KSC Report for: "Mitigating soybean root and seedling diseases in Kansas." (Year 2)
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Introduction:

From 2012 to 2020, SDS, Fusarium diseases, charcoal rot, and Phytophthora root rot cost Kansas soybean farmers an average of 7.8% of production per year. Through past KSC support, the Row Crops Pathology Lab at KSU has taken a leadership role in soybean root health research with an emphasis on diseases. Our priorities are the discovery of plant resistance, management strategies, and new disease information to help Kansas producers increase their yields and profits over the long-term.

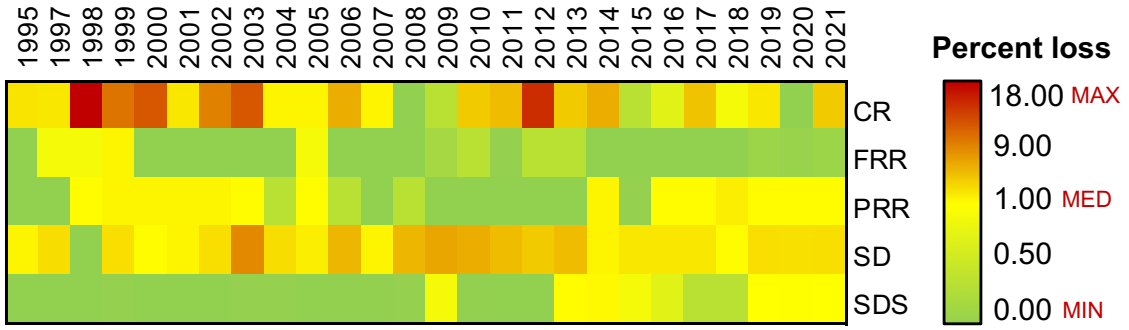

Figure 1. Heat map of relative losses for soybean root diseases from 1995 to 2021. **CR** = charcoal rot, **FRR** = Fusarium root rot*, **PRR** = Phytophthora root rot, **SD** = seedling disease, **SDS** = sudden death syndrome. *Fusarium root rots are probably underdiagnosed & underreported.

Objectives & Activities:

1. Discover resistance to fungal diseases: SDS, Fusarium diseases, and charcoal rot.

So far, three methods are being tested to assess resistance to these diseases in soybean germplasm: rolled-towel, layer-cake, and seed-plate assays. Most efforts have focused on these in vitro/growth chamber/greenhouse assays for the purpose of finding higher throughput methods to discover resistance, rather than relying on long-term and environmentally confounded field studies.

SDS screening using a culture filtrate/toxin assay has occurred on both commercial, public, and breeder materials for senescence and toxin reactions. Senescence occurs in seedlings that are clipped and placed in sterile-distilled water. In some cases, the seedlings remain green and receive a lower rating (1-2) compared to when they become senescent (3-6). Similarly, when clipped seedlings are placed in culture extracts of *F. virguliforme*, the seedling remains green and receive a low rating (1-2) compared to toxin-affected seedlings that wilt, display SDS-like symptoms, or become necrotic receive a higher rating (3-6). An example of some senescence and toxin reactions in public materials is shown in Figure 2.

Screening for *Fusarium* seedling disease reactions was started during this year of the project and is in progress. A rolled-towel pathogenicity screening is being used to test multiple isolates of several different seedling-associated *Fusarium* spp. for impacts on germination and seedling root length. This study will continue in the next year of the project.

Public

GENOTYPE	Senescence					Toxin reaction					Median	
Seedling	*1*	*2*	*3*	*4*	*5*	*1*	*2*	*3*	*4*	*5*	S	T
AR Osage	2	1	1	1	4	3	4	6	2	2	2.0	3.0
AR R09-430	1	2	6	1	1	3	1	4	4	2	2.0	3.0
AR UA 5014C	6	1	1	1	1	2	4	2	5	4	2.0	4.0
AR UA 5414RR	2	1	2	3	4	5	5	6	5	6	4.5	5.0
LD06-7862	1	6	1	1	1	3	5	4	4	3	3.0	4.0
LS09-1920	2	2	5	4	5	2	2	6	6	2	3.0	2.0
K12-1348	1	2	5	1	1	3	2	5	6	4	2.5	4.0
K12-1355	6	5	5	6	6	6	6	6	6	6	6.0	6.0
K12-2333	1	6	1	1	1	6	2	2	6	2	2.0	2.0
K13-1615	6	6	6	6	6	3	3	6	3	2	6.0	3.0
K4313	1	6	1	2	1	2	2	3	2	1	2.0	2.0
KS3406	2	2	2	4	4	6	6	4	5	6	4.0	6.0
KS4117	3	3	4	1	1	4	6	5	6	3	3.5	5.0
KS4313	6	2	3	6	2	6	6	4	4	4	4.0	4.0
Morgan	1	6	3	3	6	6	6	6	5	6	6.0	6.0
Ripley	2	1	2	1	2	2	3	4	4	5	2.0	4.0
S13-10590C	6	6	6	6	6	6	6	6	6	6	6.0	6.0
S13-1805C	4	6	4	6	5	6	5	6	6	5	5.5	6.0
S13-1955C	6	2	6	6	6	2	6	6	6	6	6.0	6.0
S13-2743C	6	6	6	6	6	5	5	6	6	3	6.0	5.0
S13-3851C	6	6	2	1	5	6	6	2	4	5	5.0	5.0
S14-9051R	4	4	1	6	6	4	4	2	6	5	4.0	4.0

Figure 2. Senescence and *F. virguliforme* toxin reactions of public soybean varieties using the SDS culture filtrate/toxin assay. (Left) An example of the culture-filtrate (toxin) assay; Left seedlings are in sterile-distilled water and righthand seedlings are in culture filtrate. (Right) The responses for five seedlings are shown for senescence (sterile, distilled water) and culture filtrate (toxin). Median values for senescence and toxin reactions are shown in the right columns. Values between 1 and 2 indicate "non-senescent" and "toxin resistance," whereas values between 3 and 6 indicate "senescent" and "toxin sensitive," respectively.

A seed-plate assay. cut-stem assay, and layer-cake assay have been used for charcoal rot assessment in a range of numbered genotypes from Bayer (Figure 3). This data is being analyzed and compared.

2. Evaluate the management strategies for fungal pathogens: SDS and Fusarium diseases.

During the first year, small-scale studies were conducted to determine the effect of *B. juncea* against the SDS and Fusarium seedling/root pathogens. *B. juncea* extracts were obtained from vegetative, flowering, and senescent plants and were tested against *F. virguliforme* and other *Fusarium* spp. directly to see if the cover crops exert any antifungal activity on their own. In general, the plant extracts themselves do not show much pathogen inhibition.

Cover crops studies: Unfortunately, *Brassica juncea* cover crops failed in southeast Kansas this spring, and therefore this part of the project could not be completed.

Figure 3. Seed plate (left), cut-stem (middle), and layer-cake (pot-based; right) assays for charcoal rot.

Fungicides: Testing of strobilurin fungicides have dominated our experiments in the lab so far. In this regard, we have tested azoxystrobin, picoxystrobin, pyraclostrobin, and trifloxystrobin against *F. virguliforme* (the causal agent of SDS) and *F. proliferatum* (a common soybean seedling pathogen) (Figure 2). So, far the *F. proliferatum* isolates we've tested on azoxystrobin do not appear to differ in their sensitivity to this fungicide active ingredient. However, the average EC50 threshold across the isolates tested is 18.5 μg/mg. However, one *F. proliferatum* isolate, RCPL0165, exhibited significantly greater tolerance to picoxystrobin than the others. Interestingly, *F. proliferatum* isolates proved to be most sensitive to pycraclostrobin among the four strobilurins tested. However, the isolates were not different from one another.

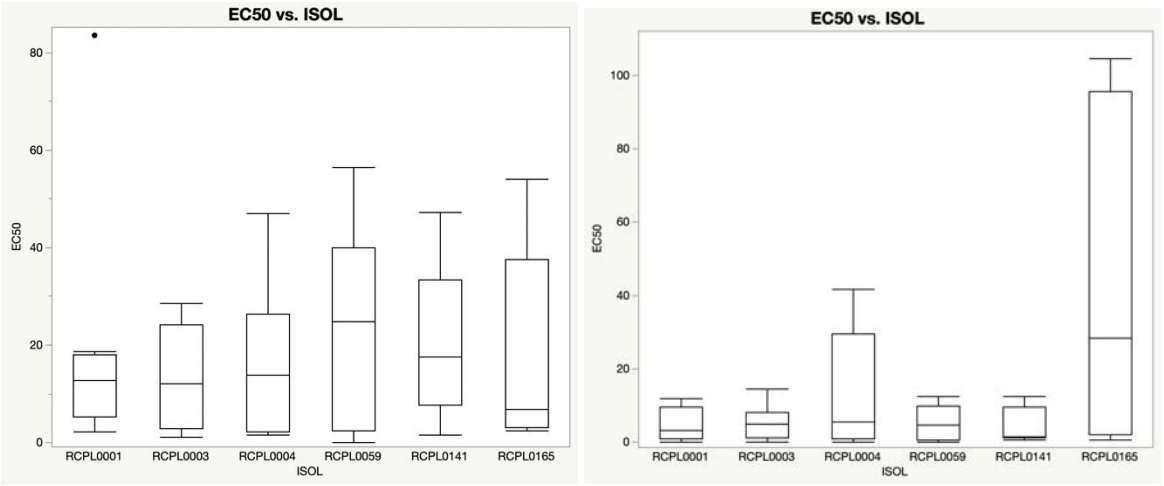

Figure 2. Response (EC50) of six *Fusarium proliferatum* isolates from Kansas soybean seedlings grown on azoxystrobin (*top left*), picoxystrobin (*top right*), pycraclostrobin (not shown), and trifloxystrobin (center).

3. Assess the impact of re-emerging root pathogens: *Phytophthora sojae* in southeastern Kansas.

We have moved beyond SE KS to collect *P. sojae* isolates. So far this year, we have collected diseased plants (Figure 3) from Riley and Nemaha counties. Currently, plant tissues are in culture to isolate pure cultures of *P. sojae*. Once enough isolates are collected, they will be maintained in the Row Crops Pathology Lab culture collection. As time allows: (i) Pathogenicity will be estimated based upon the degree of root rot caused by each isolate. (ii) Tolerance or sensitivity to metalaxyl and mefanoxam, common fungicide active ingredients, used against this pathogen, will be estimated for each isolate. (iii) If time and resources allow, SEK *P. sojae* races will be determined using soybean differentials.

In addition to plants with *Phytophthora* symptoms, plants with mixed infections including *Diaporthe* (stem canker), *Fusarium* (root rot), and *P. sojae* have been found (Figure 3). From late summer and early fall 2022 surveys, mixed infections were 20% stem canker, charcoal rot, *Phytophthora*, and *Fusarium virguliforme*; and 48% were stem canker, charcoal rot, *Phytophthora*, and *Fusarium virguliforme* in addition to stem borer and spidermite damage. Plus, there were many SCN positive plants (63%), and plants with single infections of charcoal rot (21%). Most plants from SE Kansas had some degree of disease or fungal colonization associated with them.

Figure 3. Example of dark lesions produced by *Phytophthora sojae* at the base of soybean plants (left). Mixed infections are also common that come from combinations of *Diaporthe*, *Fusarium*, and *P. sojae* (right).