

Phenotyping *Urochloa humidicola* genotypes against spittlebug *Aeneolamia varia* (Hemiptera: Cercopidae): An experimental comparison between digital image analysis and visual evaluation

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Introduction

- » Productivity of *Urochloa* pastures [syn. *Brachiaria* (Trin.) Griseb.; Soreng et al. 2015], mainly *U. decumbens* (Stapf) R.D. Webster and *U. humidicola* - *Uh* (Rendle) Morrone & Zuloaga, are limited by susceptibility to American spittlebugs complex [Hemiptera: Cercopidae].
- » Varietal resistance is the main strategy to management spittlebugs on pasture systems (Cardona and Mesa 2011).
- » To develop superior *Uh* cultivars with high resistance to spittlebugs complex a high throughput phenotyping methodology is essential.
- » Current assessments of genotype for spittlebug resistance are based on visual damage percentage (Parsa et al. 2011). This method has been successful, as shown by the high and fast genetic gain achieved by the *Urochloa* breeding program (Miles et al. 2006).
- » Digital image analysis (DIA), has been used to maximize the accuracy of data collection from quantitative traits like yield and resistance to biotic or abiotic stress (Negrão et al. 2017).

Objective

Optimize our phenotyping process in *Uh* to detect spittlebug resistance by comparing two evaluation methods under greenhouse conditions.

Materials and Methods

Plants: 24 genotypes from *Urochloa humidicola* were infested with spittlebug nymph (*Aeneolamia varia*).

Evaluation methods:

- [1] Visual evaluation: Visual damage percent (VDP; 0 to 100% of area affected).
- [2] Digital image analysis. Yellow area percentage (YAP) and Exceeds of greenness calculated using ImageJ.

Evaluation time: VDP and YAP were conducted at 0, 7, 14, 21, 28 and, 35 DAI and used to calculate respective area under disease progress curve *AUDPC1* and *AUDPC2*. $[=\sum_i^n [(X_i + X_{i-1})/2][t_i - t_{i-1}]]$

where X_i = VDS or YAP at the i th evaluation ($X_0 = 0$), t_i = time in days from infestation to the i th evaluation ($t_0 = 0$), and n = number of evaluations.

Statistical analysis:

- [1] Damage growth curve/genotype.
- [2] Lin's concordance correlation (CCC) calculated between *VDP* and *YAP* values using the *epi.ccc* function in the *epiR* package (Stevenson 2012).
- [3] Broad-sense heritability (H^2) or repeatability (Piepho and Möhring 2007) for *AUDPC1* and *AUDPC2* with AsReml 4.1 (Gilmour et al. 2015).
- [4] In order to corroborate interactions [genotypes, treatments (infested and uninfested plants) and evaluation method] a three-way ANOVA was made at 7, 14, 21, 28 and 35 days after infestation for damage percent variable.

Results

- 1) The genotypes evaluated exhibited different growth damage curves.
- 2) Lin's concordance correlation coefficient showed that digital image evaluation reproduces it with an accuracy of 0.81 (95% CI 0.79 – 0.82).

- 3) Interactions between genotype (*G*), evaluation method (*M*) and treatments (*T*; consisting on infested and no infested), showed statistically significant differences in *G-T* interaction at 21 ($F = 4.1$, $p < 0.001$), 28 ($F = 7.6$, $p < 0.001$) and 35 ($F = 7.7$, $p < 0.001$) DAI, meanwhile *G-M* and *G-T-M* were not significant, showing that both eval. methods provide similar information.
- 4) Heritability value from *AUDPC1* (visual evaluation) was 0.73, while from *AUDPC2* (DIA) was 0.76. This suggests that *AUDPC* calculated through DIA have more precision and accuracy.
- 5) ANOVA made only with infested plants data, showed statistically significant differences among genotypes for *AUDPC1* ($F = 3.8$, $p < 0.001$, $LSD_{\alpha = 0.05} = 120.8$) and *AUDPC2* ($F = 4.23$, $p < 0.001$, $LSD_{\alpha = 0.05} = 139.1$) (Fig 1).

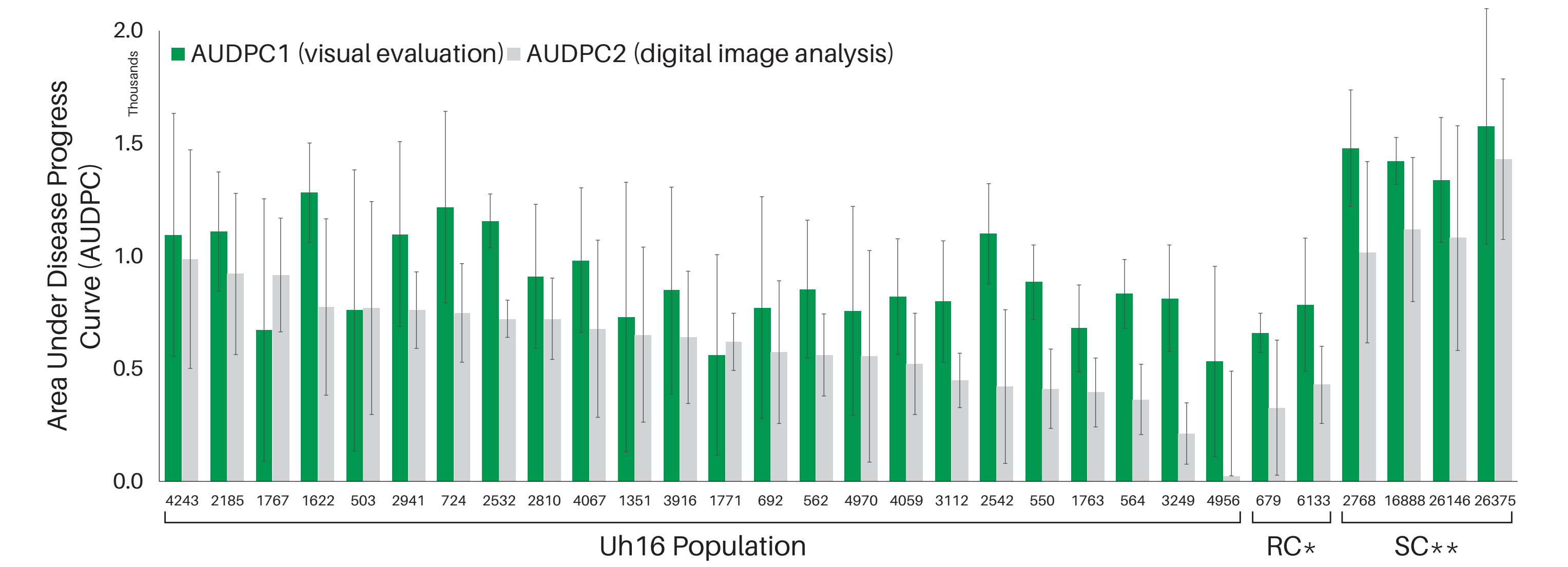


Figure 1: Area under disease progress curve value of *Uh16* genotypes evaluated against spittlebug nymph *Aeneolamia varia*. * Resistant checks. **Susceptible checks. *AUDPC* values correspond to the subtraction between *AUDPC* of infested plants and *AUDPC* of non-infested plants.

- 6) As a result of spittlebug herbivory, relative growth rate and excess of greenness decrease in all genotypes except in *Uh16/4956* (Fig 2). In this genotype, tolerance mechanism exhibited is by increase growth rate, in contrast, genotype *Uh16/1763* genotype, exhibited a second mechanism of tolerance named enhanced photo-synthesis.

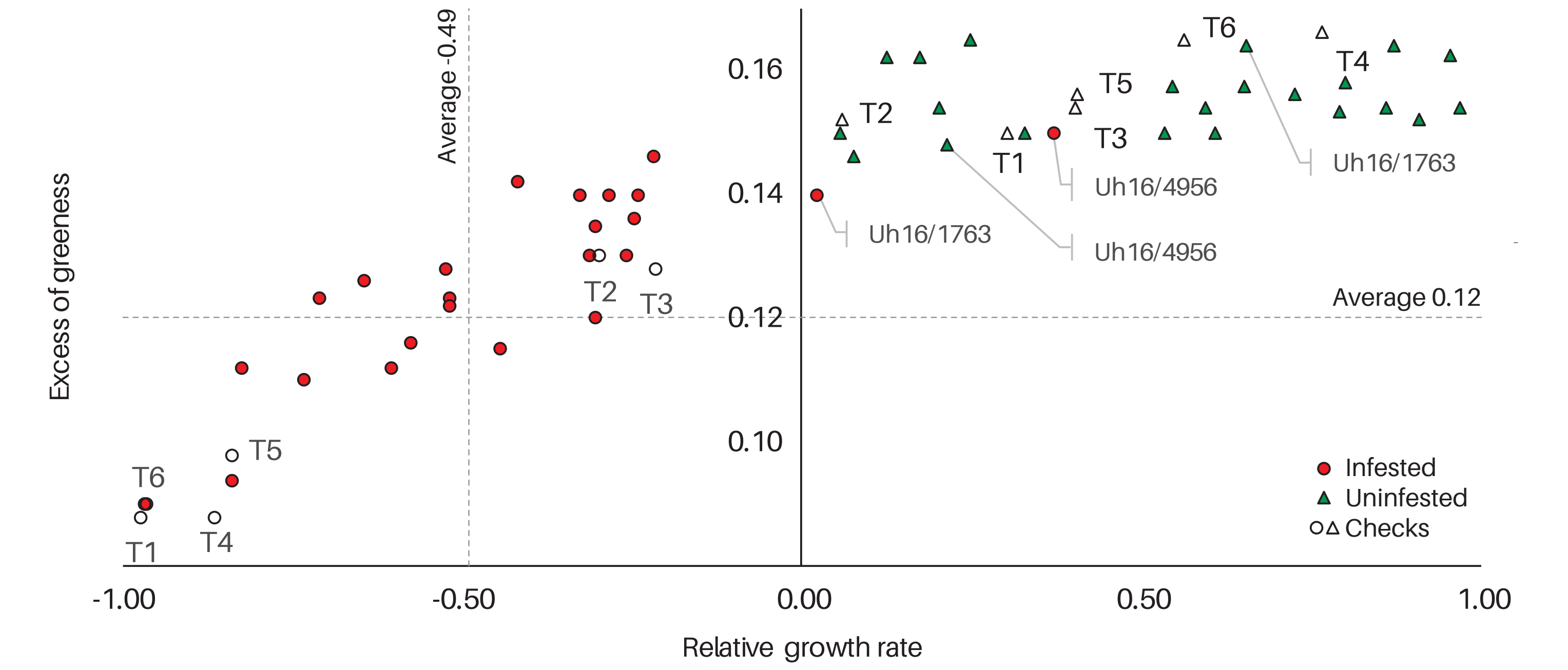


Figure 2: Relative growth rate (RGR) and Excess of green (EG) of 24 *Uh* genotypes (*Uh16* population) evaluated against *Aeneolamia varia* (nymph stage). [resistant: CIAT/679 (T2, cv. Humidicola), CIAT/6133 (T3, cv. Llanero) and susceptible: CIAT/16888 (T4), CIAT/26146 (T5), CIAT/26375 (T6), *Uh13/2768* (T1)].

Conclusion

Digital image analysis is an appropriated technique to detect spittlebug resistance in *Urochloa humidicola*. This methodology has significant advantages over visual evaluation, as: 1) high throughput technique, 2) high precision and accuracy, 3) image information is processed by a software which can work from autonomous form, 4) Statistically is similar to visual evaluation method, 5) multiple variables can be obtained in only one evaluation, 6) variables obtained have continuous values and, 7) low cost.

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