



Exploring fungal and viral pathogens associated with intermediate wheatgrass, a promising new small grain crop

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Introduction

Why Intermediate Wheatgrass?

- Intermediate wheatgrass (IWG), sold commercially as Kernza® grain, is a perennial grass.
- The perennial growth of IWG means it develops a larger, more extensive root structure than annual wheat.
- The perennial root structure of IWG helps support soil structure and retain water.

Potential for Disease

- As a new crop, disease pressure on IWG is not well characterized.
- Fusarium head blight and wheat streak mosaic virus are two of the most common and problematic diseases on annual wheat, so we want to see if these pathogens, or others, can be recovered from IWG.



Fig 1: Kernza® grain and spikes. PC: The Land Institute.



Fig 2: Standing IWG crop in Olathe, KS
PC: A. Dahal, 2021.

Objectives

- To identify different fungal pathogens in IWG
- To detect wheat streak mosaic virus from IWG using DAS-ELISA

Materials and Methods



Culturing samples in SNA plate



Mycelium growth after final subculture



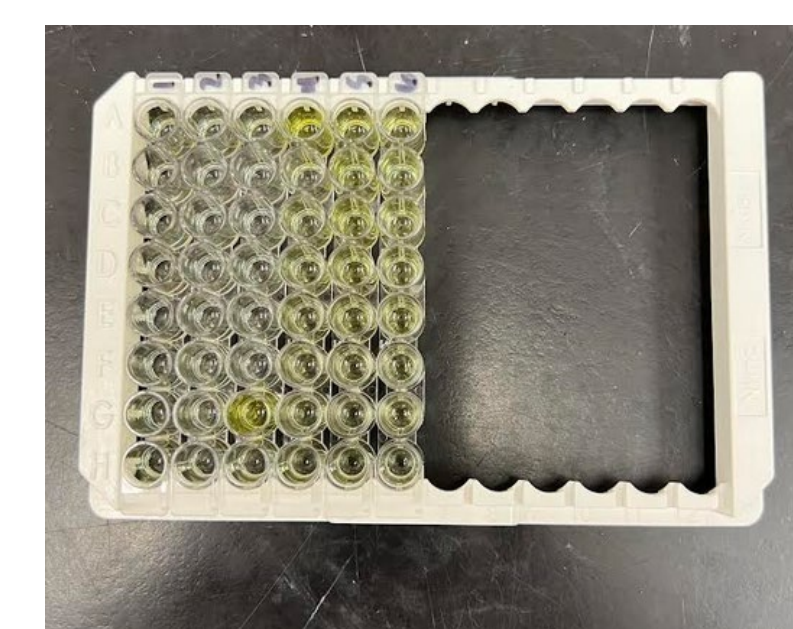
Leaf samples prepared with General Extraction Buffer



Ship to MCLAB for DNA sequencing



PCR performed on DNA extracted from mycelium



DAS-ELISA plate after testing

Fig 3: Flowchart for Pathogen identification process

Fig 4: Flowchart for DAS-ELISA

Results

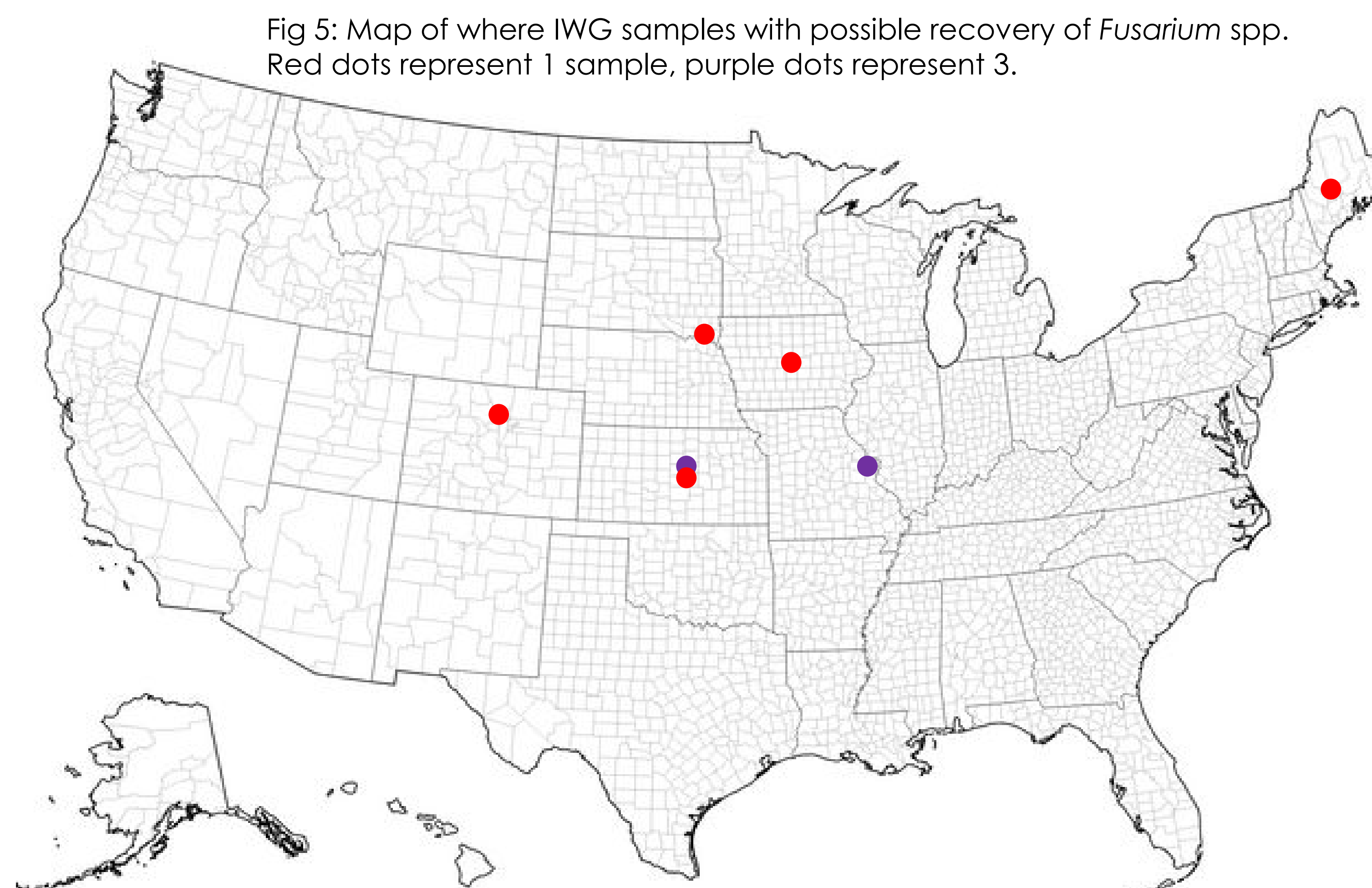


Fig 5: Map of where IWG samples with possible recovery of *Fusarium* spp. Red dots represent 1 sample, purple dots represent 3.

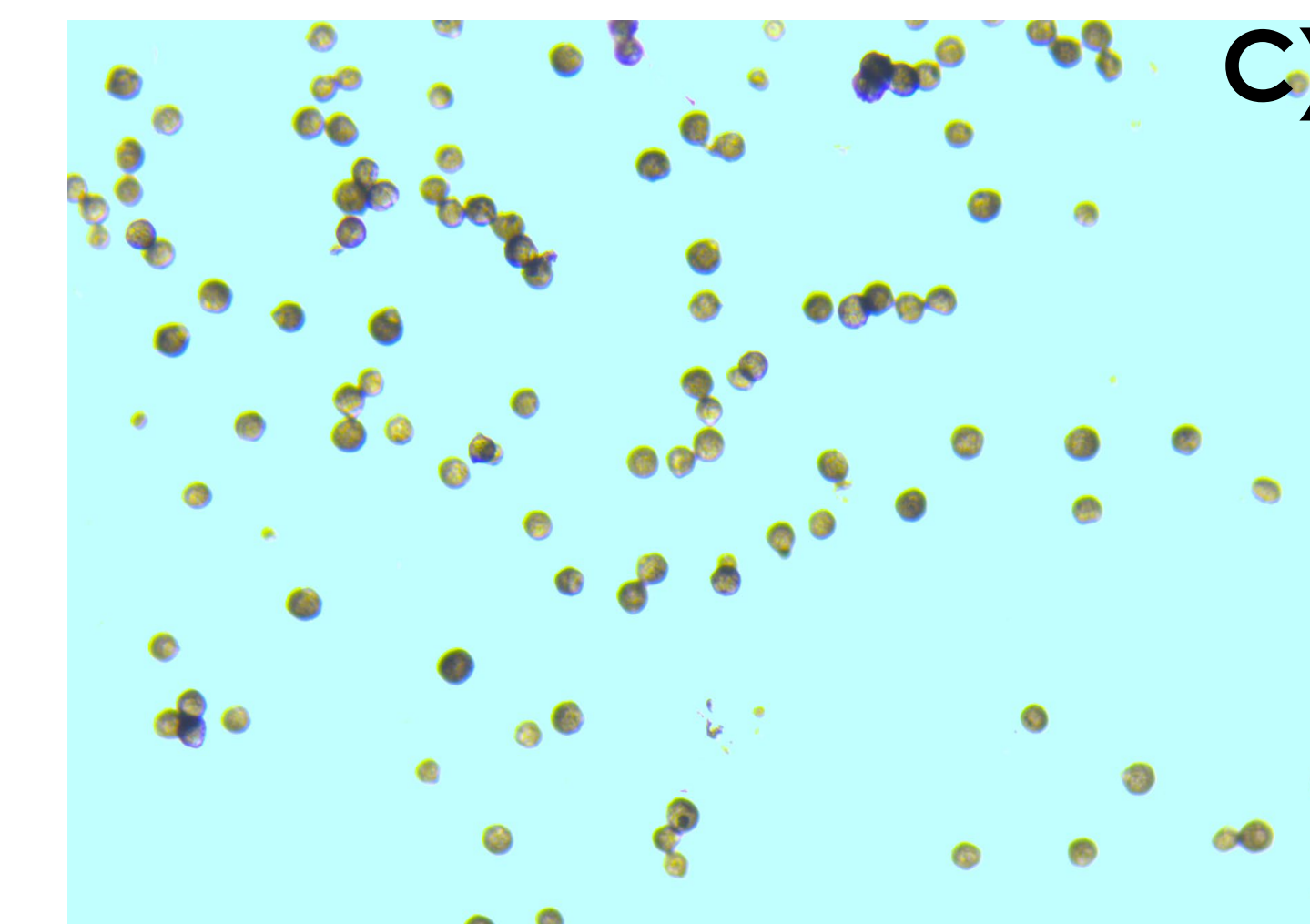
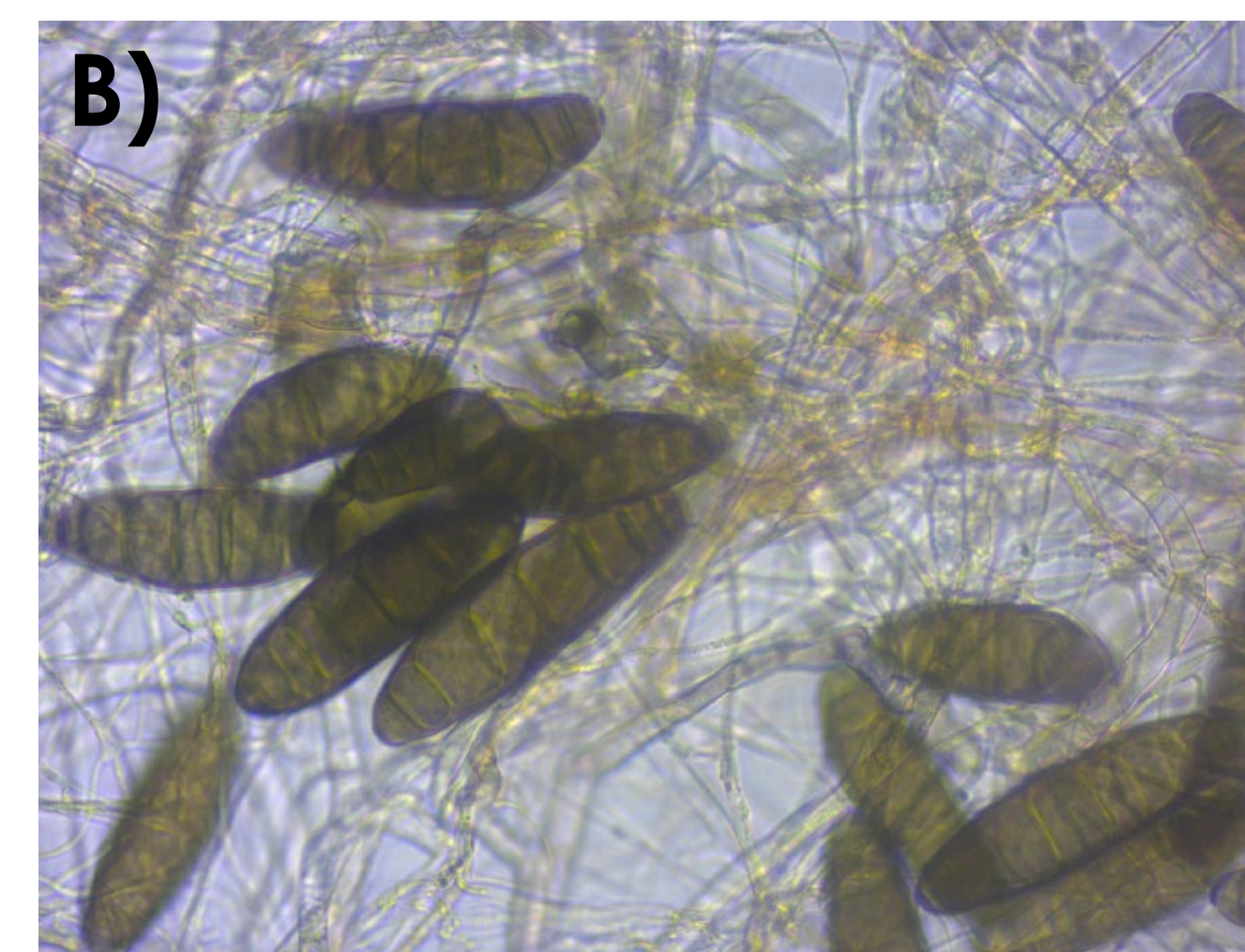


Fig 6: **A)** Conidium from sample 090 at 400X magnification
B) Conidia from sample 095 at 400X magnification
C) Conidia of sample 107 at 100X
PC: G. Webb, J.R. Noller 2023.

Table 1

	1	2	3	4	5	6
A	SYN 0.343	DNR 0.287	C11 0.408	PC 0.408	TLI1 1.142	C10 1.064
B	K02 0.255	Su55 0.219	S40 0.184	104 0.334	DNR 1.392	K12 0.96
C	K12 0.257	C10 0.249	KO1 0.195	KO1 0.584	303 1.493	K02 0.92
D	2111 0.332	B 0.227	TLI2 0.237	302 0.628	103 1.083	201 0.855
E	104 0.19	G17 0.24	TLI1 0.216	Su38 0.676	S40 0.993	Su55 0.919
F	103 0.273	Su38 0.306	302 0.205	C11 0.618	TLI2 0.637	B 0.642
G	202 0.353	304 0.336	PC 0.339	G17 0.663	301 0.511	SYN 0.779
H	201 0.4	303 0.261	301 0.339	2111 0.48	304 0.524	202 0.439

Table 1: Left, DAS-ELISA results. Highlighted cells represent samples that registered as positive.

Table 2: Right, Sample ID, origin, and suspected fungal pathogen based on morphological and DNA results

Table 2

ID#	Sample Origin	Grower/Civic Science	Morphological ID	ITS Region ID	Agreement
088	MO	TLI	<i>Bipolaris</i> spp.	<i>Bipolaris</i> spp.	Yes
089	MO	TLI	<i>Bipolaris</i> spp.	N/A	N/A
090	MO	TLI	<i>Fusarium</i> spp.	<i>Fusarium</i> spp.	Yes
093	KS	TLI	<i>Bipolaris</i> spp.	<i>Bipolaris</i> spp.	Yes
094	IA	Civic Science	<i>Bipolaris</i> spp.	<i>Alternaria</i> spp.	No
095	KS	Civic Science	<i>Bipolaris</i> spp.	<i>Bipolaris</i> spp.	Yes
096	KS	TLI	Unknown	<i>Didymella</i> spp.	N/A
101	KS	TLI	<i>Bipolaris</i> spp.	<i>Bipolaris</i> spp.	Yes
105	ME	Civic Science	<i>Fusarium</i> spp.	<i>Fusarium</i> spp.	Yes
107	SD	Civic Science	<i>Epicoccum</i> spp.	<i>Epicoccum</i> spp.	Yes
111	CO	Civic Science	<i>Epicoccum</i> spp.	<i>Epicoccum</i> spp.	Yes

Summary and Future Work

- Current commercially available DAS-ELISA tests for WSMV have the potential to identify infection from IWG samples, but levels are low.
- High recovery of *Bipolaris* spp. indicates the possibility of pathogenicity on IWG.
- Future studies using Koch's postulates with isolates should be done to demonstrate pathogenicity.
- Some samples recovered *Fusarium* spp., indicating the possibility of FHB on IWG
- Direct PCR (Jeon et al., 2023), used here to shorten the time between culturing and DNA analysis, seems to be an effective solution.

Acknowledgements



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References

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- <https://www.honestlymodern.com/kernza/>
- Jeon, H., Kim, J., Yang, J., Son, H., and Min, K. 2023. Application of direct PCR for phylogenetic analysis of *Fusarium fujikuroi* species complex isolated from rice seeds. *Front. Plant Sci.* 13:1093688.