Personal Information and Commercial Confidential Information [CCI] redacted

STUDY TITLE

2016 Field Efficacy of Potato Event Z6 against *Phytophthora infestans* (Late Blight)

AUTHORS

[Personal information redacted]

REPORT DATE

04 November 2019

STUDY NUMBERS

[CCI]
[CCI]

QUALITY CONTROL STATEMENT

This report was reviewed to assure that it accurately reflects the raw data of this study. The raw data were audited for compliance with the protocol, study notebook, and Standard Operating Procedures where applicable.

Signed	11/4/2019
[Personal information redacted]	Date
Quality Control Reviewer	

CERTIFICATION PAGE

We, the undersigned, declare that, to the best of our knowledge, this report provides an accurate evaluation of data in this study.

Signed

[Personal information redacted]	11-04-2019
Author	Date

Signed

[Personal information redacted]	11-4-2019
Author	Date

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SUMMARY

Objective: To evaluate the field efficacy of potato event Z6 against foliar late blight (*Phytophthora infestans)*.

Methods: Z6 and Snowden were grown at two sites in the United States during the year 2016. The experimental unit was the plot, consisting of five to ten plants in a row, with five replicates. Each plot was bordered on either side by at least one spreader row of a conventional variety susceptible to late blight. When the potato plants reached row closure, spreader rows were inoculated with the *P. infestans* strain US-23.

The percentage of foliar area affected in each plot was estimated. Plants were evaluated approximately once per week from inoculation until either plants senesced or reached 100% foliar infection. Foliage affected over time was graphed, and the area under the disease progress curve (AUDPC) was calculated using a trapezoidal function. The resulting AUDPC values are dimensionless and are a relative measurement of the cumulative late blight infection over a period of time. The AUDPC was statistically analyzed using a linear mixed model, and differences between means were considered statistically significant when p-value ≤ 0.05. Lower AUDPC values mean less foliar damage by late blight.

Results: Z6 showed a significantly lower AUDPC when compared to Snowden. The AUDPC for Z6 had a mean and standard deviation of zero. These results reflect consistently negligible development of late blight symptoms in the foliage of Z6.

Conclusion: The results demonstrated that the foliar late blight protection trait in Z6 is effective against *P. infestans* strain US-23.

INTRODUCTION

The J.R. Simplot Company has developed the potato event Z6. This event was developed using *Agrobacterium*-mediated transformation. The potato variety Snowden was transformed with pSIM1278 to generate event V11. V11 was transformed with pSIM1678 to generate Z6. Z6 contains the late blight resistance gene, *Rpi-vnt1*. This gene in Z6 confers foliar protection against certain strains of late blight. Details on the plasmids, the down regulated or expressed genes, and the intended traits are shown below (Table 1).

Table 1. Plasmids, Genes, and Traits

Plasmid	Gene	Intended Trait	Method
pSIM1278	*Asn1*	Reduces asparagine (contributes to low acrylamide potential)	RNAi
	Ppo5	Reduces black spot	RNAi
	R1	Lowers reducing sugars (contributes to low acrylamide potential)	RNAi
	PhL	Lowers reducing sugars (contributes to low acrylamide potential)	RNAi
pSIM1678	*Vlnv*	Lowers reducing sugars (contributes to low acrylamide potential)	RNAi
	Rpi-vnt1	Late blight resistance	Protein expression

STUDY OBJECTIVES

The purpose of this study was to evaluate the field efficacy of Z6 against foliar late blight (*Phytophthora infestans*) compared to Snowden.

STUDY DATES

Experimental start date: May, 2016
Experimental end date: September, 2016

KEY STUDY PERSONNEL

[Personal information redacted]

MATERIALS AND METHODS

Test and Control Materials

Z6 containing the *Rpi-vnt1* gene was evaluated for field efficacy of the late blight protection trait, compared to the non-transformed parental variety, Snowden.

Additional conventional Atlantic potatoes were provided by the Principal Investigators at each site and planted as spreader rows between plots. These rows were part of the field plot design but were not scored. In the spreader rows, plants were inoculated with *P. infestans* to allow the disease to spread uniformly throughout the field. Atlantic is susceptible to late blight and thus an appropriate variety for this purpose.

Appropriate handling procedures were used to prevent inadvertent mixing of the study materials. These steps included securely packaging the materials in appropriate containers and maintaining chain of custody records for the materials.

Study Sites

Test and control plants were grown at the two locations detailed in Table 2.

Table 2. Study Sites

Site State	Site County	Trial Design[1]	Planting Date	Inoculation Date	Inoculum Strain
MI	Ionia	RCB, 5 reps	06/13/2016	07/28/2016	US-23
PA	Centre	RCB, 5 reps	06/09/2016	08/15/2016	US-23

[1]RCB=Randomized Complete Block design (number of blocks was equal to the number of reps)

Experimental Design

Each plot consisted of five to ten plants in one row approximately 5 to 10 ft long, respectively, with a spacing between plants of approximately 12 in. Rows were spaced approximately 36 in apart. Each plot was bordered on either side by at least one row of Atlantic, a conventional variety susceptible to late blight. These rows were designated as spreader rows and were not scored. Each plot was identified through the use of a stake labeled with the plot number assigned in the plot randomization.

Plot Management during the Growing Season

Pest control (with the exception of fungicide applications), maintenance practices, and irrigation were consistent with regional agronomic practices for potato production. The entire trial at each site was treated with the same agronomic inputs, pesticide, and fertilizer applications to ensure uniformity from pre-season through harvest. Any fungicide treatment used did not affect *P. infestans* infection and was discussed with and approved by the Study Coordinator before application.

Plot Inoculation

Spreader rows were inoculated with *P. infestans* after row closure with zoospores at a concentration of approximately 2.1×10^5 sporangia/ml. The inoculum was prepared by the Principal Investigator. After inoculation, plots were irrigated regularly to induce a humid environment and facilitate spread of disease.

Foliar Infection by *Phytophthora infestans*

Foliar evaluations were carried out by scientists with experience in evaluating late blight infection in the field. Foliar infection of plots were determined by estimating the percentage of foliar area affected. Plants were evaluated approximately once per week from inoculation until either plants senesced or reached 100% foliar infection. Foliage affected over time was graphed, and the AUDPC (shown below in the yellow area of Figure 1) was calculated. The AUDPC is a commonly used method by plant pathologists to represent this type of data and was calculated using the trapezoidal method described in Madden et al., 2007. The resulting AUDPC values are unitless numbers and are relative measurements of late blight infection over the course of the growing season. The AUDPC was subjected to statistical analysis to determine the effect of the late blight resistance gene (R-gene) on foliar late blight protection in the test event.

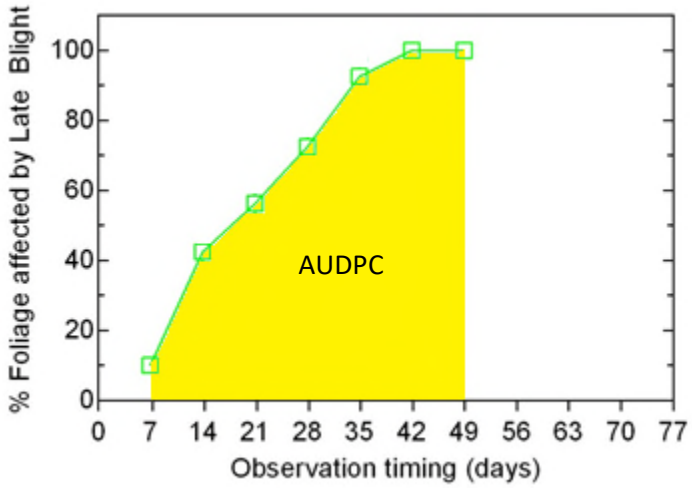

Figure 1. Example of Area under Disease Progress Curve (AUDPC)

Statistical Methods

The statistical analysis was performed by Simplot using JMP 12.2 (SAS Institute, Cary NC) . Data were analyzed using the following linear mixed model:

$Y_{ijkl} = \alpha_i + \beta_j + \gamma_{k(j)} + (\alpha\ \beta)_l + \varepsilon_{ijkl}$

α = mean of treatment (fixed)
β = effect of site (random)
γ = rep[site] (random)
ε = residual random error

Where α_i denotes the mean of the i^{th} treatment (fixed effect), β_j denotes the effect of the j^{th} site (random effect), $\gamma_{k(j)}\gamma_{k(i)}$ is the random rep effect (within site), $(\alpha\ \beta)_{ik}$ denotes the interaction between the i^{th} treatment and random k^{th} site effect, and ε_{ijkl} is the residual random error. A significant difference was established with a p-value < 0.05.

RESULTS

Late blight infection was significantly reduced in Z6 compared to Snowden (Table 3). The percent foliage affected by late blight over time after inoculation at each site (Figure 2 to Figure 3) supports the conclusion that the late blight R-gene, *Rpi-vnt1*, confers protection in Z6 and is efficacious in the foliage.

Table 3. Area under Disease Progress Curve (AUDPC) for Z6 and Snowden across Sites

Variety	Mean AUDPC	Standard Deviation	N	P-value[1]
Snowden	1381.8	581.6	20	**<u><0.0001</u>**
Z6	0	0	20	-

[1]P-values indicating significant differences between test and control are in bold and underlined.

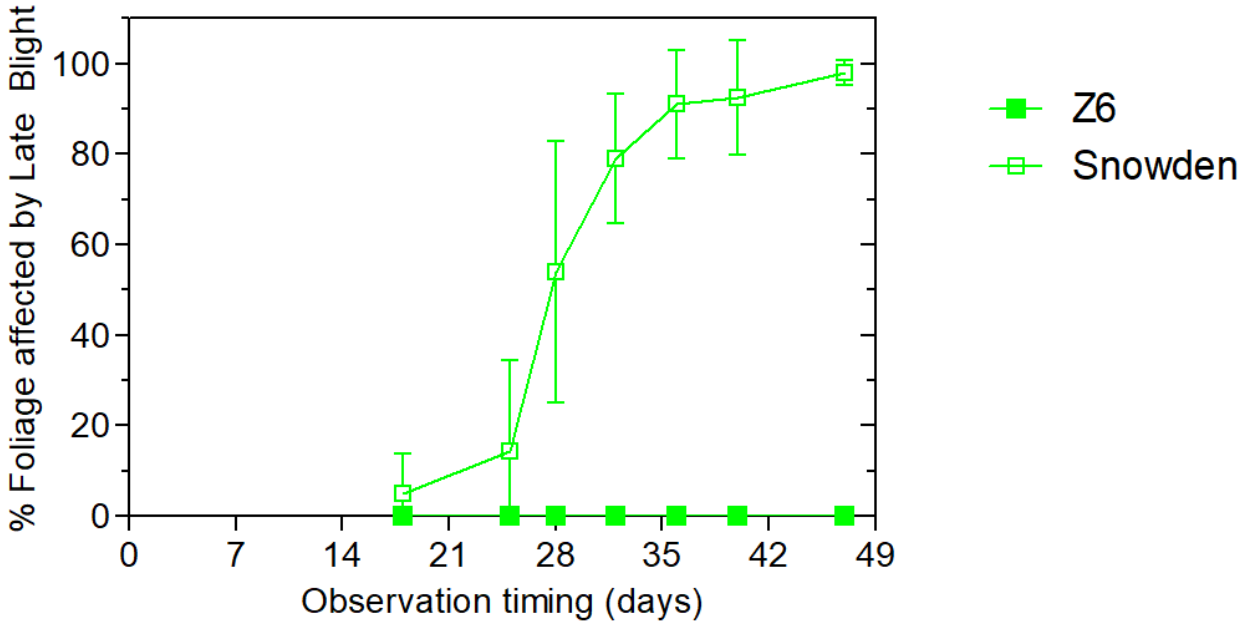

Figure 2. Percent Foliage Affected by Late Blight Strain US-23 for Z6 in Michigan

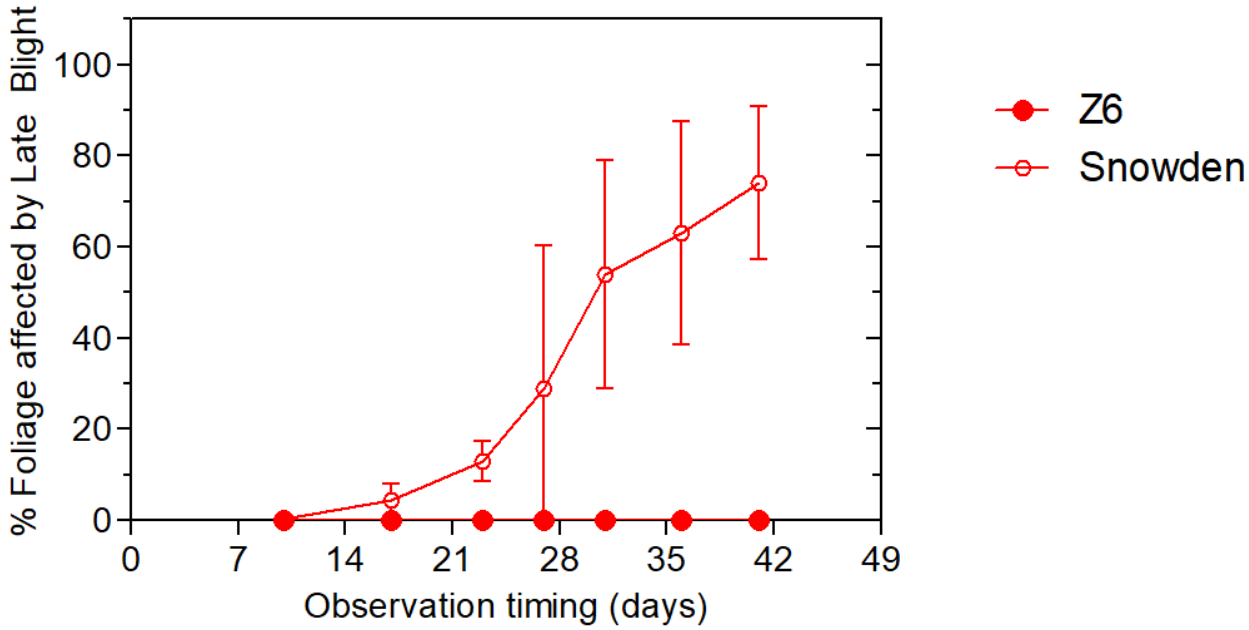

Figure 3. Percent Foliage Affected by Late Blight Strain US-23 for Z6 in Pennsylvania

CONCLUSION

A significant reduction in late blight foliar infection was observed in Z6 compared to Snowden, demonstrating efficacy of the late blight protection trait.

REFERENCES

Madden, L.V., Hughes, G., and van den Bosch, F. (2007). Temporal Analysis I: Quantifying and Comparing Epidemics. In The Study of Plant Disease Epidemics, Madden, L.V., Hughes, G., and van den Bosch, F., eds. (American Phytopathologoical Society (APS Press)), 63–116.