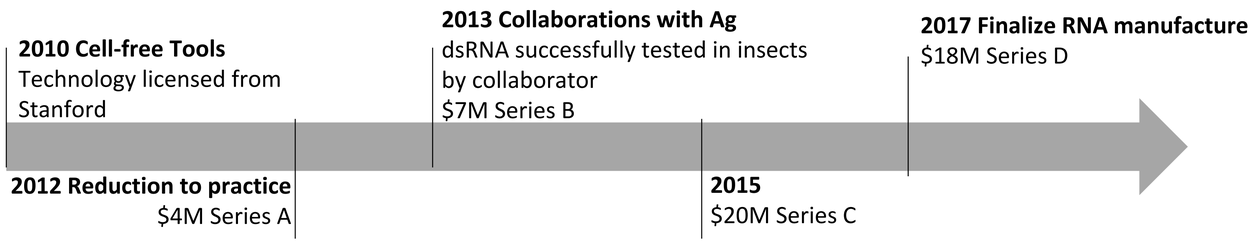


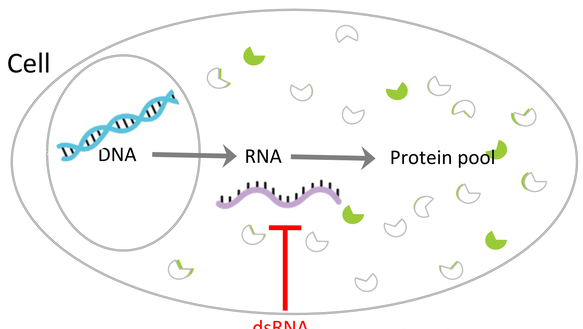
Enabling the RNA revolution; Cell-free dsRNA production and control of Colorado potato beetle

Bridey Maxwell (bmaxwell@greenlightbio.com), Doug Boyes, Jo Tang, Thais Rodrigues, Suresh Desai, Drew Cunningham, Karthikeyan Ramachandriya, James Abshire and Carole Cobb.
GreenLight Biosciences @ Medford MA, St Louis MO, and RTP NC

RNA interference holds great potential as a new non-transgenic tool for crop protection and trait improvement because of its ability to very selectively downregulate gene expression in many different organisms, such as insects, plants, nematodes, and viruses. RNAi via transgenic delivery has been successfully used to protect crops from viruses and to introduce desirable traits. Recent examples include SmartStax® PRO (corn rootworm protection), Vistive® Gold (modified oil), Innate® potato (reduced browning/acrylamide) and Rainbow Papaya (viral protection). However, large-scale and cost-effective production of dsRNA for non-transgenic delivery remains a challenge. Currently, dsRNA molecules are primarily synthesized through *in vitro* transcription (IVT) and fermentation both of which are costly and have inherently limited scalability hindering the commercial viability of dsRNA crop protection. At GreenLight Biosciences, we have developed a cell-free RNA production platform that overcomes the historic challenges of purity, cost, and rapid scale-up performance found with conventional RNA synthesis. Our platform delivers the same bioactivity and specificity as IVT-based dsRNA with a projected cost of <$0.50/g dsRNA and scalability to metric tons. Our cell-free platform breaks down RNA from a low-cost polymeric RNA-containing substrate such as yeast into nucleoside monophosphate (NMP) building blocks and rebuilds these into the RNA product of interest using proprietary enzymes, a DNA template, and a low-cost energy source. Due to the cell-free nature of the platform, all the components, except for the DNA template, are independent from the RNA product type. This means when novel targets are discovered, the platform can easily prepare corresponding dsRNA products at scale by simply preparing a new DNA template. We have shown our process to produce highly bioactive dsRNA with our 2018 field trials for our upcoming product; Colorado Potato Beetle dsRNA insect control. We are currently expanding our Crop Protection portfolio to other insects, fungi and plant viruses.

Advantages of RNAi

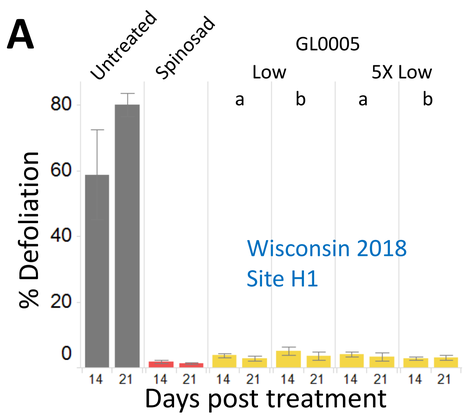
New pest control MOA	RNAi can be used to control insects, weeds and fungi by selectively knocking out essential gene activity
Specificity	RNAi only affects target species and is innocuous to other insects, animals and plants
Low toxicity	RNA is biodegradable, safe to nontarget species and sustainable
Rapid development	Rapid development and testing of new candidate RNA formulations Non-transgenic regulatory path as a Bio-pesticide (18-24mo)

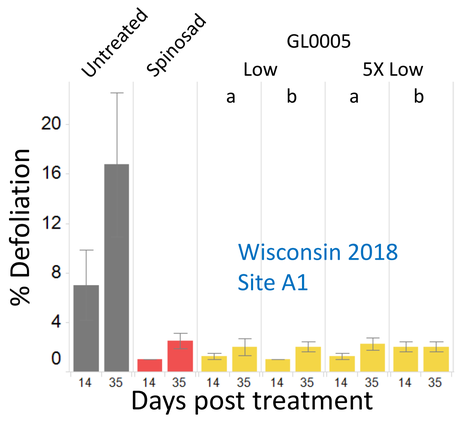


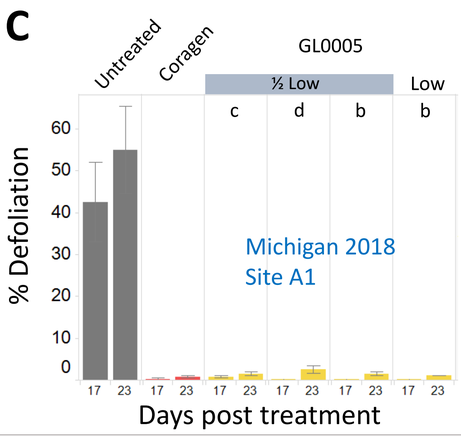
Specific gene control

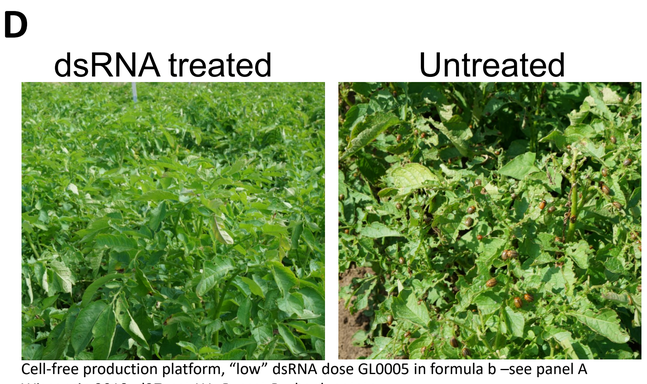
Control of Colorado potato beetle in the field

The dsRNA GL0005 provides protection from CPB defoliation in four independent field tests at our low dose which enables competitive costs vs. the current control products. Data for three sites are shown here. **A** At our WI H1 site we saw control similar to Spinosad at both a low and 5X-low dose formulated in two ways “a” and “b”. This was repeated at our WI A1 site (**B**). **C** at our MI site, we saw GL0005 providing protection at half our low dose in formulation b as well as two others; c and d. Three novel leads also showed similar efficacy in this trial (not shown). **D** WI H1 trial images for GL0005 in formulation “b” at the low dose; see data set in panel A.







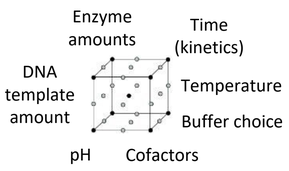


Cell-free production platform, “low” dsRNA dose GL0005 in formula b –see panel A
Wisconsin 2018, d27, set H1, Russet Burbank potato.

Achieving RNAi at cost and scale for agriculture

Platform comparison

	Chemical Synthesis	In vitro Transcription	Fermentation	GreenLight Cell-Free
Source of Nucleotides	Chemically synth.	Purified	Biomass	Biomass
Source of DNA template	NA	Purified	Biomass	Biomass
Source of Polymerase	NA	Purified	Biomass	Biomass
Product Length Limitations	Yes (< 100 base pairs)	No	Some	No
Cost of Goods ($/g)	$100,000/g	$1,000/g	$1.00/g	<$0.50/g
Scalability	mg-mg	mg-g	kg	kg-MT
Capital Intensity	High	Med	High	Low



✓Cost effective, scalable, flexible
✓True platform: Single manufacturing process → multiple products
✓Proprietary, patent pending

dsRNA products can finally achieve a commercially competitive cost per application ($/acre)

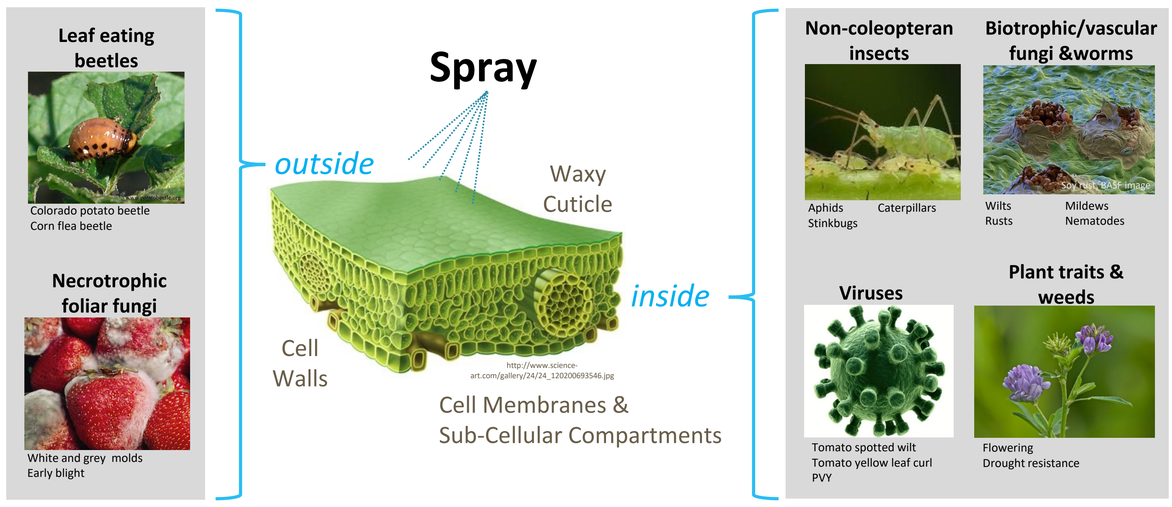
Rapid optimization of the cell-free platform

Technical Levers	Q1 2017	Today
Cost Driver 1. Minimize Fermentation Cost of Producing ‘Ingredients’		
•Enzyme fermentation productivity (g enzyme/L ferm./h)	1X	4-10X
•DNA template fermentation productivity (g plasmid/L ferm./h)	1X	2X
Cost Driver 2. Maximize Recovery & Stability of ‘Ingredients’		
•Overall recovery of enzymes (g/g)	1X	1.5X
•Overall recovery of DNA template (g/g)	1X	1.5X
Cost Driver 3. Maximize Productivity by Optimizing the Reaction ‘Recipe’		
•Depolymerization reaction yield (g NMPs/g cellular RNA)	1X	2X
•Production reaction yield (g dsRNA/g NMPs)	1X	4X
•Product per DNA template (g dsRNA/g template plasmid)	1X	20X
•Product per enzymes (g dsRNA/g enzyme)	1X	5X
COGs ($/gram dsRNA) for 100 MTA Plant	**> $4.00**	**< $0.50**

Strain Engineering & Fermentation Development

Recovery Process Development & Biochemistry

Pathway Engineering & Biochemistry

GreenLight Crop Protection platform

At GreenLight, we are committed to improving agricultural productivity to meet the demands of a growing population with sustainable crop protection solutions.
Our goals are to: ❶Be the leading producer of dsRNA, ❷Be a leading innovator of RNAi crop protection products in the areas diagramed below; Bio-insecticides, Bio-fungicides, Bio-nematicides and Bio-herbicides, and ❸Be a trusted partner for growers. We can achieve all this through a combination of breakthrough technology, consistent focus and dedication.