# Screening of entomopathogenic fungi from West Central Nebraska against key pests of corn

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## Introduction

- The western corn rootworm (WCRW), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is a highly adaptable pest and cases of resistance to Bt crops and insecticides in the Corn Belt have been reported (1, 2, 3, 4).
- Integrating biological control into the scope of management practices against the WCRW might help us suppress populations and delay resistance issues.
- Entomopathogenic fungi have been tested against the WCRW with low to moderate mortality rates (5, 6, 7, 8).
- Any control applied to the soil against the WCRW may also impact secondary targets that spend part of their life cycle in the soil such as the western bean cutworm, *Striacosta albicosta* Smith (Lepidoptera: Noctuidae).
- Nebraska is currently the third largest maize grower in the country and WCRW and WBC are two of the state's biggest pests.

## Research Objective

Screen entomopathogenic fungi (EPF) from irrigated commercial corn fields against the WCRW for potential biological control and screen selected strains against the WBC.

## Fungal Strains Background

Native entomopathogenic fungi were collected in 2014 and 2015

High WCRW-pressure sites (n= 4)

- Commercial corn
- Cry3Bb1 or Cry3Bb1+Cry34/35Ab1
- Continuous corn (>5 yrs)
- Center pivot irrigation

Low WCRW-pressure site (n= 1)

- 1st year corn in 2014

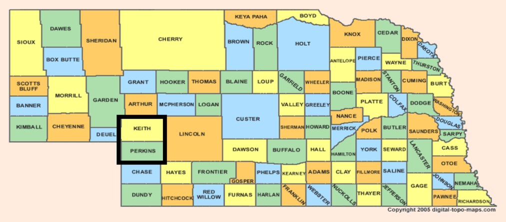


Fig.1. Locations of research fields in west central Nebraska.

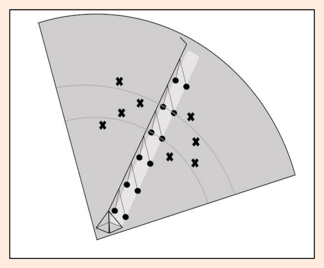
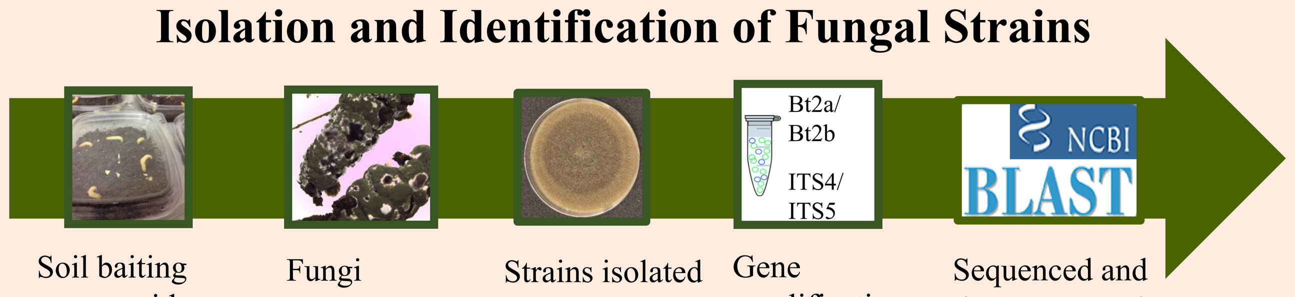


Fig.2. Map of soil sampling inside fields.

### Isolation and Identification of Fungal Strains




Soil baiting assays with *G. mellonella* and *T. molitor.* — Fungi recovered from insect cadavers. — Strains isolated in selective media. — Gene amplification using ITS and Bt primers. — Sequenced and then compared to GenBank database for ID.

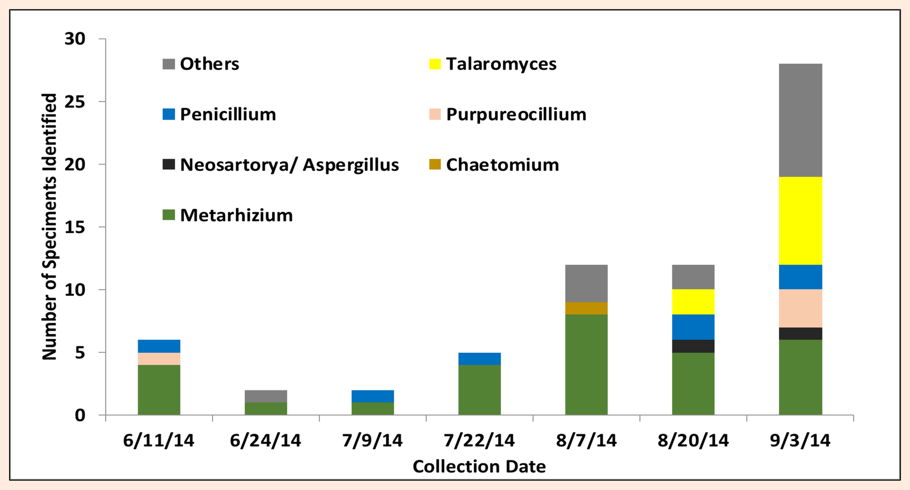



Fig. 3. Diversity and seasonality of fungal strains isolated (n=67) from soil baiting assays in 2014.

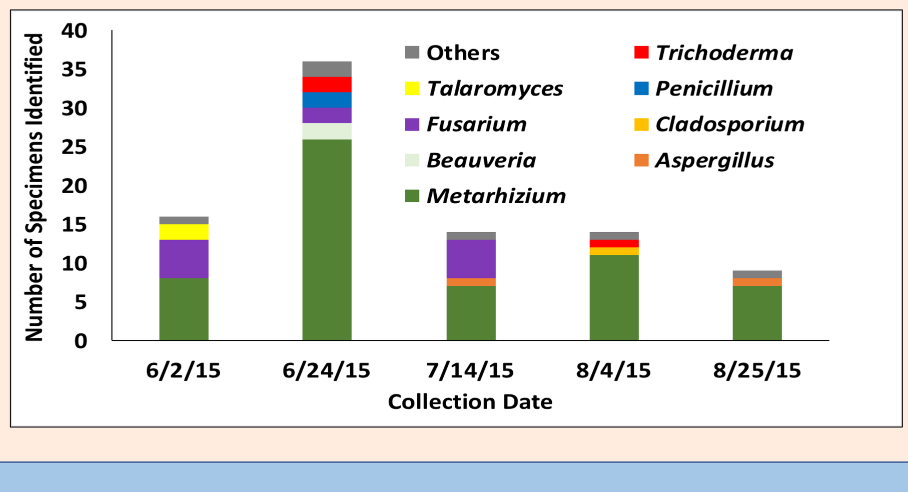
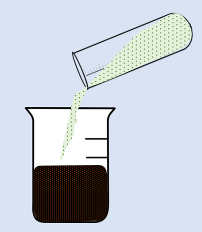



Fig. 4. Diversity and seasonality of fungal strains isolated (n=89) from soil baiting assays in 2015.

## Bioassay Methods

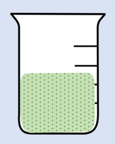
Conidia from 14-day old plates were washed with 0.1% Tween 80 and inocula were adjusted for viability. All bioassays cups were sandwiched between café-trays lined with moist paper towels and then placed into an incubator set at 65% RH, 26.3 ± 0.5°C.

**WCRW soil assay**



- 48 fungal strains tested from Figs. 3 and 4 at 1x $10^7$ spores/gram of soil or maximum obtained concentration.
- Inoculated soil dispensed into three 2-oz cups, each with three 3-day-old corn seedlings.
- Inoculum incorporated into sterile soil at 25% water holding capacity (WHC). Controls received 0.1% Tween 80.
- Ten third instar larvae added/cup. Total of 30 larvae/strain.
- Mortality checked at 9 days and corrected with Abbot's formula.
- Analysis: least square means (Tukey's adjustment) in Rstudio glm package.

**WCRW dipping assay**



- 15 strains tested.
- Ten third instar larvae dipped in 1 x $10^7$ spores/ml for 5 secs. Controls in 0.1% Tween 80. Total of 30 larvae/strain.
- Larvae placed in 2-oz soil cups with sterile soil at 25% WHC with three 3-day-old corn seedlings.
- Mortality checked at 7 days and statistical analysis performed as described above.

**WBC assay**

- 11 strains tested at 1 x $10^7$ spores/ml or maximum obtained concentration.
- 3 ml of inoculum/prepupae sand cup. Controls received 0.1% Tween 80.
- Total of 15 or 16 larvae/strain.
- Prepupae mortality checked at 9 days and corrected using Henderson-Tilton's formula (non-uniform population).

## Results

**Table 1. Western corn rootworm (WCRW) and western bean cutworm (WBC) corrected mortality from bioassays. Fungal growth represents visual confirmation of mycosis in cadavers.**

| | | WCRW soil assay | | | WCRW dipping assay (All 1.00E+07 spores/ml ) | | WBC assay | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Species | Spores/ gram | % Corrected Mortality | Fungal growth | % Corrected Mortality | Fungal growth | Spores/ ml | % Corrected Mortality | Fungal Growth |
| E998 | *Aspergillus flavus* | 1.00E+07 | 0 | - | - | - | - | - | - |
| Botanigard | *Beauveria bassiana* | 1.00E+07 | 16.1 | Y | 30 | Y | 1.00E+07 | 26.7 | N |
| E1040 | *Beauveria bassiana* | 1.00E+07 | 7.4 | N | - | - | - | - | - |
| E312 | *Chaetomium* sp. | 2.70E+05 | 32 | N | - | - | - | - | - |
| E126 | *Cladosporium halotolerans* | 4.24E+06 | 2.6 | N | - | - | - | - | - |
| E1060 | *Cladosporium sp.* | 6.74E+06 | 7.4 | Y | - | - | - | - | - |
| E651 | *Clonostachys sp.* | 9.63E+06 | 2.6 | N | - | - | - | - | - |
| E648 | *Fusarium oxysporum* | 1.00E+07 | 18.5 | N | - | - | - | - | - |
| E999 | *Fusarium solani* | 6.93E+06 | 2.7 | N | - | - | - | - | - |
| E1034 | *Metarhizium anisopliae* | 2.7E+06 | 12.8 | Y | - | - | 2.70E+06 | 26.7 | N |
| E213 | *Metarhizium anisopliae* | 3.3E+06 | 48.1 | Y | 23 | N | - | - | - |
| E1033 | *Metarhizium anisopliae* | 7.5E+06 | 25.9 | Y | - | | - | - | - |
| **E1089** | *Metarhizium anisopliae* | 1.0E+07 | **76**** | Y | 13 | Y | - | - | - |
| E1093 | *Metarhizium robertsii* | 1.4E+05 | 37 | Y | 27 | N | - | - | |
| **E1000** | *Metarhizium robertsii* | 5.8E+05 | **68**** | Y | 13 | N | 4.20E+06 | 26.7 | Y |
| **E653** | *Metarhizium robertsii* | 8.7E+05 | **56 **** | Y | - | - | - | - | - |
| E1030 | *Metarhizium robertsii* | 1.9E+06 | 52 | Y | 27 | Y | 1.00E+07 | 13.3 | N |
| **E645** | *Metarhizium robertsii* | 2.3E+06 | **64**** | Y | - | - | - | - | - |
| **E1016** | *Metarhizium robertsii* | 2.5E+06 | 40 | Y | **37*** | Y | - | - | - |
| **E380** | *Metarhizium robertsii* | 4.2E+06 | **51 **** | Y | 13 | Y | 1.00E+07 | 40 | Y |
| E211 | *Metarhizium robertsii* | 4.7E+06 | 15.4 | N | - | | 1.00E+07 | 57.3 | |
| E1090 | *Metarhizium robertsii* | 4.8E+06 | 18.5 | Y | - | - | - | - | - |
| E136 | *Metarhizium robertsii* | 6.6E+06 | 33.3 | Y | - | - | - | - | - |
| **E138** | *Metarhizium robertsii* | 7.1E+06 | **59.3**** | Y | - | - | - | - | - |
| E161 | *Metarhizium robertsii* | 8.3E+06 | 38.5 | Y | 23 | Y | 1.00E+07 | 40 | Y |
| **E1022** | *Metarhizium robertsii* | 8.3E+06 | **53.8 **** | Y | 17 | Y | 1.00E+07 | 40 | N |
| E1005 | *Metarhizium robertsii* | 1.0E+07 | 11.1 | Y | - | - | - | - | - |
| **E1026** | *Metarhizium robertsii* | 1.1E+06 | **60**** | Y | 30 | Y | 1.00E+07 | 21.8 | N |
| E1056 | *Metarhizium robertsii* | 2.7E+06 | 44 | Y | 20 | N | - | - | - |
| E328 | *Metarhizium robertsii* | 5.6E+06 | 52.7 | Y | 13 | N | 1.00E+07 | 20 | Y |
| E322 | *Metarhizium robertsii* | 5.8E+06 | 7.4 | N | - | | - | - | - |
| E374 | *Metarhizium robertsii* | 1.0E+07 | 7.4 | Y | - | - | - | - | - |
| E1095 | *Metarhizium* sp. | 7.1E+05 | 29.6 | Y | - | - | - | - | - |
| E1038 | *Metarhizium* sp. | 4.4E+06 | 42.7 | Y | 27 | Y | - | - | - |
| E314 | *Neosartorya* sp. | 1.00E+07 | 6.7 | N | - | - | - | - | - |
| E368 | *Penicillium bilaiae* | 1.00E+07 | 7.4 | N | - | - | - | - | - |
| E212 | *Penicillium griseofulvum* | 1.00E+07 | 18.5 | Y | - | - | - | - | - |
| E172 | *Penicillium janthinellum* | 9.63E+05 | 15.4 | N | - | - | - | - | - |
| E1035 | *Penicillium* sp. | 1.00E+07 | 10.7 | Y | - | - | - | - | - |
| E166 | *Penicillium* sp. | 1.00E+07 | 16.7 | N | - | - | - | - | - |
| E370 | *Pseudogymnoascus destructans* | 1.16E+06 | 5.1 | N | - | - | - | - | - |
| E393 | *Pseudogymnoascus pannorum* | 1.00E+07 | 11.5 | N | - | - | - | - | - |
| E376 | *Pseudogymnoascus* sp. | 1.00E+07 | 48 | N | 13 | N | 1.00E+07 | 28.9 | N |
| E378 | *Purpureocillium lilacinum* | 1.00E+07 | 20 | Y | - | - | - | - | - |
| E325 | *Taifanglania* sp. | 1.00E+07 | 0 | - | - | - | - | - | - |
| E646 | *Talaromyces pinophilus* | 3.73E+06 | 7.4 | N | - | - | - | - | - |
| E390 | *Talaromyces* sp. | 1.00E+07 | 10.3 | N | - | - | - | - | - |
| E315 | *Talaromyces trachyspermus* | 5.78E+05 | 3.7 | Y | - | - | - | - | - |
| E331 | *Talaromyces trachyspermus* | 7.70E+06 | 0 | - | - | - | - | - | - |

**Fig. 5.** Healthy (left) and *Metarhizium* infected (right) WCRW larvae.


**Fig. 6.** Healthy (left), newly infected (middle) and late *Metarhizium* infected (right) WBC prepupae.

## Results Summary

**WCRW soil assay**

- All but three strains (E998: *A. flavus*, E325: *Taifanglania* sp., and E331: *T. trachyspermus*) had greater than zero WCRW corrected mortality.
- Many strains exhibited poor sporulation and/or germination, thus inoculum concentrations varied significantly.
- However, strains with lower spore concentrations were still able to kill WCRW.
- Mean negative control mortality was 12.2%.
- Strains with "**" (E1089, E1000, E653, E645, E380, E138, E1022, E1026) were significantly greater than control mortality before Abbot's correction at ($p < 0.05$).

**WCRW dipping assay**

- All negative control larvae were alive in the end of the study and strain mortality ranged from 13-37%.
- Strain with "*" (E1016) was significantly different than control mortality at ($p < 0.10$).

**WBC assay**

- Negative control mortality was 6.2% and corrected strains' mortality ranged from 13.3- 57.3%.
- Low availability of insects didn't allow for replications and statistical analysis.

## Discussion

- This research indicates that native EPFs are capable of causing WCRW and WBC mortality.
- In Iowa, native *M. anisopliae* and *B. bassiana* from the soil caused similar WCRW mortality levels (8).
- There is a significant knowledge gap on what other EPFs are pathogenic to the WCRW besides *Metarhizium* and *Beauveria.*
- Some genera tested herein are not exclusively entomopathogenic, e.g., *Fusarium* (plant pathogen) and *Trichoderma* (biofungicide), but rather contain species that have shown entomopathogenicity or toxins against certain insects.
- There is an overlap (May-July) in which larvae and pupae of both WCRW and WBC are present in the soil at the same time and a strain that can be used for both species simultaneously may benefit fields in which both pests are a problem.
- Next step will be to explore the feasibility of *M. robertsii* strains for the control of both pests in field trials, which will establish ground work for the integration of EPF in maize IPM.

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