Potato psyllid vector density and zebra chip disease
Arash Rashed, Christopher M. Wallis, Anna K. Wallingford, Fekede Workneh, Li Paetzold & Charles M. Rush
arashed@uidaho.edu
Department of Plant, Soil, and Entomological Sciences, University Idaho, Aberdeen R & E Center

Introduction
Zebra chip disease (ZC) is associated with ‘Candidatus Librribacter solanacearum’ (Lso), and is transmitted by the potato psyllid Bactericera cockerelli Sulc (Hemiptera: Triozidae). Previous studies have shown that ZC is also associated with shifts in some phenolics, reducing sugars, and amino acids (e.g. Wallis et al 2013-phytopathology). Another study has shown that infection level is influenced by the number of vectors feeding on the plant tissue (Rashed et al. 2012-phytopathology). The relationship between vector number, symptom severity, Lso titer, and their associated physiological responses in different potato cultivars, however, is yet to be investigated. The present study is set to address this shortfall by quantifying 15 amino acids, 4 sugars, and 17 phenolic compounds following Lso infection in two different cultivars.

Methods

**Cultivars:**
• Red La Soda
• Russet Norkotah

**Infecive Psyllid Densities: 5, 15, and 30 per cage**

**Replication: 5 cages per cultivar**

**Cage:**
- Plant 2
- Plant 3
- Plant 1
- Plant 4

**Release point**

Figure 1: Plant arrangement within each experimental cage

**Symptom Scores**

![Symptom severity scores](image)

DNA Extraction and Quantification
- Slightly modified DNeasy® Plant Mini protocol
- Applied Biosystems 7500 Real-Time PCR System (taqman probe)
- Comparative Ct method (ΔΔCt): In relation to a calibrator and an endogenous 18s RNA control

Biochemical Analyses
- Phenolic compounds: Methanol extractions of tuber tissue injected and separated by a Shimadzu high-performance liquid chromatography system.
- Amino acids: PBS buffer extracts of tuber tissues analyzed by a commercially-available kit from Phenomenex, using a Shimadzu GC-FID gas chromatograph.
- Carbohydrates: PBS buffer extracts analyzed by a Shimadzu HPLC equipped with 300 mm x 7.8 mm Supelco C-611 carbohydrate column and a refractive index detector (RID-10 from Shimadzu).

Results

**i Lso quantity and symptom severity**

![Graph showing Lso quantity and symptom severity](image)

**ii Changes in amino acids**

![Graph showing changes in amino acids](image)

**iii Changes in sugars**

![Graph showing changes in sugars](image)

**iv Changes in phenolics**

Changes in 17 phenolic compounds were evaluated in Red La Soda and Russet Norkotah, following Lso infection with different insect densities. After Lso infection, the activity levels of all phenolic compounds increased in response to infection in both cultivars, with the exception from dichlorogenic acid 1 in the russet. There was a significant cultivar-by-density interaction (MANOVA; Pillai’s Trace, F= 2.20, P= 0.003), indicating that the response was cultivar dependent. In the red cultivar some phenolic activity levels were associated with vector numbers. These compounds were cryptochlorogenic acid, cryptochlorogenic acid 2, protocatechuc acid hexoside, dichlorogenic acid 2, flavanol glucoside (deriv 1 and 3), and methyl salicylate dimer.

Conclusions

- Lso titer in tuber tissue showed an increasing trend with the increase in vector density. This correlation was statistically significant in Russet Norkotah (Spearman Rho, \( r \) = 0.57, \( P = 0.03 \). Symptom severity was not statistically different among density treatments when only plant #1 was analyzed. Including all 4 plants within a cage resulted in a significant difference between 5-psyllid and 30 psyllid treatments.

- In both cultivars elevated phenolic levels were associated with Lso presence.

- Changes in some amino acids and carbohydrates were variety specific. This indicates that production quality following processing may also vary among clipping varieties. Further studies are needed to investigate variety-dependent responses to infection.

- In Several cases, our failure to detect statistical differences was due to limited sample sizes. This study has been repeated and results are currently being analyzed.

Acknowledgements

We thank J. Gray, P. Garrett, J. Arthur and undergraduate assistants in Dr. Rush’s laboratory for their help during this study.

Figure 2: Symptom severity scores

Figure 3: A) Mean Lso titer in Red La soda and Russet Norkotah cultivars infested with 5, 15 and 30 infective psyllids. B) Mean symptom severity scores in the red and russet cultivars infested with 5, 15, and 30 psyllids. A and B reflect data obtained from ‘plant 1’s’ only. C) Mean symptom severity among all 4 plants within cages. Error bars represent 95% CI. Statistical comparisons are based on ANOVA and post hoc Tukey’s.

Figure 4: Changes in amino acid levels in, A) Red La Soda, and B) Russet Norkotah. Valine, leucine, isoleucine, proline, and phenylalanine increased in both varieties following Lso infection. Glutamine, ornithine and tryptophan showed elevated levels in Russet Norkotah, but not in Red La Soda.

Figure 5: Variation in sugar concentrations in, A) Red La Soda, and B) Russet Norkotah. In both cultivars there was an increase in fructose concentrations in response to Lso infection (Red: F \( 3,16 \) = 6.97, \( P = 0.003 \); Russet: F \( 3,16 \) = 16.40, \( P <0.001 \). In the Russet there was also an increase in glucose concentration (\( F 3,16 = 5.90, P = 0.006 \). However, in Red La Soda variation in glucose levels was non-significant (\( P = 0.07 \)).

Figure 6: Changes in sugars in response to Lso infection in, A) Red La Soda, and B) Russet Norkotah.