

Diagnostic Testing for Kansas Wheat Viruses

Landon Trout¹, Chandler Day², Carla Redila³, John Fellers³, Judy O'Mara²

¹Agronomy Department Kansas State University, ²Plant Pathology Department Kansas State University, ³USDA-ARS Manhattan Kansas



Introduction:

- Wheat streak mosaic virus (WSMV), High Plains wheat mosaic virus (HPWMoV), and *Triticum* mosaic virus (TriMV) often infect wheat together as a complex.
- Since HPWMoV, and TriMV often infect wheat plants at lower titers, less sensitive tests may not detect infection.
- Enzyme linked immunosorbent assay (ELISA) tests are more cost effective and less labor intensive than reverse transcriptase polymerase chain reaction (RT PCR) tests.
- This study investigated the sensitivity of ELISA tests compared to RT PCR based tests and determined the regional presence of viruses in infected wheat samples.



Fig. 1 A). Single Infection of TriMV on the left and WSMV on the right
B). Sample collection at Rocky Ford research center

Methods:

- 63 total wheat leaf samples were taken from 9 varieties and 4 counties in Kansas. Upper leaves from the plant at milk/soft-dough stage.
- Double antibody sandwich ELISA tests (Agdia) were performed according to protocol. Samples were tested for WSMV, TriMV, HPWMoV, plus other viruses including Soil borne wheat mosaic virus and Barley yellow dwarf virus. (**Fig 2.**)
- ELISA positive samples were further tested using RT PCR. First strand cDNA was synthesized using Superscript IV (ThermoFisher).
- Primers specific for WSMV CP, TriMV CP, and HPWMoV RNA1. Positive controls were verified for virus presence by sequencing.
- PCR amplicons were separated using 1% agarose-1XTAE gel electrophoresis.

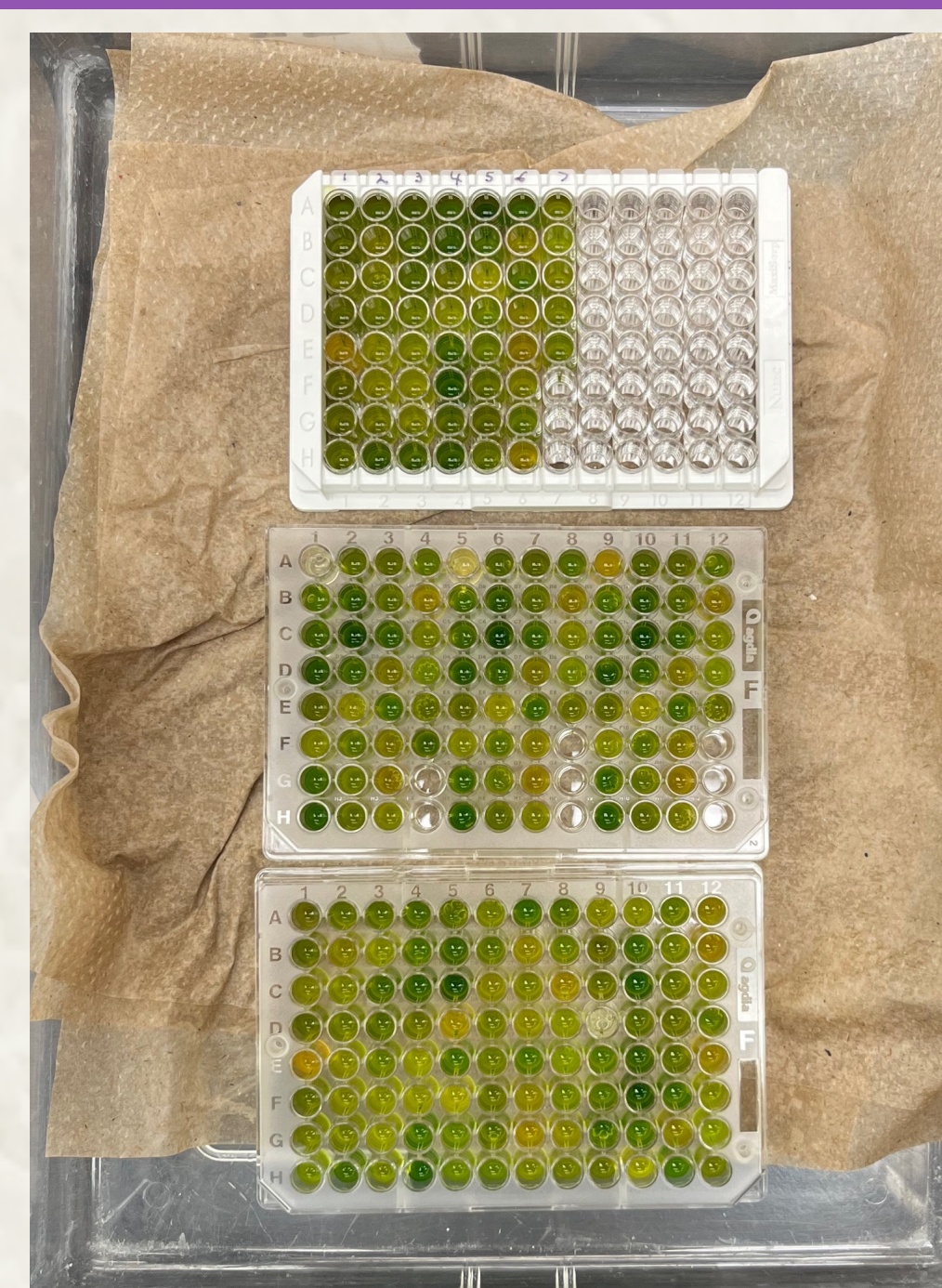


Fig. 2

Fig. 3 ELISA results for WSMV; yellow indicates positive for infection

R.6	1	2	3	4	5	6	7	8
A	WS + 3.628	9 0.218	22 3.089	36 OVRFLW	46 0.239	68 0.234	87 0.217	
B	WS - 0.176	10 0.213	25 0.201	37 0.239	48 3.561	70 OVRFLW	88 OVRFLW	
C	2 OVRFLW	11 0.254	26 2.828	38 0.327	49 0.244	74 0.283	95 3.213	
D	3 0.224	14 0.262	27 3.875	39 0.225	52 0.225	76 0.346	91 0.187	
E	5 0.206	16 OVRFLW	28 3.566	42 0.339	58 0.197	77 0.346	84 0.19	
F	6 1.835	18 OVRFLW	31 OVRFLW	43 0.312	63 0.23	78 0.276		
G	7 0.221	19 0.222	33 3.873	44 3.971	65 0.231	84 0.246		
H	8 0.2	20 1.391	34 0.21	45 0.213	67 0.223	86 0.216		

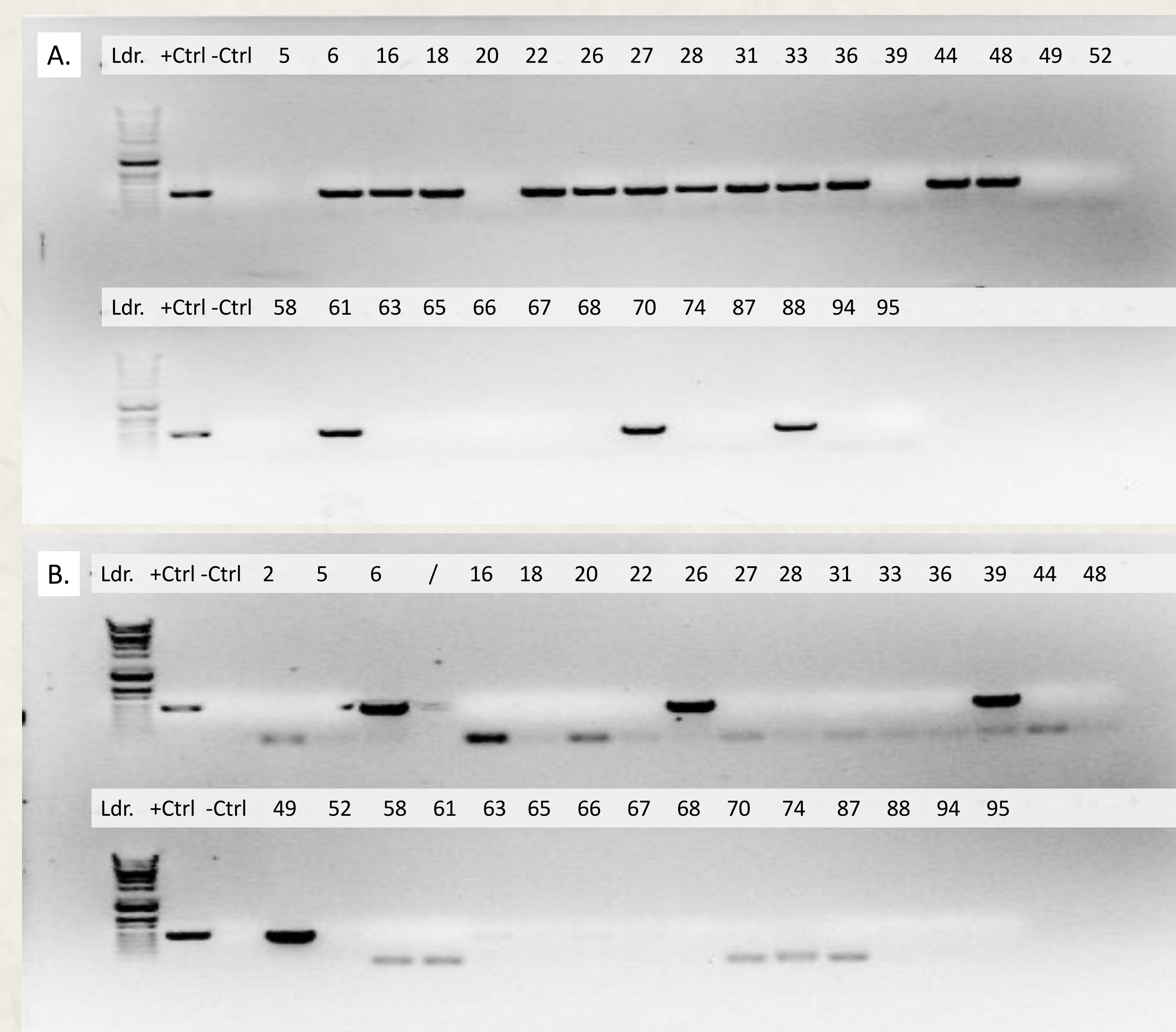


Fig. 4 Agarose Gel (1%) electrophoresis of PCR verification of virus infected plants. **A).** WSMV CP **B).** TriMV CP

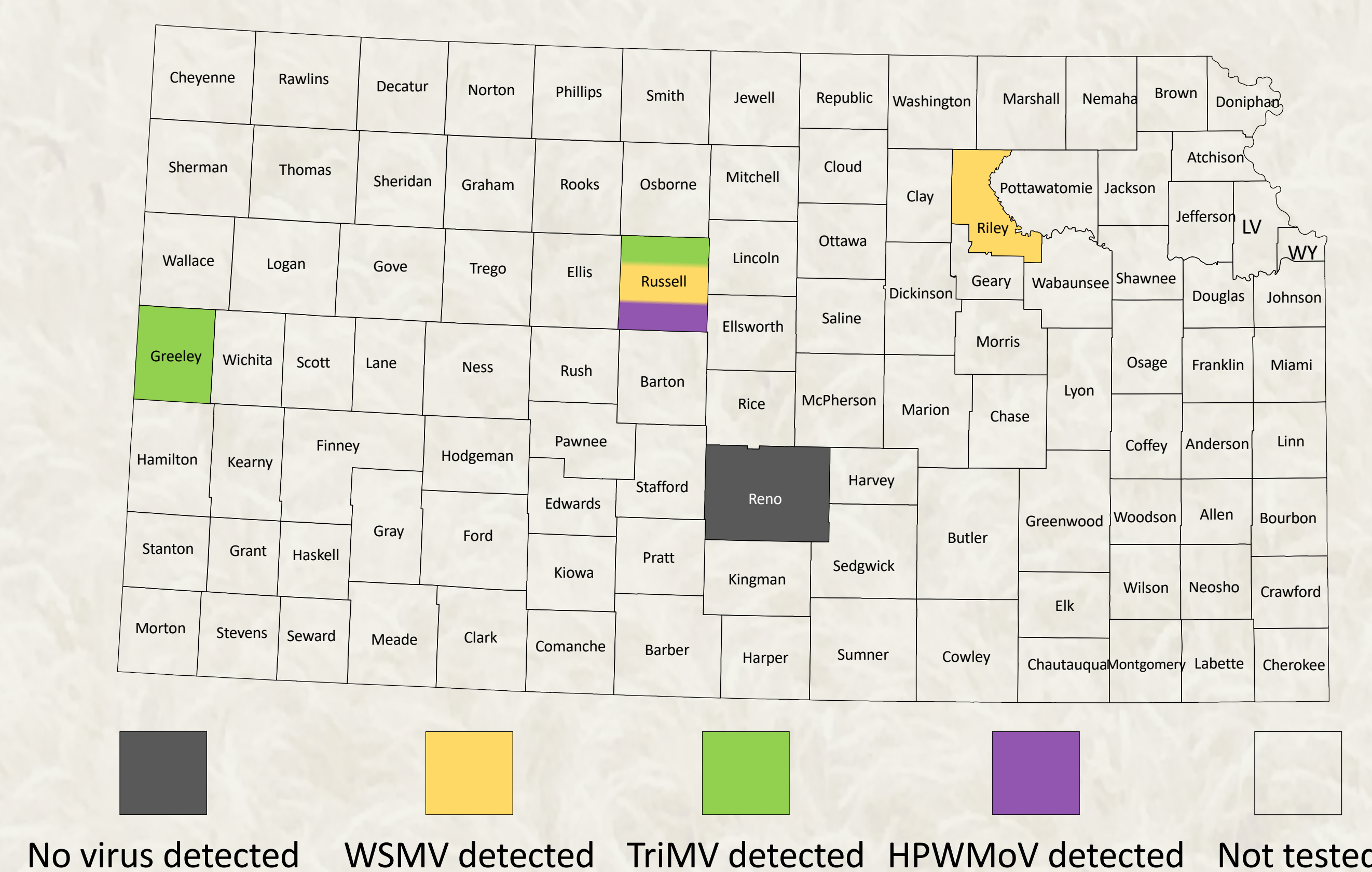


Fig. 5 Kansas state map showing counties where samples were taken. Colors indicate what viruses were detected.

Results:

- Out of 63 total samples, 32 tested negative for any virus.
- 80.6% of ELISA positive results matched with RT PCR positive results, including two false positives and four false negatives.
- Two samples indicated a single TriMV infection with no detectable WSMV, both samples came from Greeley county, one sample was Hamilton wheat and the other was Dallas wheat.
- Three samples indicated a double infection with TriMV and WSMV, all three samples were Joe wheat.
- One sample indicated a double infection with WSMV and HPWMoV via ELISA however, this was refuted by PCR testing. PCR indicated a different sample had a WSMV-HPWMoV double infection that ELISA did not detect.

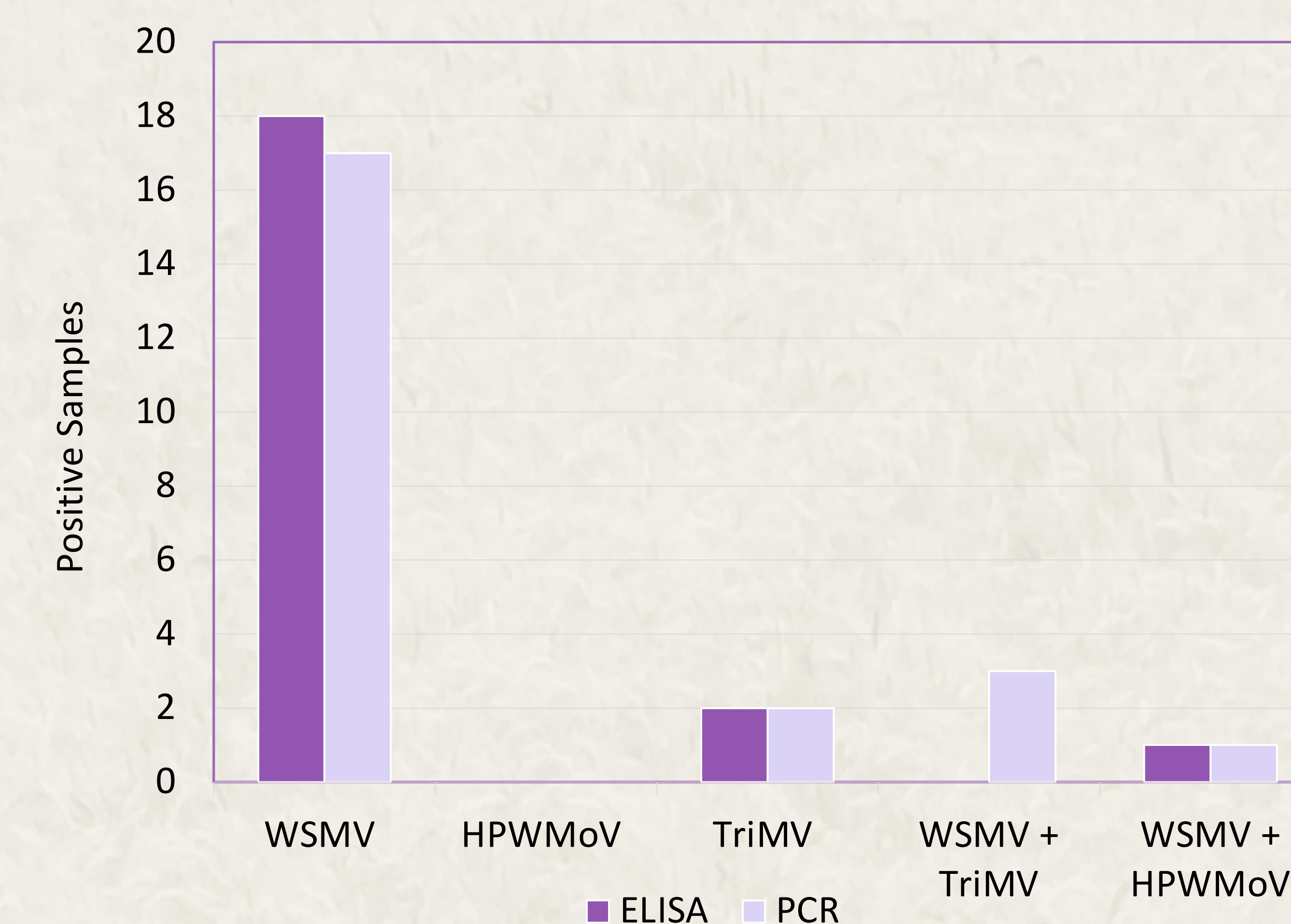


Fig. 6 Number of samples that tested positive for each virus as a single infection and as a multiple infection. *note the WSMV + HPWMoV positive was from two different samples in ELISA and PCR*

Discussion:

Five of the six inconsistencies between testing methods occurred on plants with two viruses detected via RT PCR. This suggests ELISA based testing is more likely to give false results when testing for TriMV and HPWMoV as a double infection with WSMV. This suggests that ELISA testing is less sensitive than RT PCR at detecting virus in plant tissue with coinfections, possibly due to lower virus titers. When considering only single infections, ELISA and RT PCR positive results match except for one WSMV false positive. This was most likely due to cross contamination within the ELISA plate during the wash step.

The only detected WSMV-TriMV double infections were found in 3 samples of Joe wheat in Russell county. Joe is known to have the resistance gene *Wsm2*. The only detected TriMV single infections were found in samples of Dallas and Hamilton wheat from Greeley county. Both Dallas and Hamilton carry close molecular markers to *Wsm2*. This suggests a possible link between the *Wsm2* gene and an increase in TriMV titer.