

Species diversity of Soybean Sudden Death Syndrome pathogens in Kansas

### Madison Kessler Luiza Adami

Dr. Raissa D. Moura Dr. Harold N. Trick Dr. Kelsey Andersen-Onofre Dr. Martin Chilvers Dr. Rodrigo Onofre

# Background

- Sudden Death Syndrome (SDS) of soybeans:
  - Fusarium solani species complex (FSSC) clade 2.
    - Fusarium virguliforme\*, F. tucumaniae, F. brasiliense and F. crassistipitatum:
  - 1993: First report of SDS in Kansas.
  - 2019: First report of *F. brasiliense* present in Michigan (2014 and 2016).
- Soilborne disease;
- Primary inoculum:

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- Infested soil with chlamydospores;
- Soybeans and corn crop residue.

Species Complex F. langsethiae 53436 Fusarium F. sporotrichioides 3299 armeniacum 6227 Phylogeny F. poae 26941 F. sambucinum 1370 venenatum 66329 praegraminearum 39664 sambucinum 19 genes nseudograminearum 28062 F. culmorum 25475 55.140 nt F. asiaticum 26156 nearum 31084 23.668 PIC compactum 13829 E transvaalense 31008 - F. longipes - 4 13317 - F. longipes - 1 13368 TC = 48.41F. avwerte 25410 - F. chlamydosporum 13444 chlamydosporum relative TC = 0.563- F. nelsonii 13338 \*\_\_\_\_\_\_F. scirpi 66328 best ML tree: F. equiseti 66338 incarnatum-equiset -F. irregulare 31160 94/1.0 -927514.615 -E hainanense 66475 graminum 20692 heterosporum F. nurragi 36452 -F. torulosum 2274 99/1. tricinctum F. avenaceum 5493 tricinctum 25481 . anguioides 25385 concolor 13459 concolor F. verrucosum 22566 binda 25539 babinda F. beomiforme 25174 burgessii hostae 29888 redolens F. redolens 22901 F. newnesense 66241 F. newnesense 508 newnesense oxvsporum 34936 oxysporum 32931 oxysporum foetens 38302 \*\_\_\_\_F. gaditjirrii 45417 lvarnte 54252 miscanthi 26231 nisikadoi <sup>L°</sup> F. niskadoi 25179 commune 28387 F. fujikuroi 5538 -F. sacchari 66326 -F. thansinum 22049 F. verticillioides 2095 fujikuroi xylarioides 25486 86/1.0 Fusarium sp. 52700 -. guttiforme 22945 90/1. circinatum 2533 - F. continuum 66286 torrevae -F. zanthoxyli 66285 F. stilboides 20429 100/0.9 lateritium 13622 lateritium sarcochroum 20472 buharicum 13371 buharicum F. sublunatum 13384 81/0.99 buxicola 36148 buxicola cvanostoma 5399 F. rusci 22134 F. staphyleae 22316 staphyleae F. zealandicum 22465 albosuccineum 20459 decemcellulare 13412 decemcellulare illudens 22090 - Clade F. virguliforme 31041 - Clade 2 neocosmosporiellum 22166 91/0.99 F. ambrosium 62606 solani 19 Fusarium sp. 62590 AF-6 Clade 3 falciforme 43529 F1 ttenii 458 albidum nematonhilum 54600 E ventricosum 25729 ventricosum F. domesticum 29970 12 - F. dimerum 20691 dimerum <50/<0.50 F. penziaii 20711 V ditissima 20485 Neonectria onectria sp. 2250 (sister taxon inoa 20487 - Beauveria bassiana outgroups Trichoderma brevicompactum

(Geiser et al., 2021)

0.05

# Samples collection

Kansas counties where samples were collected.



2022 Season – 6 counties

2023 Season – 12 counties



### 2021 Kansas Soybean Production







# Fusarium isolation

• SDS-symptomatic soybean plants collected across Kansas:



Malachite Green Agar

26 °C in the dark for 4-5 days





## *Fusarium* – Pure culture

Single Hyphal Tip Isolation



Potato Dextrose Agar





Picture Source: Madison Kessler

### – Diversity Analysis of *Fusarium* isolates associate with SDS in Kansas

County	Total <i>Fusarium</i> -like Isolates #
Shawnee	6
Jefferson	97
Barton	11
Riley	12
Republic	2
Total	128



# **DNA Extraction and PCR Amplification**

• 113 Fusarium virguliforme-like isolates and 15 Fusarium-like isolates:



- Target genes:
  - Broad screening for Fusarium Species Complex and FSSC clade 2: Translation elongation factor-1α (EF-1α) for all Fusarium isolates.
  - Screening for FSSC clade 2 species: 28S-18S ribosomal RNA intergenic spacer (IGS) only for FSSC species.



## TEF tree – isolates distribution in different species complex



The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 60% bootstrap replicates are collapsed. This tree is rooted on the sequences of *F. oxysporum* f. sp. *pisi* from the National Center for Biotechnology Information GenBank (NRRL 36228).



# TEF tree – isolates distribution in different species

### complex

FOSC

FSSC Clade 2	<b>RBO</b> : isolates from this work. <b>NRRL</b> : USDA-ARS Culture Collection
FSSC Clade 11	
FSSC Clade 3	
FSSC Clade 5	
FFSC	

Other *Fusarium* species are present in symptomatic plants

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# IGS tree -

Fusarium virguliforme

**RBO**: isolates from this work.

**NRRL:** USDA-ARS Culture Collection

### F. virguliforme is most likely causing SDS in Kansas

All *F. virguliforme*-like strains had 100% identity with NRRL36900 and NRRL31039 (*F. virguliforme* NCBI deposit).



Hasegawa-Kishino-Yano model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 60% bootstrap replicates are collapsed. This tree is rooted on the sequences of *F. oxysporum* f. sp. *pisi* from the National Center for Biotechnology Information GenBank (NRRL 36228).

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### – Diversity Analysis of *Fusarium* isolates associate with SDS in Kansas

County	Total <i>Fusarium</i> -like Isolates #	<i>F. virguliforme</i> isolates #
Shawnee	6	3
Jefferson	97	90
Barton	11	9
Riley	12	11
Republic	2	0
Atchison	0	0
Total	128	113

