A Bacterial Biosensor for Detecting Early-Season Nitrogen Health in Cereal Crops

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Introduction

Fertilization with nitrogen is necessary for high corn yields. Growers commonly provide a large, single dose of **nitrogen (N) fertilizer** to fields at or just before planting**.** However, because young corn plants do not have the capacity to absorb large amounts of N, much is removed from the field as runoff prior to plant uptake resulting in economic and environmental losses **(Figure 1A)**. If a grower practices **split-N application (i.e. "side-dressing")**, a second application is supplied to the field later in the season when the plants are between knee-height and flowering. At this point in the season the plant has a well-developed root system, and a higher demand for N **(Figure 1B)**.

Side-dressing may result in greater profit for growers and less environmental damage, but **only if the rate and timing of the side-dress is carefully selected based on the crop's needs** [1]. There are commercial technologies which provide side-dress recommendations (e.g. GreenSeeker^{TM}, GreenIndex^{TM}), but many are not able to accurately and reliably determine plant health. Here we describe an alternative, low cost biosensor-based N test (**GlnLux**) which uses single leaf punch samples for analysis **(Figure 2)**.

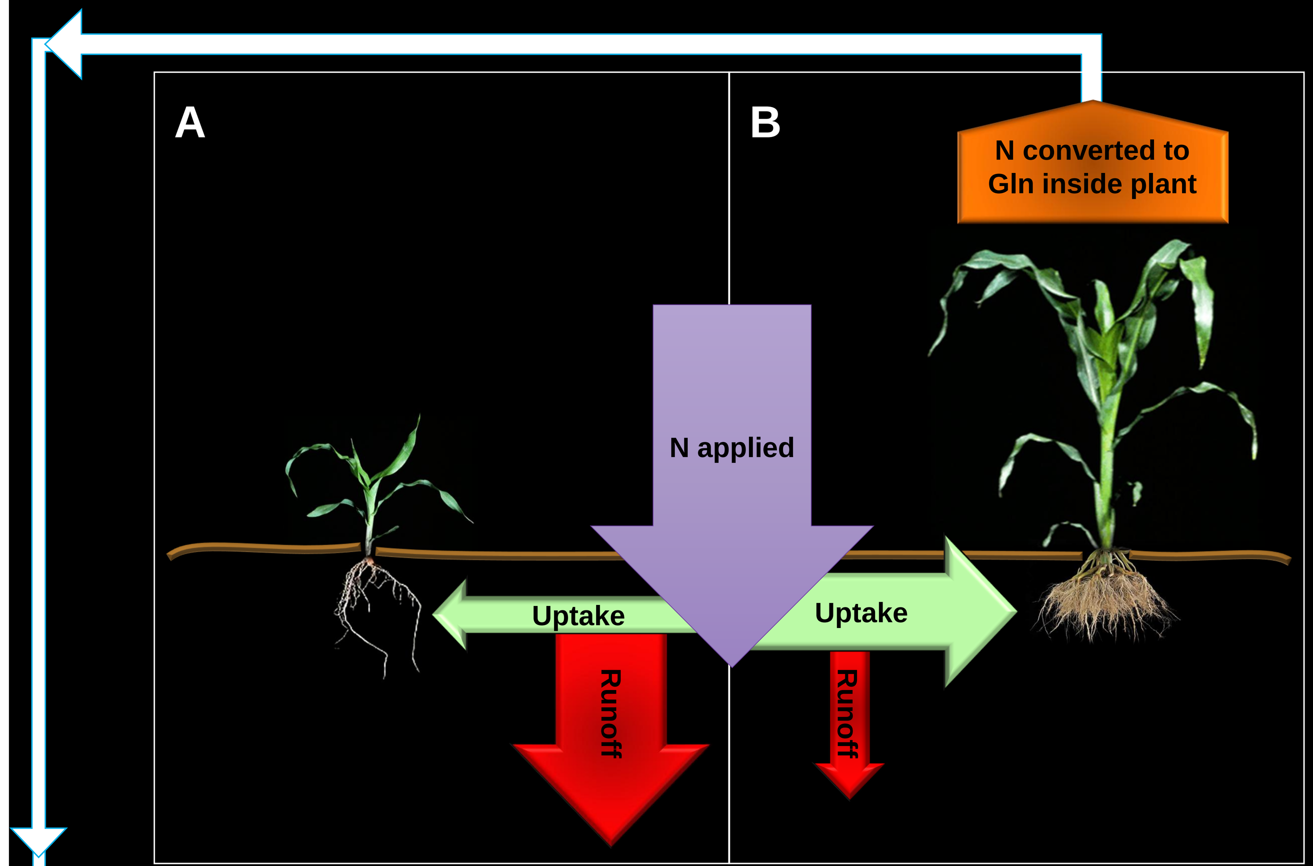


Figure 1. Image comparing the relative rates of N uptake by the plant (represented in green) and N runoff (red) when N is supplied as a single application **(A)** versus a side-dress later in the growing season **(B)**.

The GlnLux Biosensor

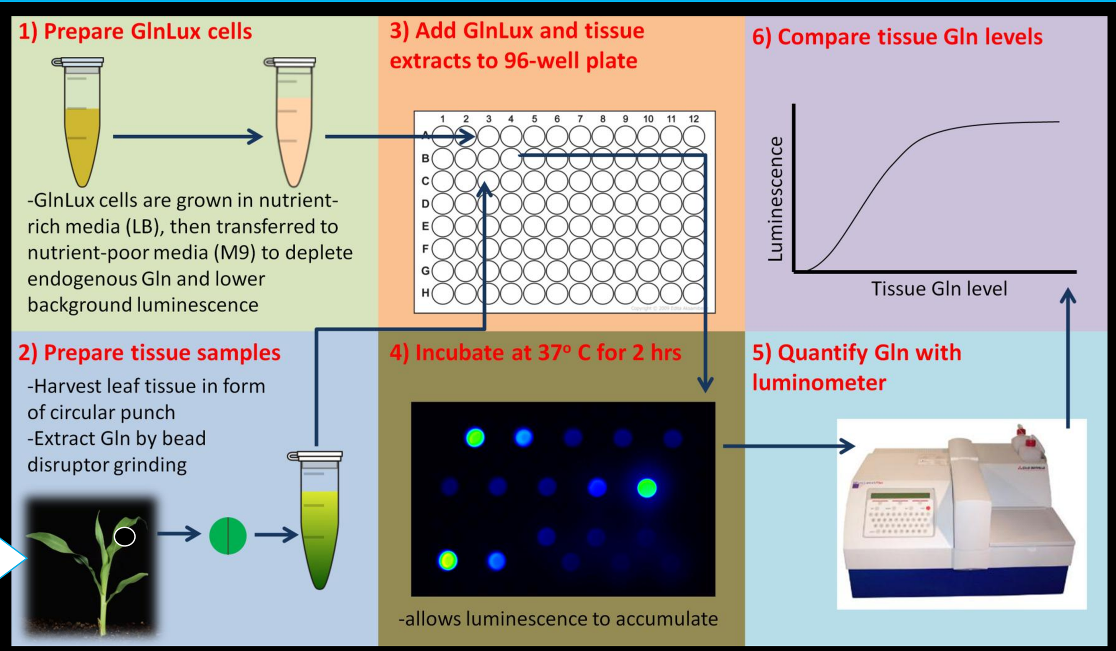


Figure 2. Methodology for estimation of tissue free-Gln levels using GlnLux. Tissue samples are prepared from small, circular leaf punch disks, and then co-incubated with GlnLux cells at 37° C after which their luminescence is quantified with a luminometer.

We have designed a new side-dress recommendation test which evaluates the internal nitrogen health of young corn plants at the side-dress growth stage **(Figure 2)**. N is assimilated into amino acids (primarily glutamine, **Gln**) after uptake by roots **(Figure 1B)** for growth and shuttling to different tissues. Our lab has engineered and patented a strain of *Escherichia coli,* named the **GlnLux biosensor** [2], to detect Gln within plant tissue. GlnLux is an auxotroph that has an absolute requirement for exogenous Gln to grow, upon which it luminesces due to an introduced *lux* operon. When the biosensor is exposed to free Gln from leaf tissue punches, it releases light **(Figure 2)**. Light intensity has been shown to correlate with N application rate and yield **(Figures 3,4)**. The GlnLux leaf punch test enables inference of plant health and a side-dress rate recommendation.

Methodology and Results

The objective of the field experiment was to determine if GlnLux biosensor readings taken during the growing season correlate with N application rate and various measurements of end-season crop health including yield, dry biomass, biomass N%, and harvest index **(Table 1)**. In 2014, the experiment consisted of two corn field locations **(Figure 5)** in which multiple rates of N fertilizer were applied pre-plant. Plant leaf tissue was sampled for biosensor analysis at four different growth stages: **V3**, **V6**, **V12**, and **V14 (Figure 6).** At location #1, commercially-available side-dress recommendation methods were also tested at several growth stages **(Figure 7)**.

Figure 6. In corn, vegetative growth stages are named according to the number of fully-emerged leaves. In this study, corn at **V3** (three fully emerged leaves), **V6** (six leaves), **V12** (12 leaves), and **V14** (14 leaves) were sampled with the GlnLux biosensor methodology for analysis of plant nitrogen health. DAS = days after seeding.

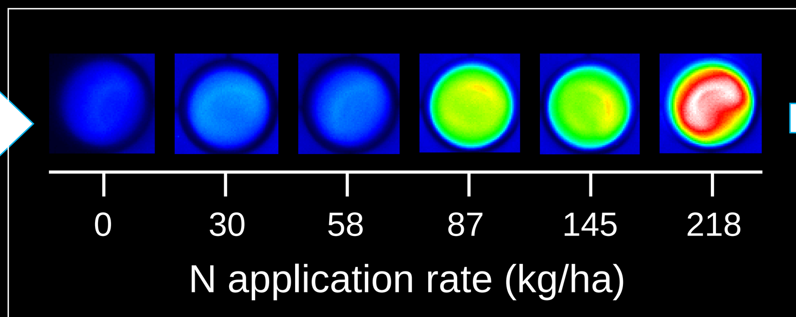


Figure 3. Corn tissue samples processed with the GlnLux biosensor have been shown to release luminescence in correlation with early season N application rate. Depicted are samples from the **V12 growth stage** in 2015.

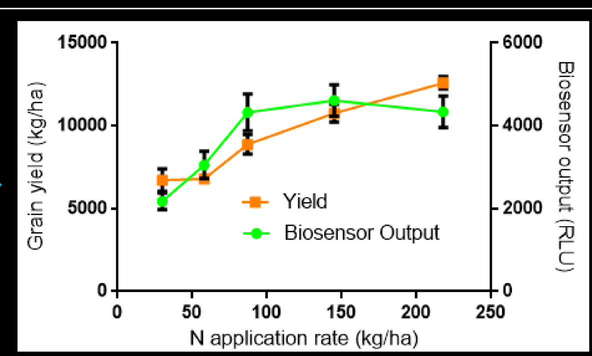


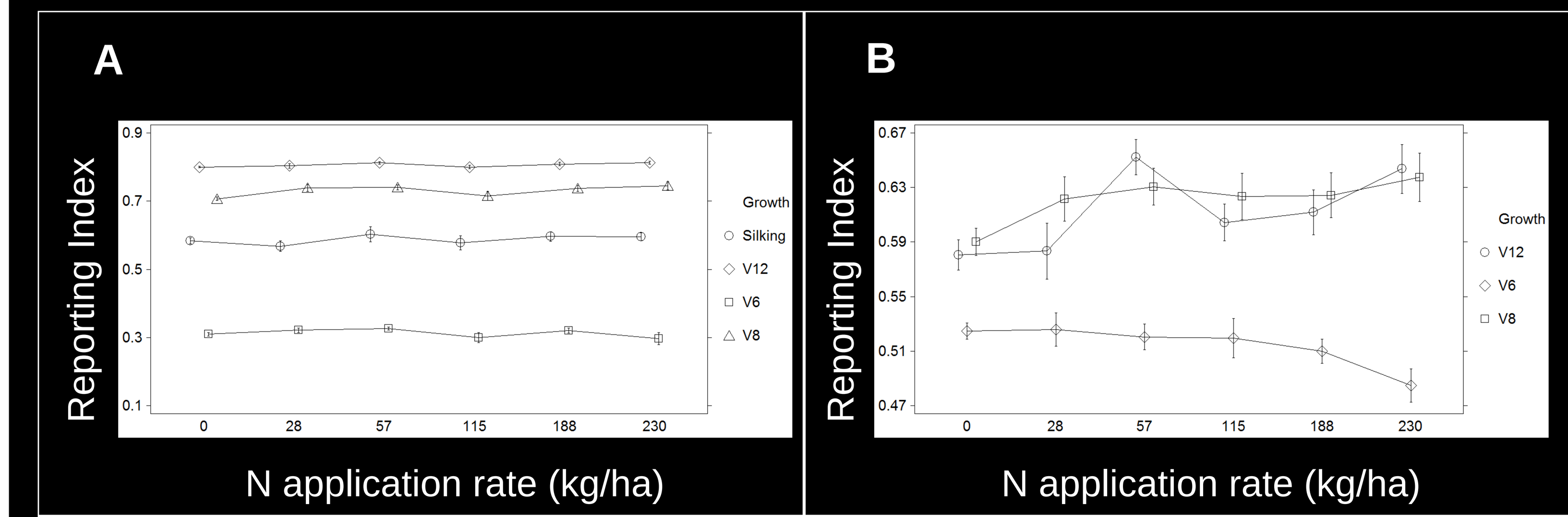
Figure 4. Corn tissue samples processed with the GlnLux biosensor have been shown to release luminescence in correlation with end-season yield adjusted to 15.5% moisture (P=0.0023). Shown are the means of six sub-samples from the **V12 stage** in 2014.

Figure 5. Field location #1 in 2015 near Elora, Ontario, Canada. Corn plants are at roughly the **V12 stage**.

	Location #1								Location #2		
	Yield (15.5% moisture)	N Application Rate	Grain N%	Stover N%	Ear Dry Weight	Grain Dry Weight	Stover Dry Weight	Harvest Index	Yield (15.5% moisture)	PSNT (soil nitrate test)	N Application Rate
V3	0.7737	0.7857	0.8135	0.0626	0.6269	0.635	0.284	0.9873	0.6071	0.1857	0.4034
V6	0.9357	0.4679	0.619	0.5748	0.7299	0.7046	0.503	0.9279	0.504	**0.0312**	0.2261
V12	**0.0023**	**0.0082**	**0.0086**	0.0945	**0.0028**	**0.0026**	**0.0123**	**0.0014**	**0.0233**	**0.0199**	**0.0121**
V14	**0.0001**	**0.0012**	**0.0001**	0.1904	**0.00001**	**0.00001**	**0.001**	**0.00001**	0.0702	**0.0189**	**0.0213**

Table 1. Pearson correlation P-values of GlnLux biosensor output at four growth stages versus N application rate, soil N levels, and various end-season metrics. Correlations significant at P<0.05 are bolded.

Commercially-Available Side-Dress Recommendations



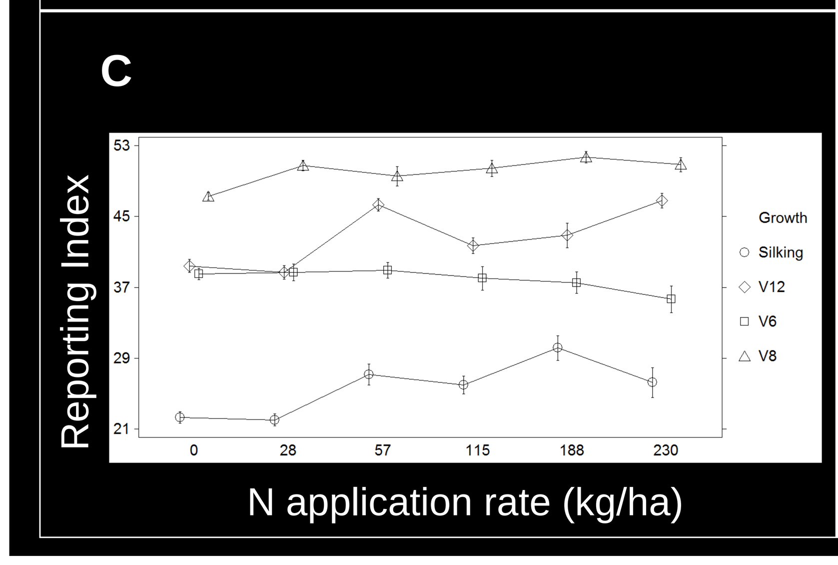


Figure 7. Current commercially-available methods of side-dress recommendation including GreenSeeker^{TM} **(A)**, GreenIndex^{TM} **(B)**, and SPAD measurements **(C)** did not correlate well with N application rate and N availability. These measurements were performed on location #1 in 2014.

Preliminary 2015 Biosensor Data

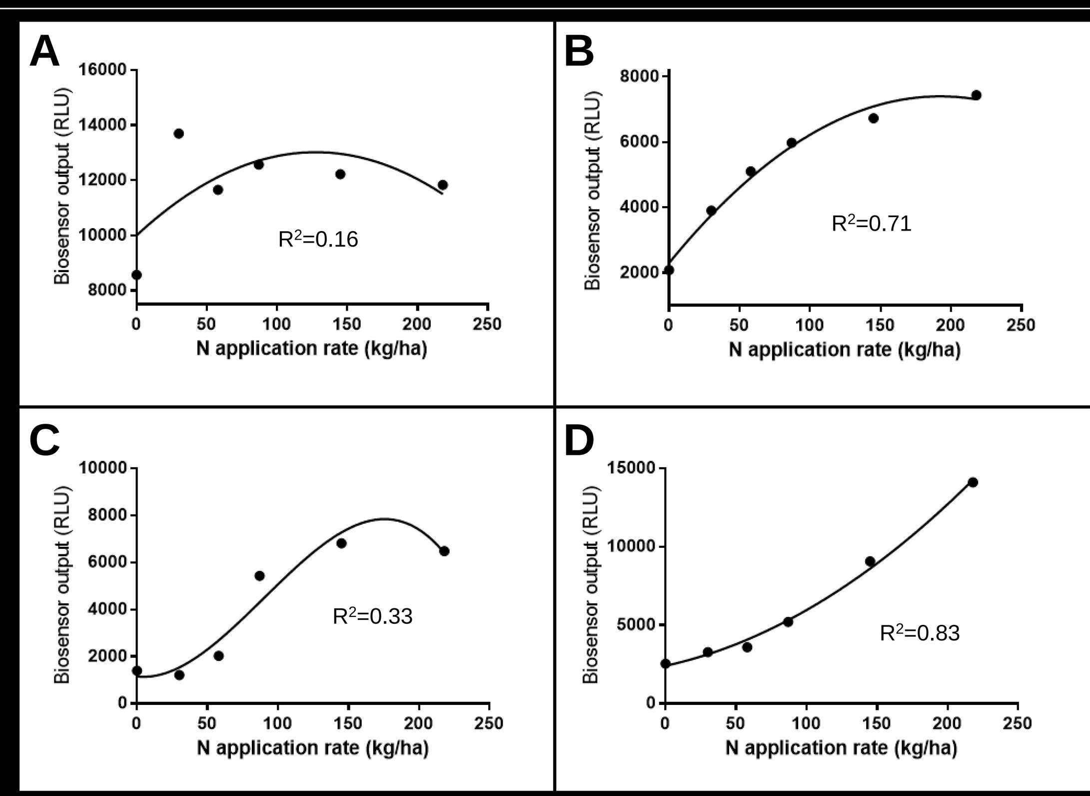


Figure 8. Preliminary data from location #1 during the 2015 growing season. Corn plants were sampled with the GlnLux biosensor protocol at **V3 (A)**, **V6 (B)**, **V12 (C)**, and **V14 (D)**. Data points represent the mean of four replicates sampled from an RCBD, each replicate consisting of six pooled subsamples. In 2015, a clear trend was observed after the **V6** growth stage **(B)**. R^2 values of non-linear models are displayed.

Conclusions and Future Experiments

As shown in **Table 1**, **Figure 7**, and **Figure 8**, the GlnLux biosensor is more reflective of soil nitrogen status, plant health, and end-season yield potential than currently available predictors including GreenSeeker^{TM}, GreenIndex^{TM}, and SPAD measurements. GlnLux data correlates with pre-sidedress soil nitrate levels **(Table 1)**. The correlation between biosensor data and these end-season measurements appear to become stronger as the season progresses, with significance first observed at the **V6** growth stage **(Table 1, Figure 8)**. The current cost of one GlnLux test is ~$1 USD including labor and reagents.

Based on these data, we believe that the GlnLux biosensor protocol may be incorporated into a decision support system (DSS) to assist farmers in determining the nitrogen needs of their crops. Future experiments will focus on completing the second year of correlations (preliminary data shown in **Figure 8**), after which a DSS will be designed. The biosensor will also be developed into a commercial test or kit, to be used by farmers of cereal grains, to whom *a priori* knowledge of the output of their fields would provide economic advantage. Such advantages include being able to predict the revenue obtained from a crop, and predict commercial agricultural costs associated with harvesting, transporting, and storing the grain. The grower may also have the capability to enter into contracts dependent upon certain yield goals (e.g. with an ethanol production facility) with a greater degree of confidence.

UID: 93288

References

1. Wang, D., Prato, T., Qiu, Zeyuan, Kitchen, N. R. and Sudduth, K. A. Economic and environmental evaluation of variable rate nitrogen and lime application for claypan soil fields. *Precis. Agric.* **4,** 35-52 (2003).
2. Tessaro, M. J., Soliman, S. S. M. & Raizada, M. N. Bacterial whole-cell biosensor for glutamine with applications for quantifying and visualizing glutamine in plants. *Appl. Environ. Microbiol.* **78,** 604-606 (2012).

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