

Introduction

- Hessian flies have caused a large amount of damage to Kansas winter wheat for over 130 years. (R. Jeff Whitworth et. al. 2009)
- Once wheat is parasitized the larva move from the leaves to the collar of the wheat stock where feeding occurs. (Dr. Ming Chen 2017)
- The feeding of the larva involves the injection of saliva. This saliva is used to fundamentally change the genetics of the wheat to benefit the larva.
- When infected the wheat increases sugar production to feed the larva.
- Not only is the function of the wheat changed but also its form. The saliva also causes malformed cell growth with perforated cell membranes. This allows for easier transfers of sugars to the feeding larva. (Dr. Ming Chen 2017)
- The end effect of the parasitism is the weakening and death of the wheat plant before the production of wheat grain can occur. (R. Jeff Whitworth et. al. 2009)

Objectives

- To identify varieties of wheat that are both resistant and susceptible to Hessian Fly parasitism through the infection of these wheat varieties.
- To analyze wheat tissue samples from two representative wheat varieties, and to compare the proteins from both resistant and susceptible wheat plants to determine the effect of the hessian fly parasitism on protein synthesis

Methods

- Six varieties of wheat ("Molly H13", "Magnum H5", "Caldwell H6", "Iris H9", "Karl 92", "Hamlet 09") were planted in a sterilized medium of vermiculite, sand, and field soil.
- These varieties once allowed to grow were infested with Hessian Fly (*Mayetiola destructor*) adults
- The Hessian Fly (*Mayetiola destructor*) larva were allowed to infect the wheat until they reached the pupal stage in the susceptible wheat. At this time the two representative varieties were chosen ("Molly H13", and "Karl 92")
- These varieties were then processed by removing the pupa and taking tissue samples in the feeding and non-feeding sites. These samples were frozen at -29C
- At a later date, the samples were crushed in the presence of a buffer to create a protein concentrate. The concentrate was vibrated, centrifuged, and then tested with a nanospectrophotometer to determine the concentration of the protein.
- The samples were then centrifuge filtered to clean them for HPLC
- once the sample volumes were equalized (25µl), they were submitted for HPLC (high-pressure liquid chromatography) so that a comparison of the protein content of the samples could be made.

Figures

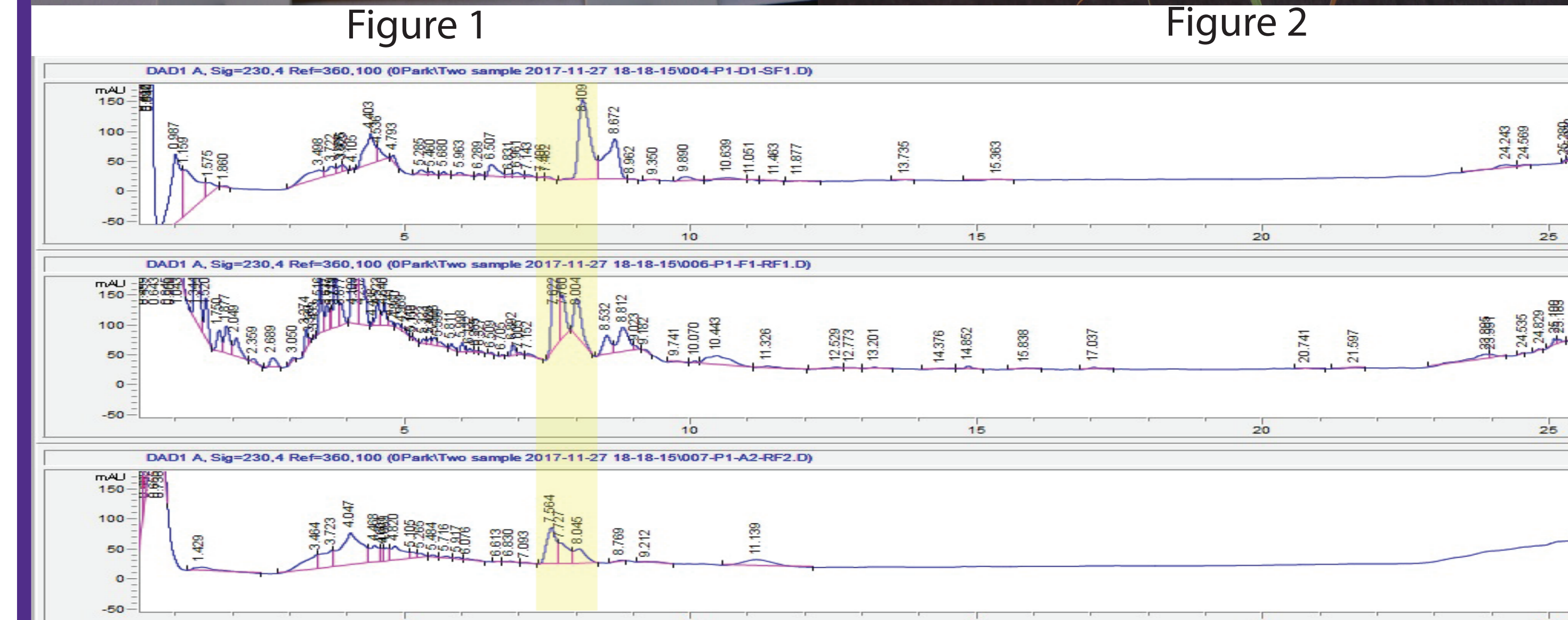


Figure 3 (above), Comparison of protein extract from the feeding sites on both susceptible and resistant wheat

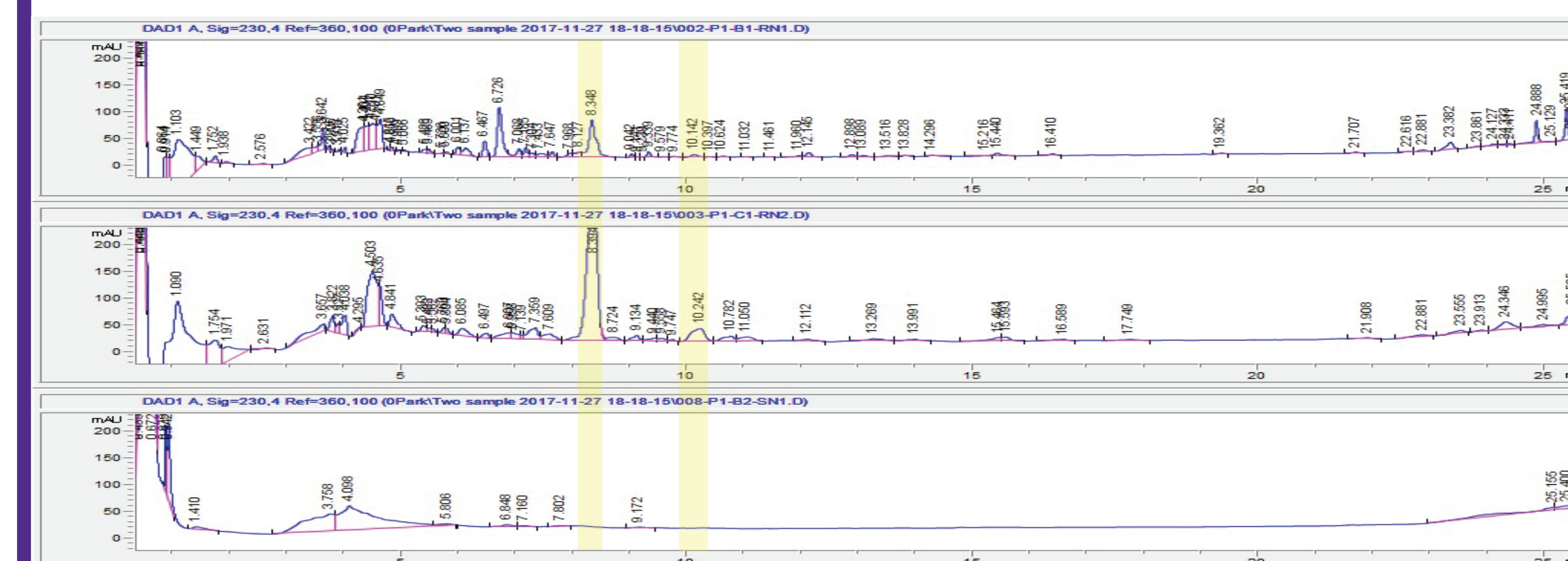


Figure 4 (above), Comparison of protein extract from the non-feeding sites on susceptible and resistant wheat

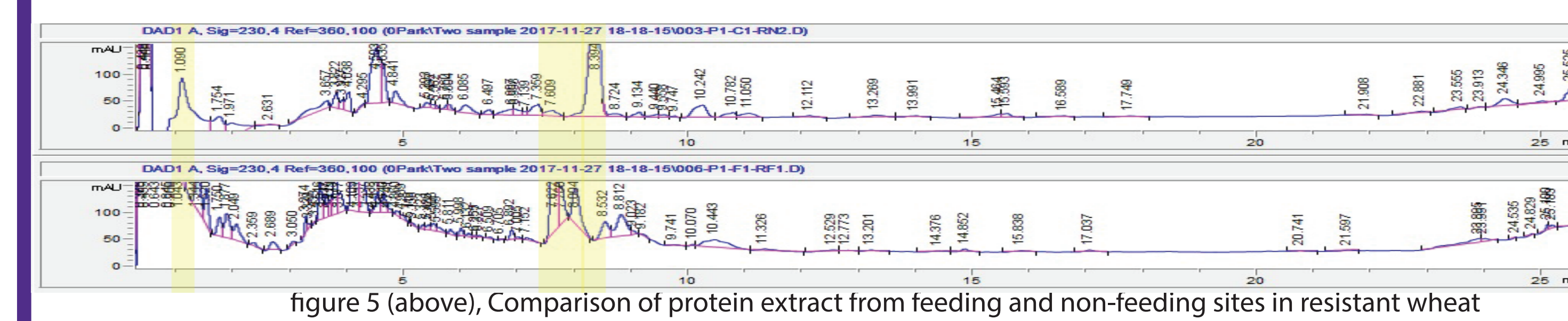


figure 5 (above), Comparison of protein extract from feeding and non-feeding sites in resistant wheat

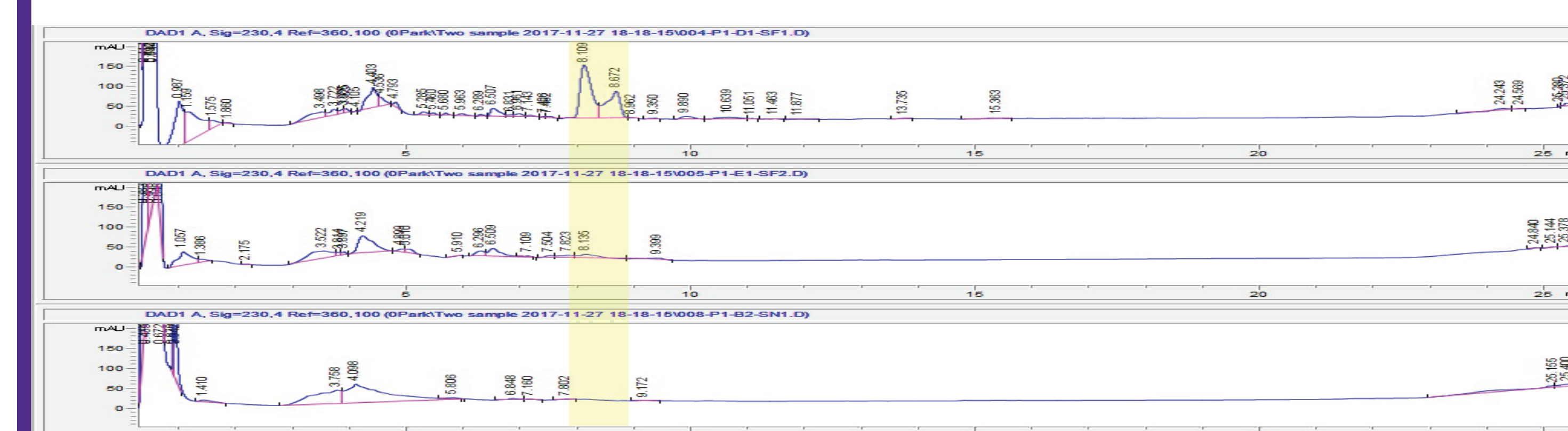


figure 6 (above), Comparison of protein extract from the feeding and non-feeding sites in susceptible wheat

Discussion

- Overall we have proven the fact that the susceptible infected wheat has undergone a genetic change due to the parasitism of the Hessian Fly (*Mayetiola destructor*). This genetic change leads to different proteins being encoded (figure 3 and 4) which causes the effects seen in figure 1 and 2, namely greener, weaker, smaller plants.
- It can also be seen in figure 5 and 6 that the proteins expressed in these regions differ in both the susceptible and in the resistant wheat samples. It cannot however be stated surely whether or not these discrepancies are caused by the feeding or attempted feeding of the Hessian Fly (*Mayetiola destructor*), due to the fact that in the sampling process we neglected to properly sample the un-infested control group.
- It can also be seen that the third chart on figure four is seemingly devoid of abundant peaks seen in the other samples. We theorize that this is caused by either the low concentration of the sample due to the volume equilization (likely), or in machine error (un-likely)
- It can also be seen that SN2 cannot be found in this experiment. This is due to an error in the HPLC that could not be reversed.
- Overall this experiment shows the importance of the choice of wheat variety in a hessian fly prone environment. With the correct variety the Hessian Fly larva will not develop past the first instar which leads to pest death with no functional or morphological changes to the wheat due to parasitism. (figure 7)

figure 7

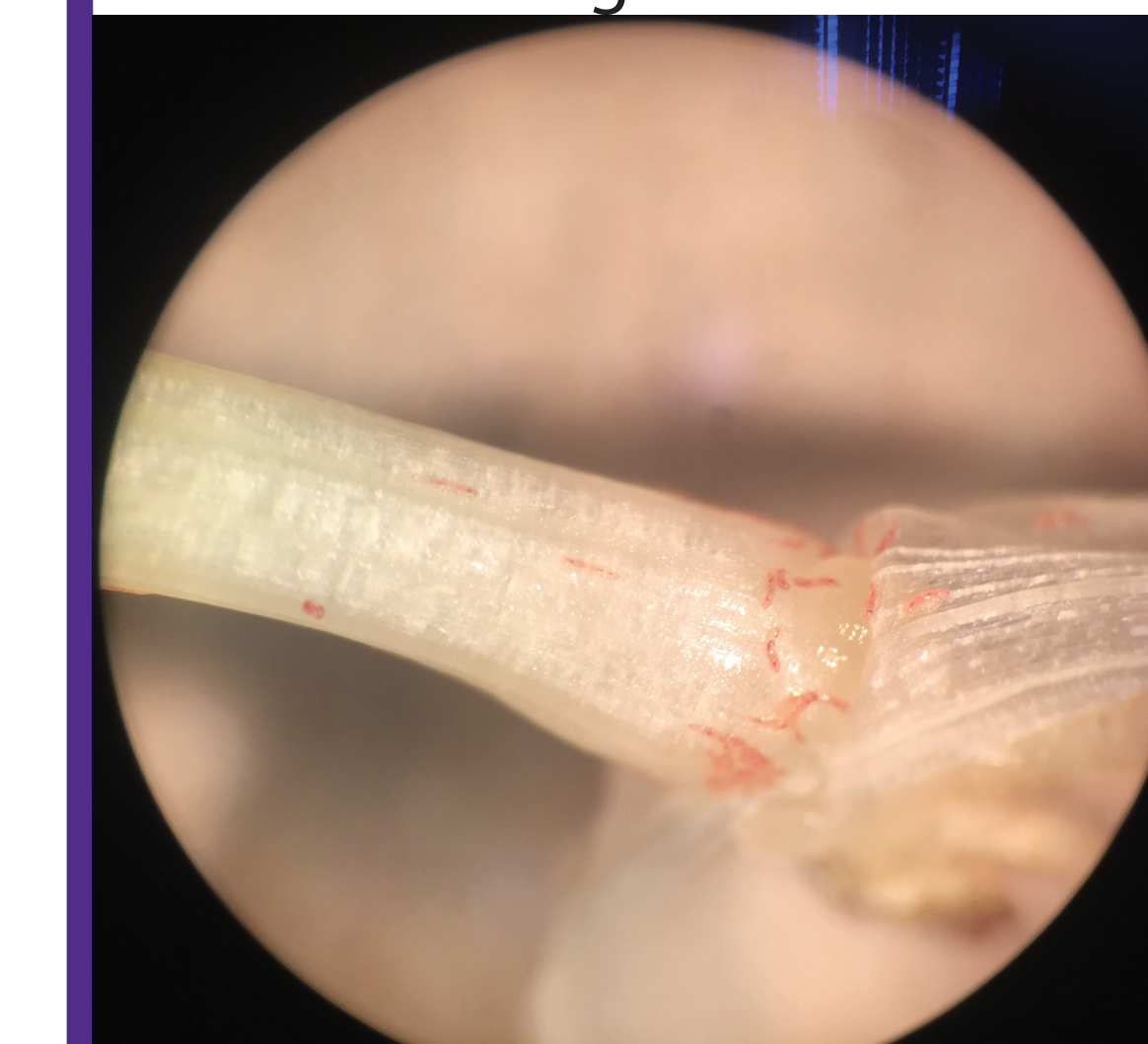


figure 8



Future Directions

- If this experiment were to be continued I would add and utilize the control. This would allow a determination to be made as to whether the discrepancies between proteins in the feeding and non-feeding sites are caused by the Hessian Fly larva, or if they are naturally occurring.
- I would also like to analyze the identity of the proteins and how they are used to cripple and kill the Hessian Fly larva. We could not do this in this experiment because we do not have the machine and it costs \$180,000
- If we knew the proteins, we could identify the genetics needed to produce these proteins. If we knew the genetics needed, we could bioengineer wheat with these genetics that also has superior grain quality and production.

References

- R.Jeff Whitworth, Phil Sloderbeck, Holly Davis, Gary Cramer (2009). Kansas Crop Pests: Hessian Fly, KSU Agricultural Experiment Station and Extension Services.
- Ming Chen PhD. (2017) Personal interview