feed for 2-3 weeks inside root
  - Physical damage and terpenes (toxic, bad taste) production
  - Damage cannot be cut away
- Pupation occurs in tuber; 8-9 generations per year
- Annual yield loss in Africa 60-100%; 5-80% worldwide

Cylas formicarius

Cylas puncticollis

Cylas brunneus

Microbial Control of Sweetpotato Weevil

PROJECT

- 3-year proof of concept project
- Goal: Identify microbes with potential to control sweetpotato weevils

TARGET

- Sweetpotato in sub-Saharan Africa
- Small-holder farmers

PARTNERS

- AgBiome – Primary grantee
- Louisiana State University – Subgrantee
- NaCRRI/NARO – Research partner
- Funding source – BMGF

International Partnership

- Biotechnology company located in Research Triangle Park, NC, USA
- Largest (50,000+) fully sequenced microbe collection
- Database tracks location, genome sequence, bioassay and field results
- Systematic, data-driven insect and other agricultural pest-screening processes

- University with Agricultural Expertise
- Baton Rouge, LA, USA
- Maintains colony of sweetpotato weevils (*C. formicarius*)
- Developed and running bioassay for testing microbes against SPW

- Ugandan National Agricultural Research Organization Institute with root crop expertise
- Namulonge, Uganda
- Maintains colonies of sweetpotato weevils (*C. brunneus* and *C. puncticollis*)
- Coordinating and conducting collection of sweetpotato environmental samples for microbe isolation

Objectives & Approach June 2016-July 2019

Objective 1
Assess Microbial Movement

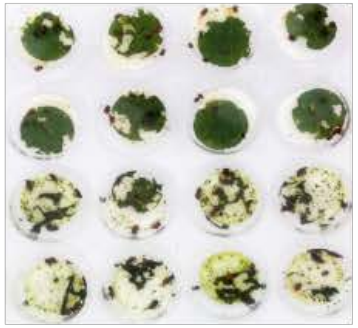
Obtain permits for strain transport between Uganda & US

Objective 2
Microbe Collection & Sequencing

16S amplicon sequencing to catalog culturable and unculturable strains

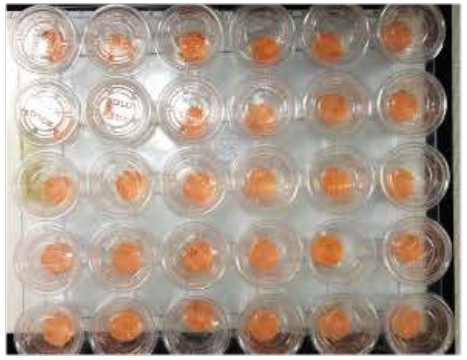
Isolate & sequence strain genomes

Objective 3
ID Active Microbes via Surrogate Screen



Colorado potato beetle & Western corn rootworm bioassays

Objective 4
Sweetpotato Weevil Bioassay



Develop bioassay & screen strains against target

Objective 1:

Microbial movement

Goal: Establish relationships among partners and obtain permits to move environmental samples to and from Africa and USA - complete

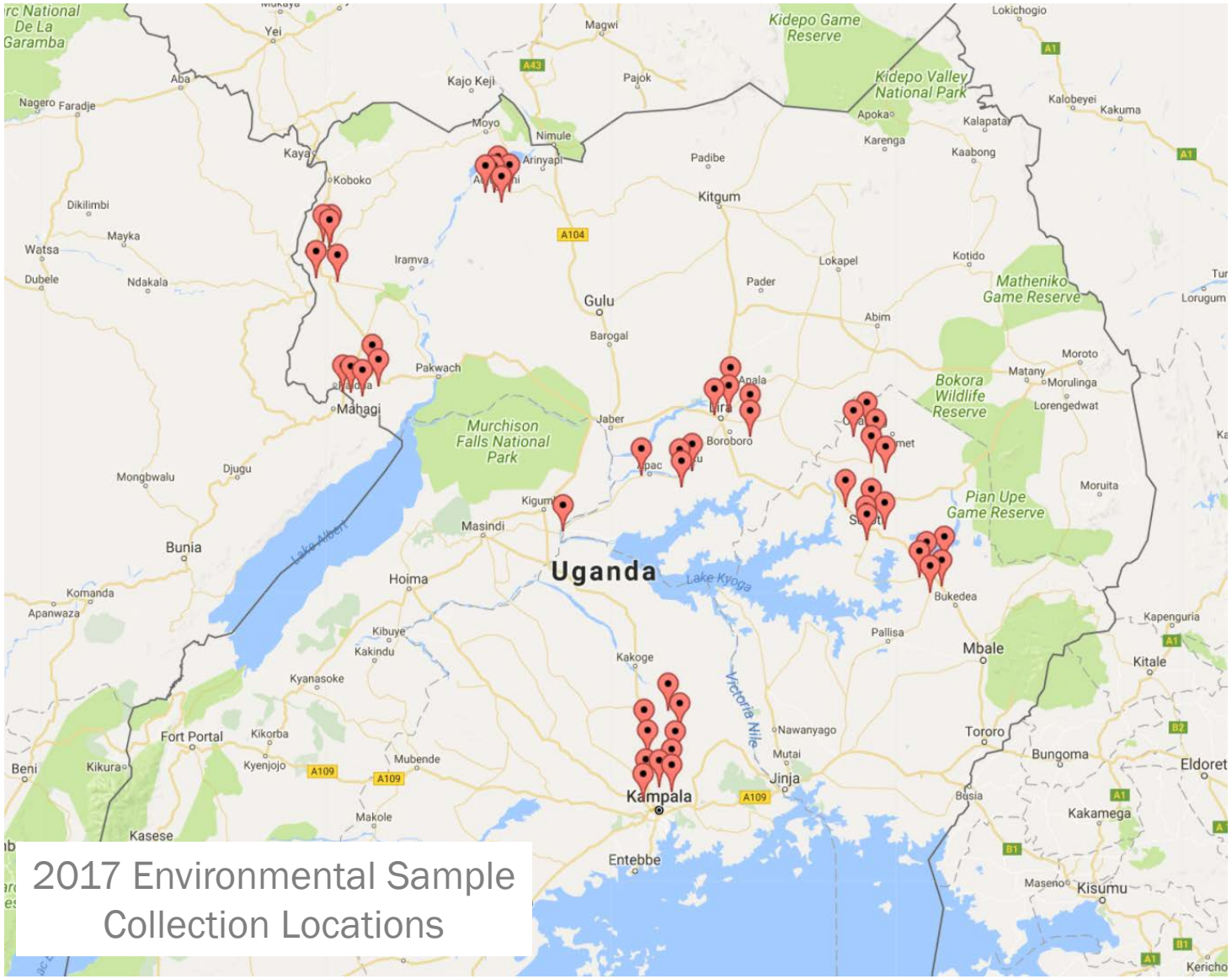
Objective 2:

Collect Environmental Samples from sweetpotatoes, isolate microbes and sequence genomes

Goal: 15,000 fully sequenced microbes that associate with sweetpotatoes

Ugandan field sampling

- Goal: Characterize microbiome in SSA country to identify active microbes that can potentially colonize sweetpotatoes and are easy to register in Africa
- 10 geographic districts
  - 5 farms/district
    - 9 samples/farm
      - Bulk soil
      - Rhizosphere
      - Sweetpotatoes
- Sample 4 times
  - August & November 2017 & 2018



2017 Environmental Sample Collection Locations


>8,000 microbes isolated & sequenced from Uganda

Colorado potato beetle

Western corn rootworm

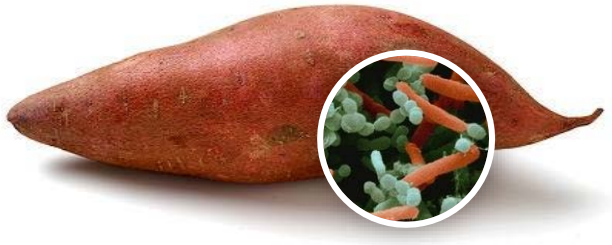
Objective 3:

Identify Actives via Surrogate* Screen

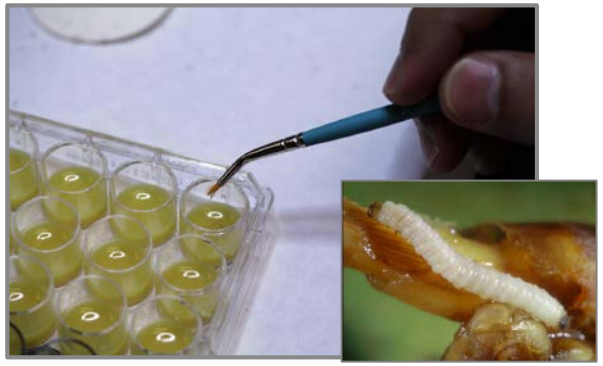
Goal: Identify >10 Coleopteran active microbes

Coleopteran Microbe Screening

Crop-associated Microbes

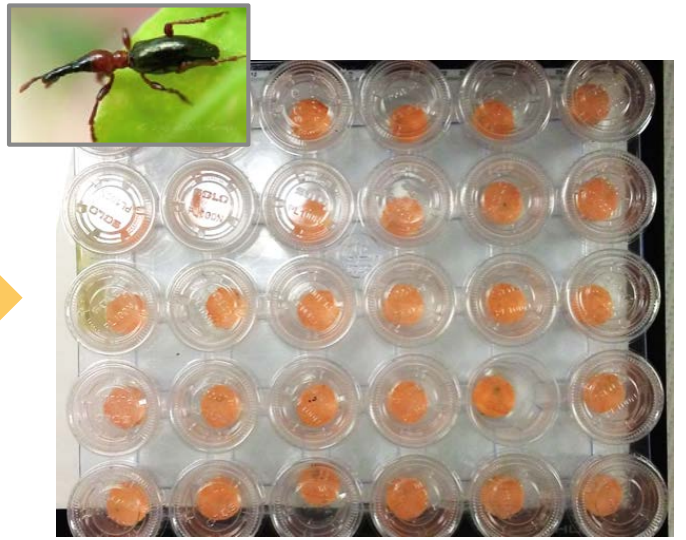


Surrogate beetle assays



CPB and WCR actives progress to SPW

Sweetpotato weevil assays



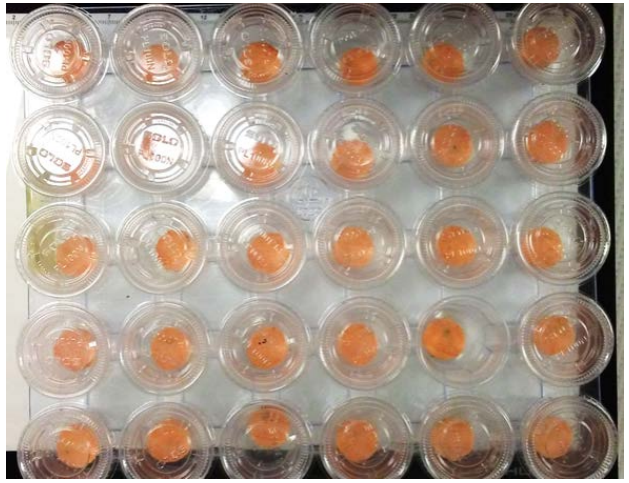
*Surrogate necessary because SPW is a quarantined pest where AgBiome is located

Results from Surrogate Screen

- Colorado Potato Beetle
  - 3,911 microbes tested against CPB
  - Three microbes confirmed active after >5 replicates
  - Three additional active microbes identified using searches of genomic data in AgBiome's microbial collection

- Western Corn Rootworm
  - 7,069 tested against WCR
  - 69 microbes confirmed active after 5 replicates

- Next steps
  - Testing of surrogate-active microbes underway at Louisiana State University
  - Active microbes from LSU screen will be tested on African species at NaCRRI



Objective 4:
Sweetpotato Weevil Bioassay

Goal: Develop robust SPW bioassay & screen actives from surrogates

SPW Bioassay Development - Larvae

- SPW larvae fed chips dipped in microbe solution with blue dye show blue dye in gut indicating feeding
- Successful development from larvae to adult on root chips

Day 1

Day 3

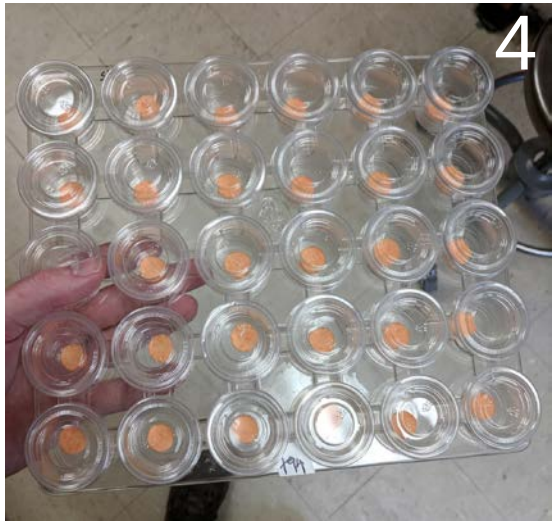
Day 5

Day 11

Day 17

SPW Bioassay Set-Up

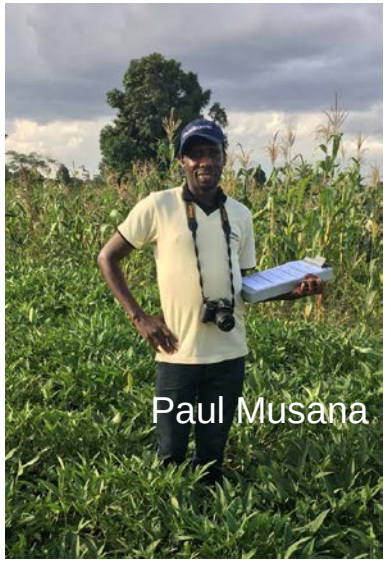
1. Root chips are soaked in microbe solution
2. Larvae are gently removed from infested roots
3. Larvae are added to chips
4. Clear cups for easy data collection
5. Chips are stored in light, temp, humidity controlled chamber
6. Results read after 4 & 8 days



Overall Project Summary

- Objective 1 - Microbial Movement
  - Agreement and permits in place
  - >800 environmental samples shipped from Uganda to AgBiome for microbe isolation
- Objective 2 - Sample Collection
  - Sampling nearly complete
- Objective 3 - Surrogate Screen
  - 75 active strains identified from CPB, WCR and AgBiome database
- Objective 4 - SPW Bioassay
  - Initial surrogate hits undergoing testing

Acknowledgements



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