QUANTIFYING SOILBORNE PATHOGENS IN ALFALFA WITH FLUORESCENT POLYMERASE CHAIN REACTION ASSAYS

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The standard test for resistance in alfalfa to *Phytophthora medicaginis* specifies that plants be classified as either resistant or susceptible based on the visual assessment of disease severity. The standard test for resistance to *Aphanomyces euteiches* uses an integer scale for scoring disease severity. The use of discontinuous classes for rating resistance limits the ability to discriminate among highly resistant plants. An alternative method for selecting the most resistant plants would be to directly quantify the amount of pathogen present in infected tissue. We have developed assays to quantify *A. euteiches* and *P. medicaginis* in infected alfalfa roots based on the detection of fluorescent-labeled amplicons that are pathogen-specific. The assays are very precise, with the correlation between detectable fluorescence and pathogen DNA quantity typically exceeding 0.98. In the case of *A. euteiches*, the assay has been used to examine the relationship between pathogen DNA quantity and disease severity in both individual plants and bulked plant samples. In all cases, resistant and susceptible check populations could be discriminated based on the amount of pathogen DNA detected in roots, and a positive and significant correlation was observed between pathogen DNA quantity and disease severity index (DSI) ratings (Table 1). Analysis of individual plants has also demonstrated positive and significant correlations between pathogen DNA content and DSI. The assay for *P. medicaginis* successfully discriminates between resistant and susceptible check populations. A significant negative correlation has been observed between the number of resistant plants in a bulk sample and the amount of *P. medicaginis* DNA detected. Multiplexing assays for both pathogens will allow for accurate identification of plants with dual resistance and also provide a means of studying pathogen population dynamics in mixed infections.

Table 1. Means comparisons between bulked samples of check populations[a] for quantity of pathogen DNA[b] and disease severity index (DSI)[c] ratings

	A. euteiches MW5-43 (Race 1)		*A. euteiches* WI-98 (Race 2)	
	ng DNA	DSI	ng DNA	DSI
Saranac	8.03 a	3.81 a	11.79 a	3.99 a
WAPH-1	1.62 b	2.59 b	9.81 b	4.0 a
WAPH-5	---	---	2.62 c	3.0 b
LSD (_ = 0.05)	1.65	0.37	1.83	0.19
_ (Prob > |_|)[d]	0.86 (<0.0001)		0.74 (<0.0001)	

[a] Saranac = susceptible to race 1 and race 2; WAPH-1 = resistant to race 1 and susceptible to race 2, and WAPH-5 = resistant to race 2.
[b] Each experiment included four bulks of 15 plants each for each population. DNA was extracted from roots of each entire bulk sample. Each bulk sample was tested with three PCR reactions using 100 ng DNA/rxn.
[c] DSI ratings: 1 = healthy – 4 = extensive necrosis and severe stunting.
[d] Spearman rank correlation (_)between pathogen DNA quantity and DSI.

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