

Functionalized Micro-Capsules: Exploration Using Antibodies and Peptides



Dow AgroSciences

Solutions for the Growing World

Acknowledgment for all the Contributors

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Why Encapsulate?

- **To extend the window of activity of the a.i.- Reldan[®]** for mosquito eradication (malaria control): 6 month activity vs. a few days for the EC
- **To lower the use rate:** N-Lock[™] has the same nitrogen stabilizing capability as the conventional formulation at ½ the rate
- **To minimize the Exposure Risk by lowering formulation toxicity:**
- **To extend the shelf-life of water-based formulations-** The AI do not hydrolyze when encapsulated
- **To potentially lower the amount of solvent relative to EC**
- **To stabilize EW formulations**

First Chapter

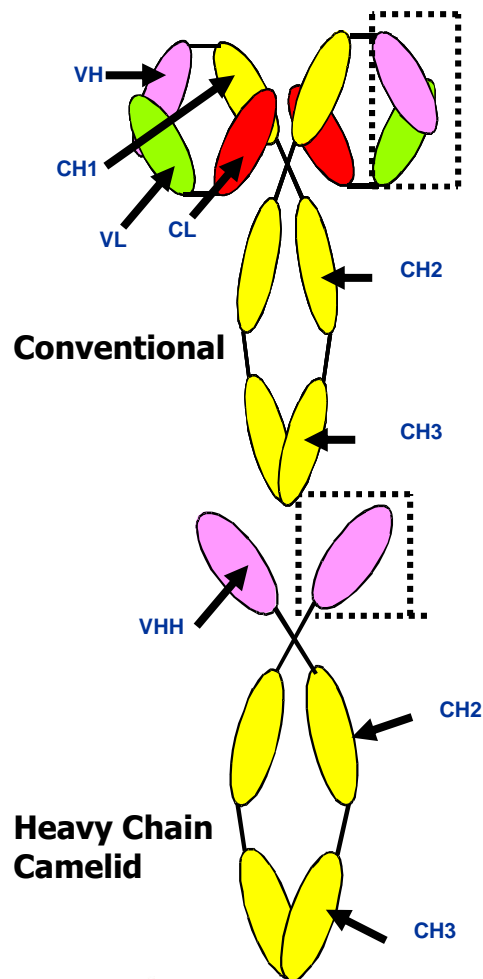
Use of Antibodies To Enhance Binding of Microcapsules To Leaf Surface

Camelids Project - Background

- Project has been carried out in the past collaboratively between DAS and National Research Council - Canada
- Premise: Utilize Camelid antibodies to selectively deliver active ingredients to a target surface – targeted delivery

Features of Camelid Antibodies

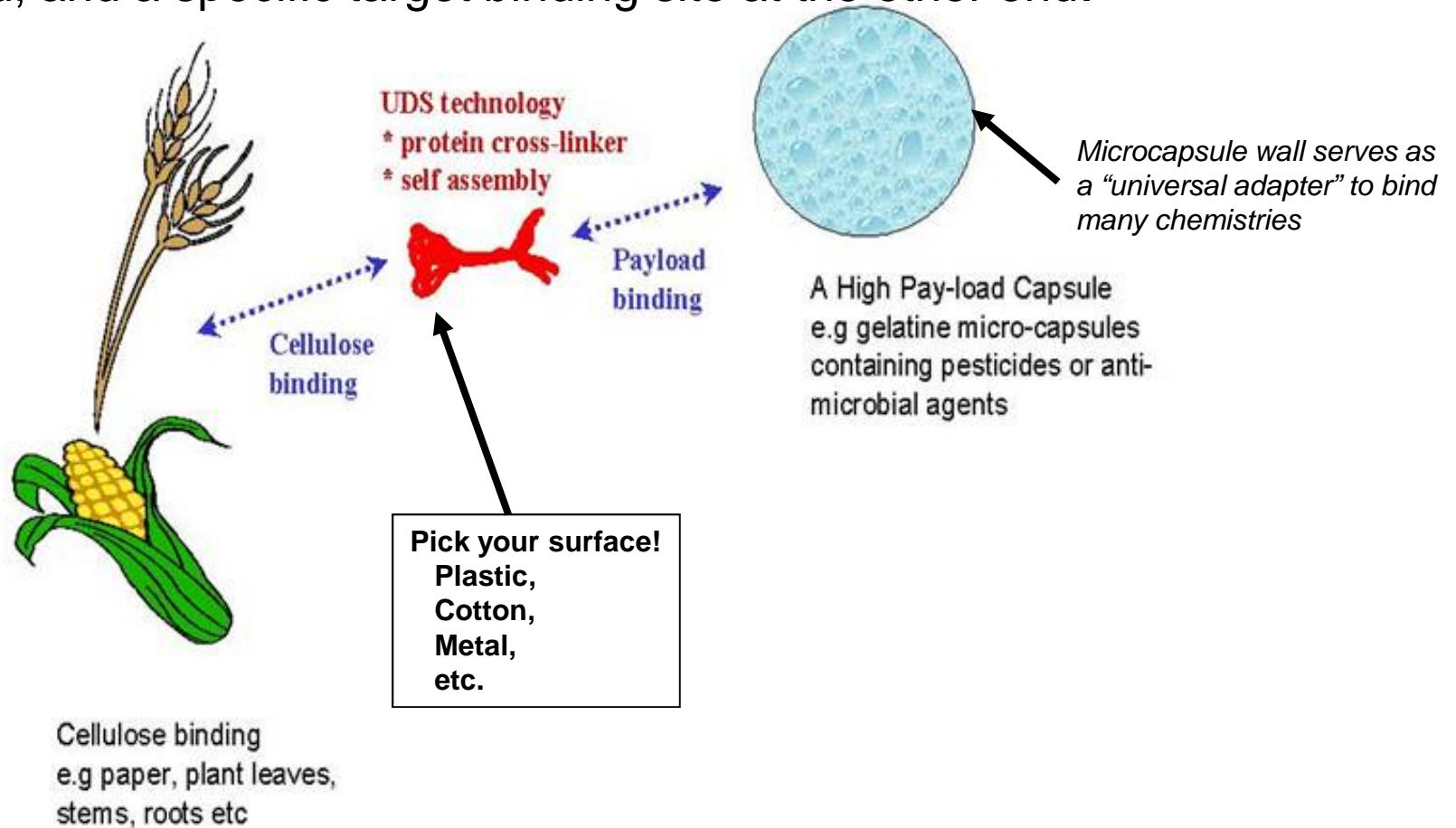
Camelids special because.....



- Found in the serum of camels and dromedary
- Novel molecule recognition domain
 - single chain-based (lack of the light chain the Y molecular structure)
- SMALL sized (heavy chain approximately 17 kD)
 - increased solubility
 - easier to clone and engineer
- Wide range of stability; tolerant to
 - heat
 - pH
 - detergents
 - protease
- Amenable to a wide range of host expression systems
 - bacteria, yeast, plants

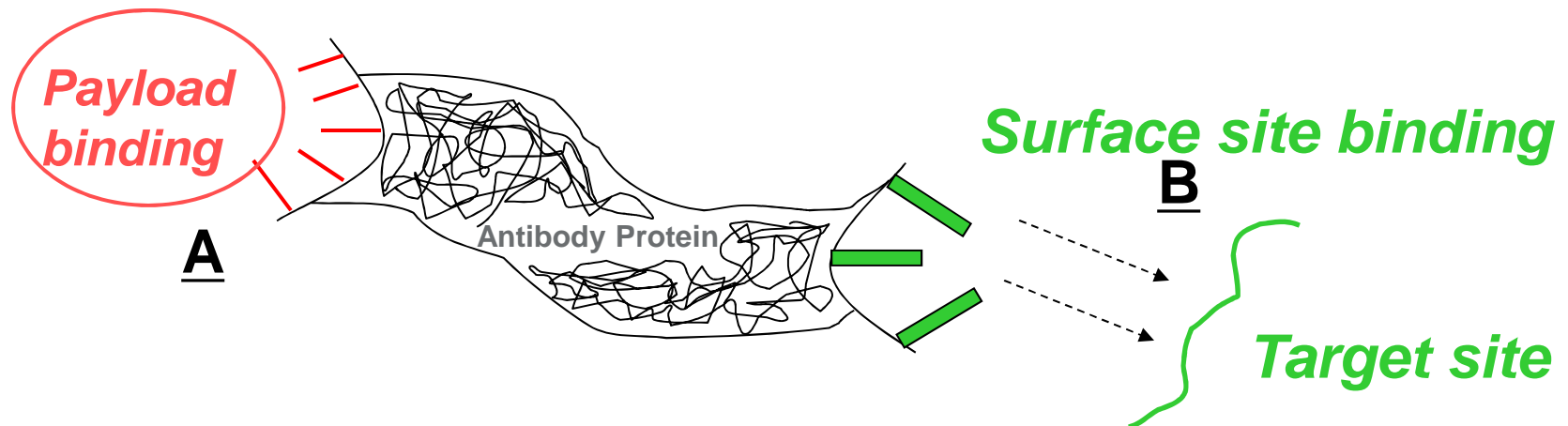
Camelid Technology

Attach antibody to a microcapsule (the payload) at one end, and a specific target binding site at the other end:



Utility of Camelid Technology for DAS

- Bifunctional “fusion” macromolecules (tethered payload delivery):



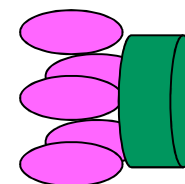
Payload particle forms:
Microcapsule chemistries – varied
Active ingredient particle
Latex
Spent yeast cells

Target binding sites:
Crop species
Weed species
Insect
Seed surface

General Approach for Proof of Concept

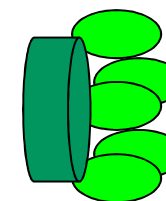
CAPSULE PENTAMER

- Pan camelid library of antibodies for binding to polyurea capsules
- Sequence antibodies that successfully bind to capsules
- Express the antibodies as pentamers in *E. coli*
- Demonstrate binding of the pentamers to the capsule



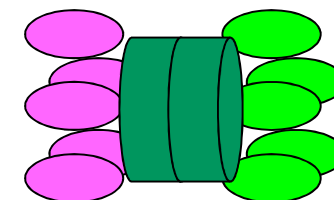
PLANT PENTAMER

- Initiate panning for antibodies that bind to whole plant (rice)
- Conduct binding/sequencing/expression for plant-binding antibodies
- Demonstrate binding of pentamers to the plant



CAPSULE + PLANT

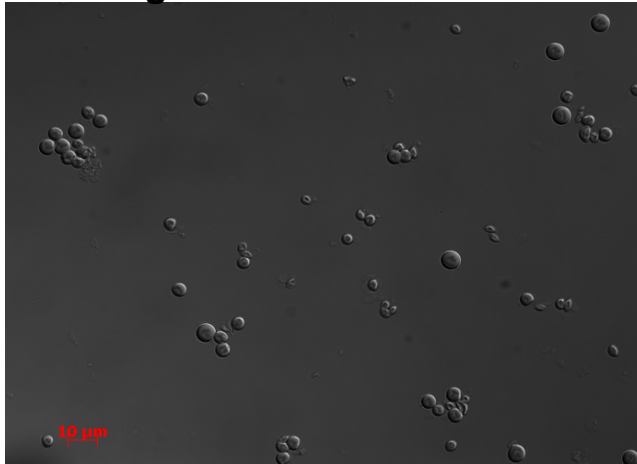
- Clone and express decamer
- Confirm decamer binding to capsule and plant



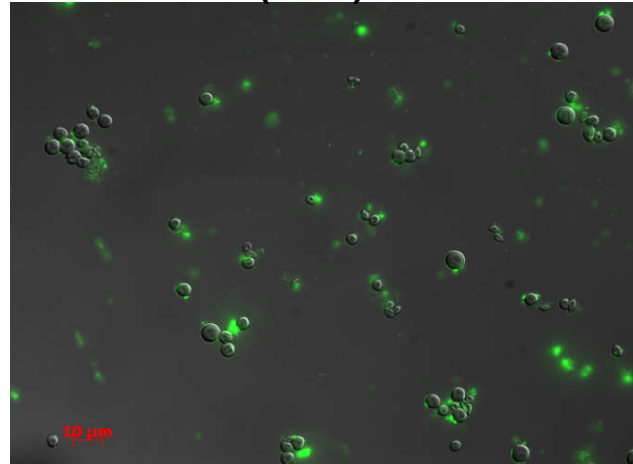
DECAMER

Capsule Binding Pentabody Bound to Capsules - Fluorescence Microscopy

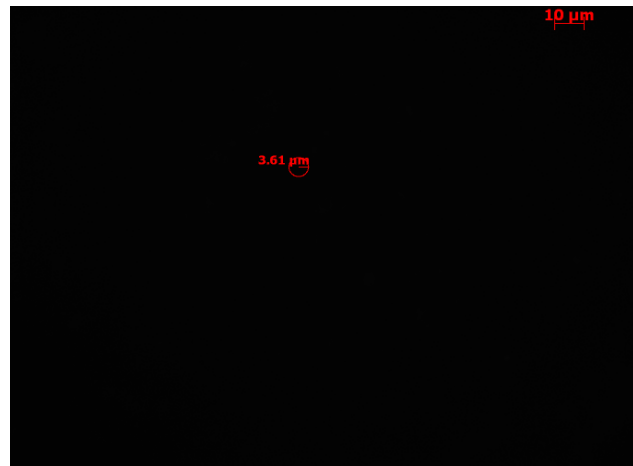
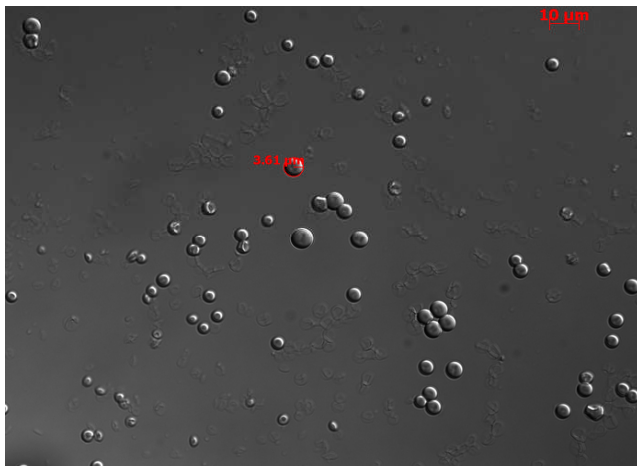
Phase light



Fluorescence (FITC)



Labeled
pentabody



Control

Panning of Plant Leaf Surface and Plant Waxes

- No observed camelid binding for
 - Stripped rice leaf wax
 - Whole rice leaf
 - Whole rice leaf treated with rinse solvent or adjuvant
 - Alternate library types (naïve vs. HVL) on any form of rice leaf wax

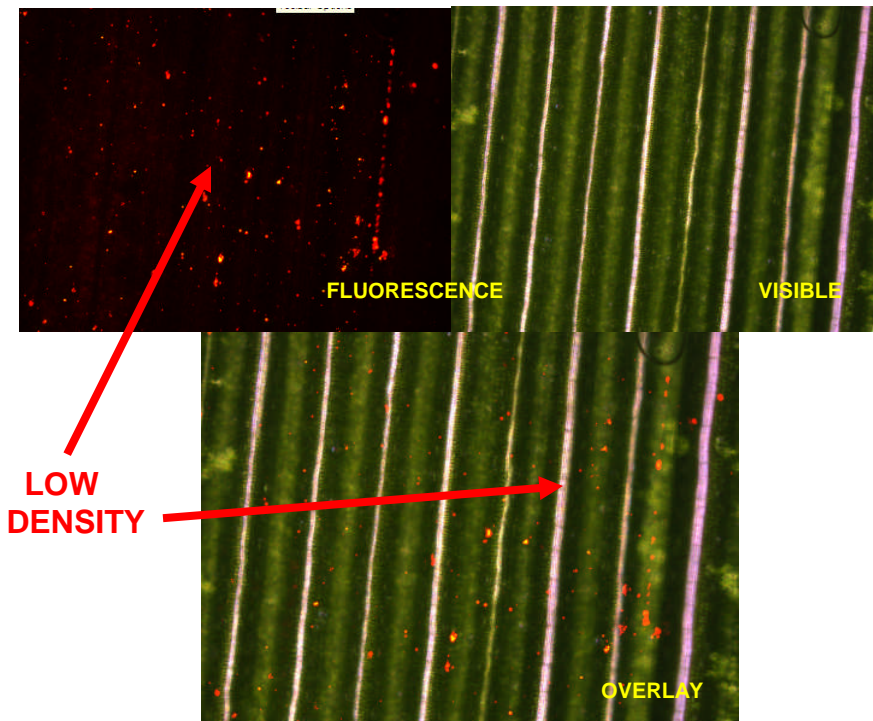


Arghhhh!!! Step back....what might be some alternative approaches??

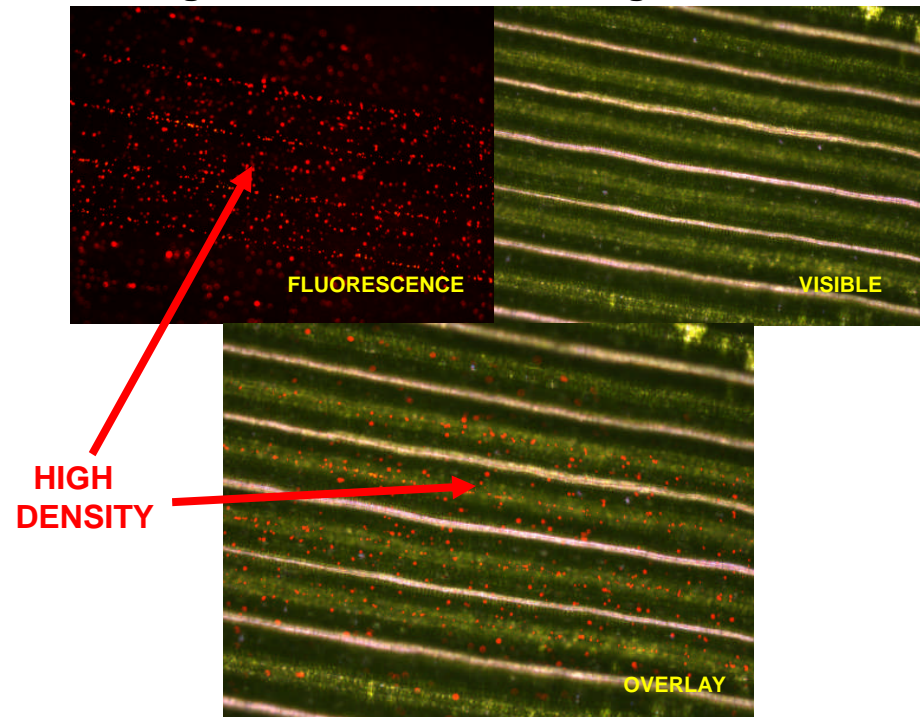
Selective Decamer Binding on Rice - Fluorescence Microscopy

PROCEDURE: + Incubate rice leaf segments with dyed GF-2020 CS +/- decamer
+ Rinse
+ Photograph

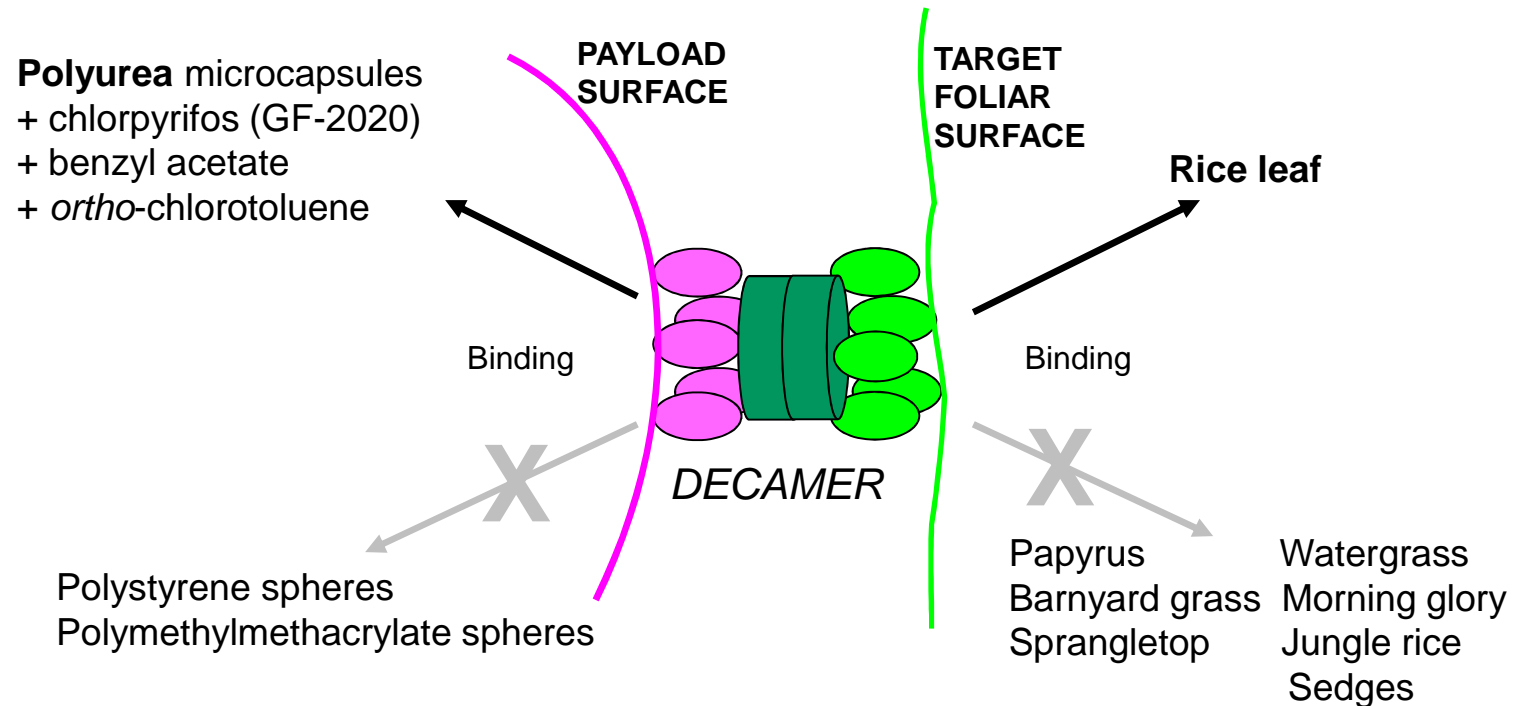
0 ug/mL DECAMER + 5000 ug GF-2020



0.1 ug/mL DECAMER + 5000 ug GF-2020



Selectivity of Protein Binding – Summarized from Lab Rinse Studies

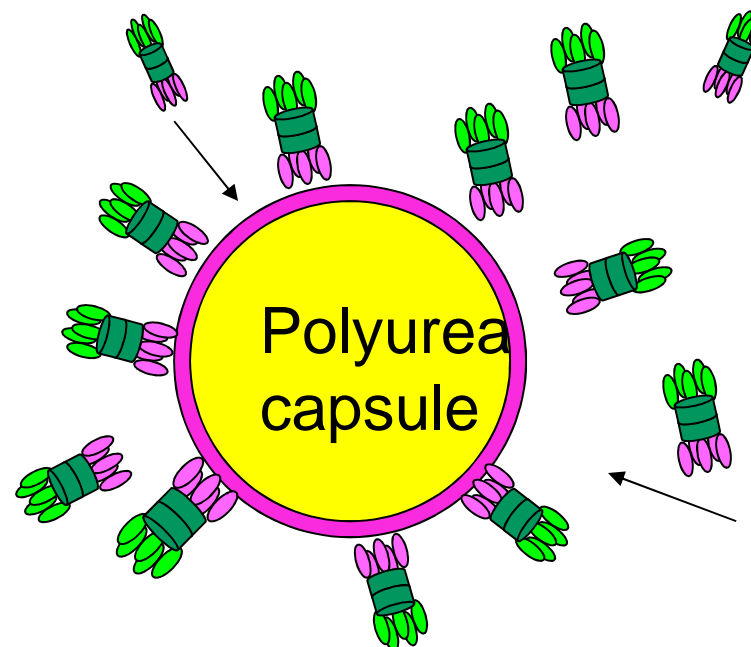


Binding sites designed for a particular surface qualitatively show binding preference for that surface:

- + polyurea binding site binds polyurea capsules
- + rice leaf binding site binds rice leaf

Binding Capacity of Capsule Surface

How much decamer can a 3.0 micron polyurea microcapsule hold?



Importance:

Can help target an effective use concentration.

Binding Capacity of Decamer onto Microcapsules

- How much decamer can a microcapsule hold?

Titration Study:

RATIO # decabody molecules/ # capsules, approx.	DECABODY 1			CAPSULES, GF-2020			Observation
	Wt.	Vol.	#	Wt.	Vol.	#	
1,000,000:1	10 ug	-	2.4 x 10E14	5 mg	5 uL	2.05 x 10E8	Significant XS decamer rinse-off
100,000:1	1 ug	-	2.4 x 10E13	5 mg	5 uL	2.05 x 10E8	50% decamer rinsed off
10,000:1	0.1 ug	-	2.4 x 10E12	5 mg	5 uL	2.05 x 10E8	No decamer rinsed off

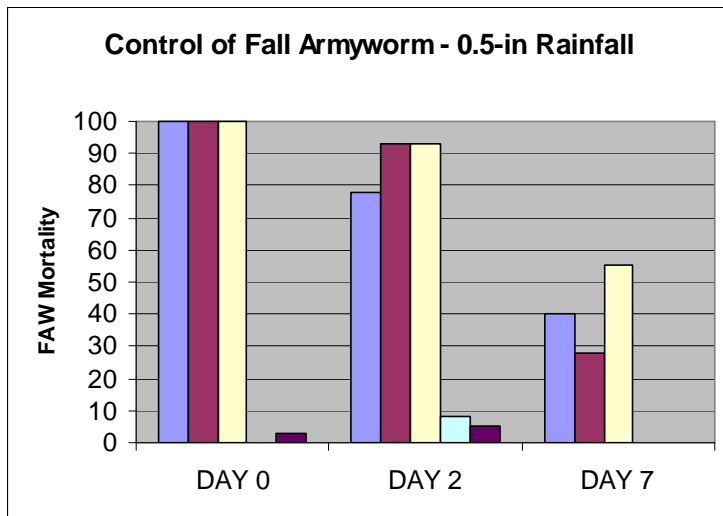
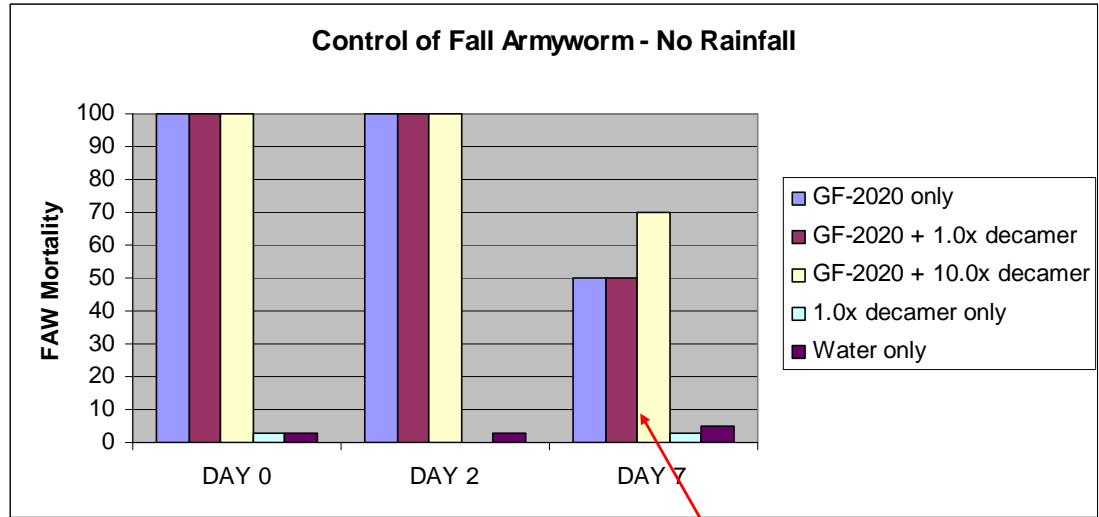
*Saturation
Point*

0.5 ug decabody
5000 ug GF-2020

Each 3-micron capsule can accommodate approximately 50,000 decabody molecules at saturation (approx 0.01 wt.% added decamer based on GF-2020).

Greenhouse Efficacy Results

The decamers were evaluated for biological activity against fall armyworm larvae under rainfall/no rainfall conditions:



The decamers provided modestly improved control of FAW larvae under rainfall/no rainfall conditions

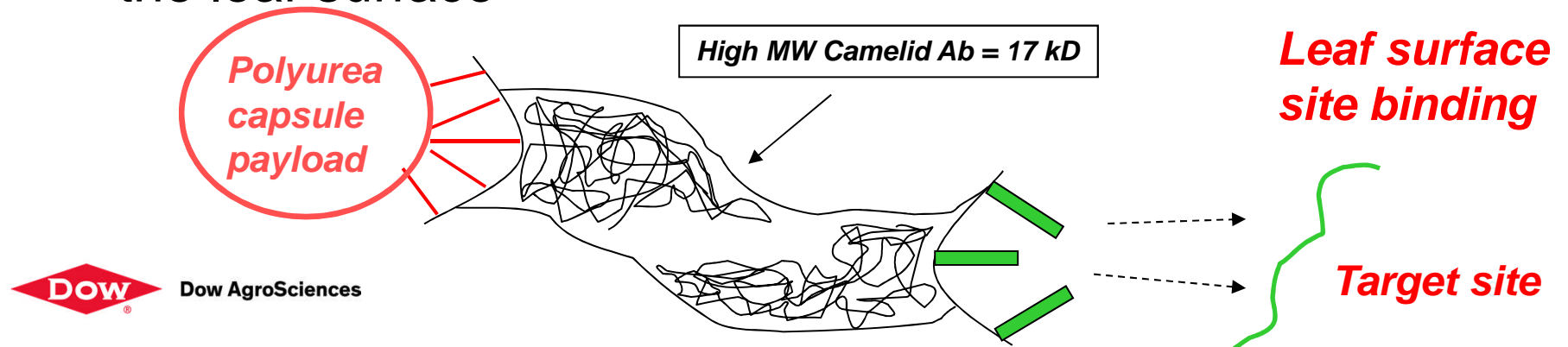
- + Decamers (4 ct.) were successfully constructed and shown to selectively bind polyurea microcapsules and rice plant leaf surface
- + A select decamer provided numerically improved (but not statistically improved) biological activity under rain/no rain conditions in the greenhouse
- + A project was initiated to characterize the utility of attaching 12-mer rice leaf binding peptides directly to polyurea microcapsules

Second Chapter

Use of Peptides To Enhance Binding of Microcapsules To Leaf Surface

Background

- In the past DAS team started a collaboration work with the Canadian National Research Council to attach Camelid antibodies to Polyurea capsules;
- Chlorpyrifos was used A.I.
- The antibody was engineered in such a way to express two different domains:
 - A binding domain for the polyurea capsule external surface
 - A domain able to anchor the complex antibody-capsule to the leaf surface



Limitations/Opportunities

- The antibody is bulky
 - Stability of its tertiary structure in presence of surfactants is unknown
- It is not easy to synthesize
- Antibody technology is not fully developed
 - It can be expensive



What if.....



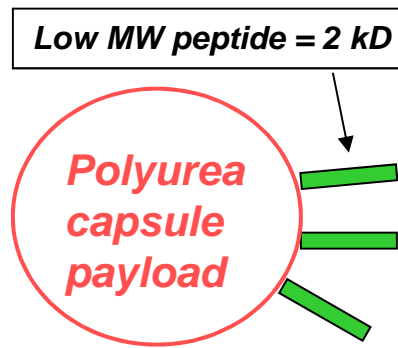
Nice and Shaved Ball



Big octopus where the head is the capsule.

CONCEPT:

Functionalize the capsule surface with simpler, lower MW binding peptide



Peptide Selection

Four binding peptides identified from past rice leaf-binding studies:

HIHRSTSHTGTP (HK-18)
HIHRGEHGPSAR (HK-19)
HHGHSPTSPQVR
HNKHLPTQPLA

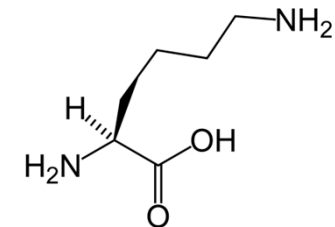


Ac-**H-I-H-R-G-E-H-G-P-S-A-R**-W-G-G-G-G-S-**K**-CONH₂

Binding Site

Spacer

Reactive Site



Lysine (K)

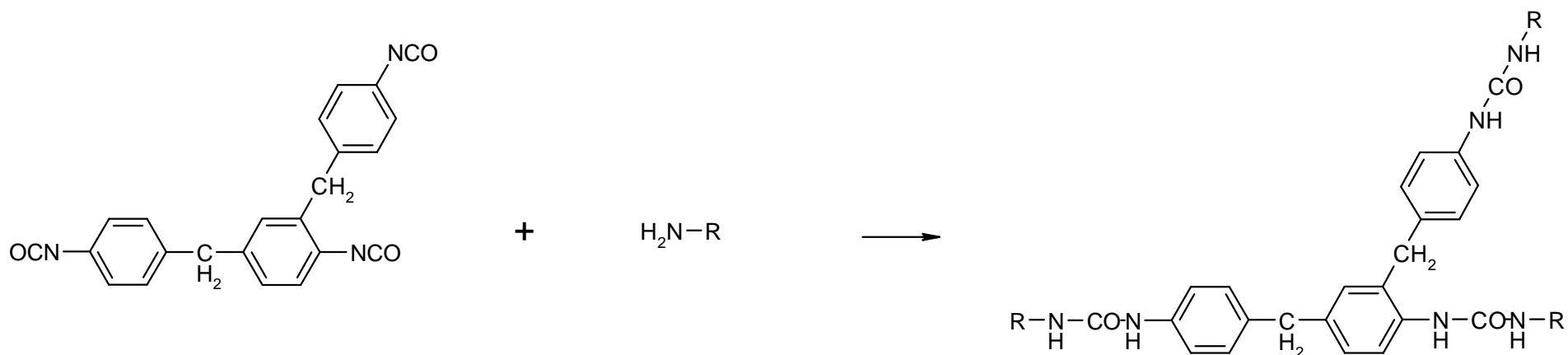
KKGGGGFLIVI (KI-11) – A peptide sequence found in transmembrane protein

KKGGGGSGLGLGSGLGLG (KG-18) – Sequence analogous to spider silk

KSGGGGSGGGGSGGGG – A scrambled sequence used as control

Interfacial Polycondensation

- Reaction occurs at organic/aqueous phase interface
- Oil-soluble isocyanate (such as Dow's PAPI 27) + water-soluble polyamine (ethylenediamine, etc.)
- Generates polyurea shell around active contained in the organic phase

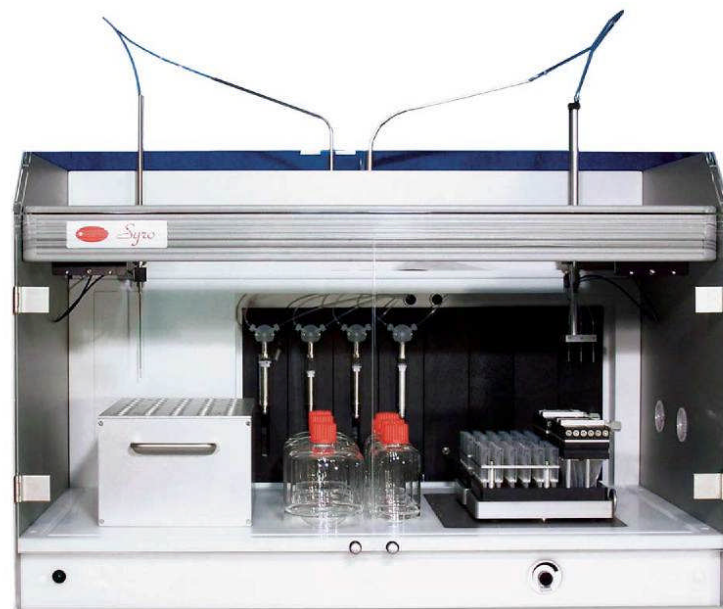
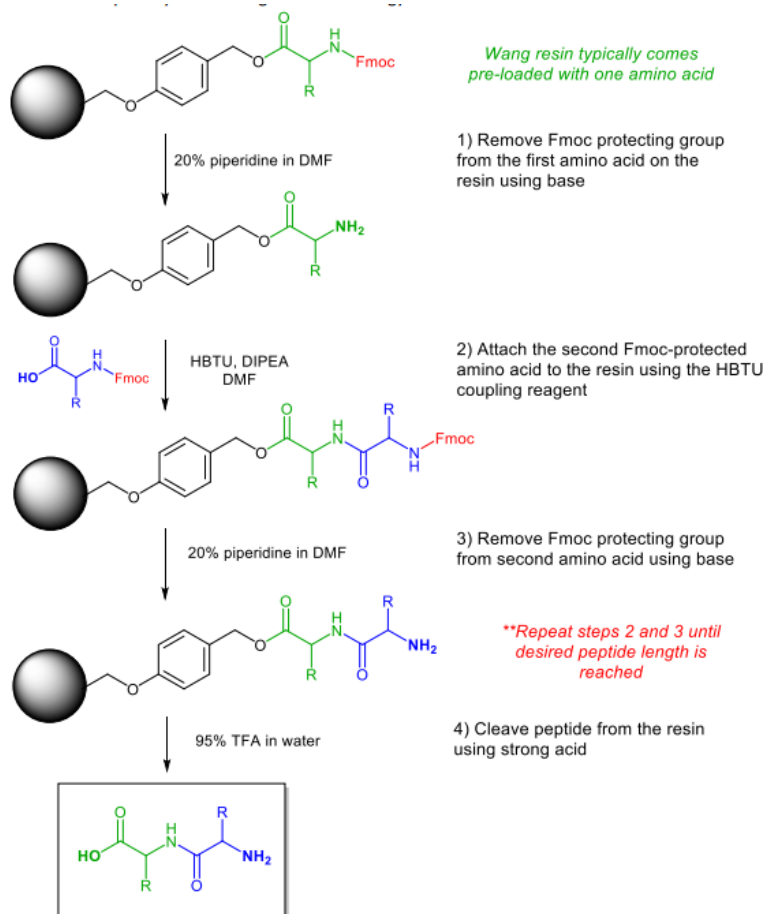


Interfacial Polycondensation Encapsulation Characteristics

- Straightforward extension of emulsion technology
- Core-shell morphology
- Very thin walls attainable
- Requires liquid core
- Release is primarily by diffusion

Peptide Synthesis

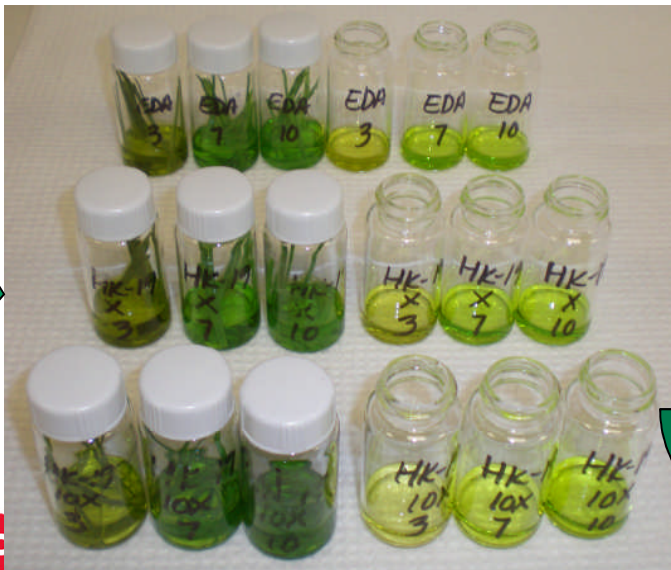
Solid Phase Synthesis



Leaf Rinses

Parafilm wrapped roots

200 ml
water +
capsules

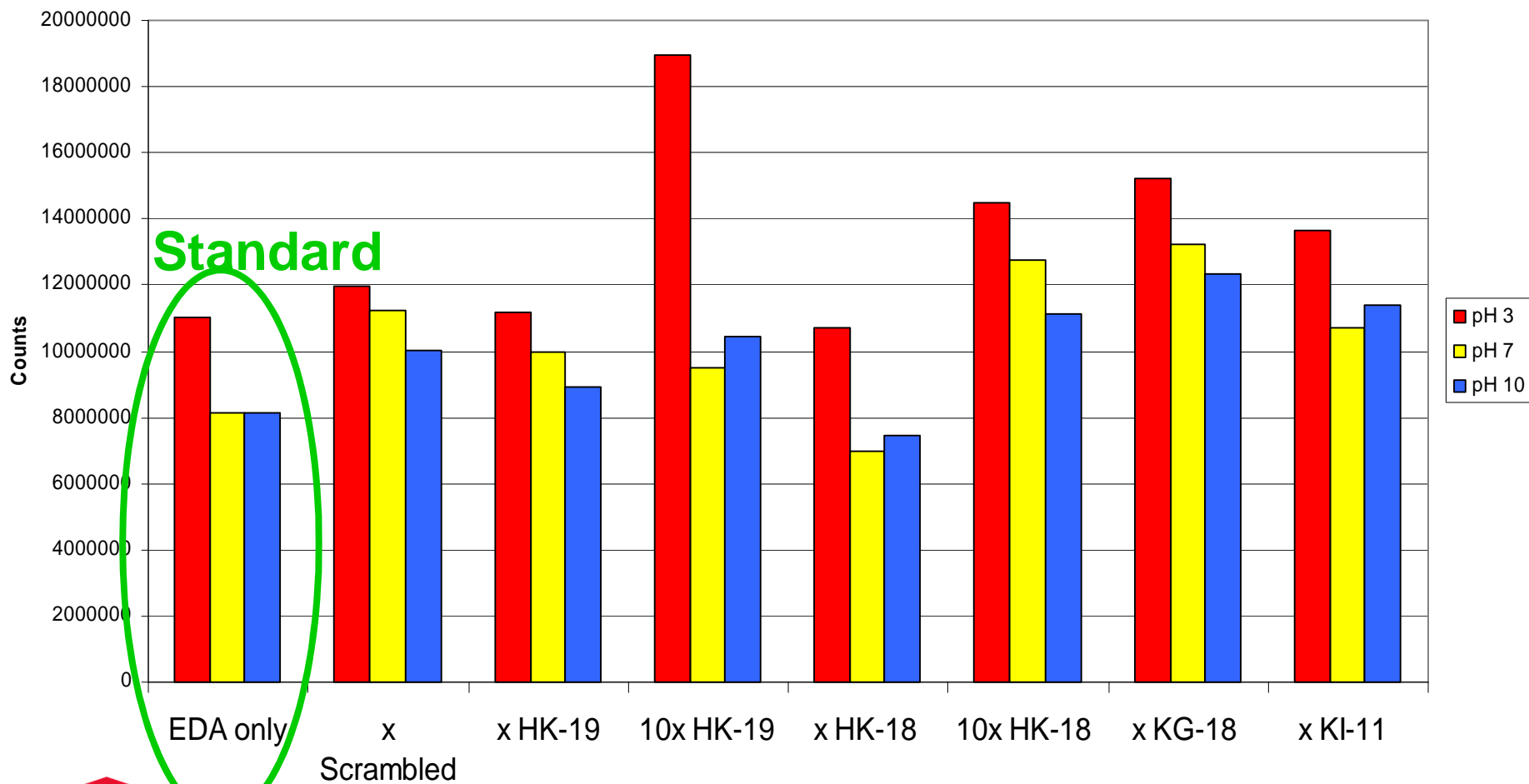


Do

POW CONFIDENTIAL - Do not share without permission

Leaf Dip MS Results

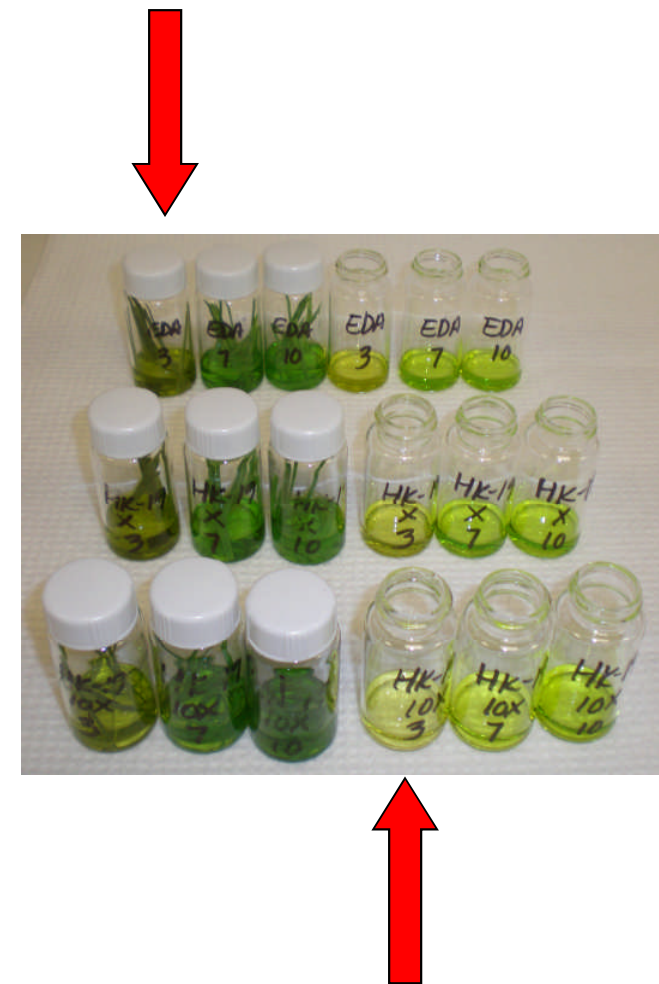
Relative Average



Dips

Conclusions

- Peptide is incorporated into capsule wall successfully
 - 100% peptide reacts with the capsule
- Capsules with peptide present show a varied increase in amount of CPF attached to rice leaf surface depending on pH
 - Acidic pH environment resulted in highest amount of capsules attached to surface but kills rice plants



Second Part of the Story

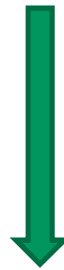
New DAS Herbicide for Rice Market

- Herbicide used to control ***Echinochloa crus-galli*** (barnyard grass)
 - Weed found in rice fields



Project Goals

- ▶ Determine Optimal Capsule Composition
 - Surfactants, Dispersants, Solvents
- ▶ Confirm that the peptide is incorporated into the capsule wall
- ▶ Evaluate if functionalized capsules are able to provide better biological activity



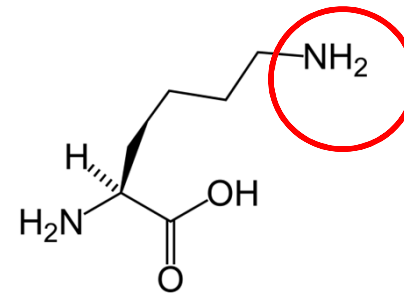
Peptide Selection

- The two peptides that showed the most promising results in the previous study were compared

The reaction for the wall formation with the peptides is made possible because lysine is one of the amino acids present in each peptide. This amino group is then free to react with isocyanate polymer

- **HIHRSTSHTGTP (HK-18)**
- **SGLGLGSGGLGLG (KG-18)- spider silk peptide**
 - **More hydrophobic peptide-thought to work well with the waxy leaf texture**

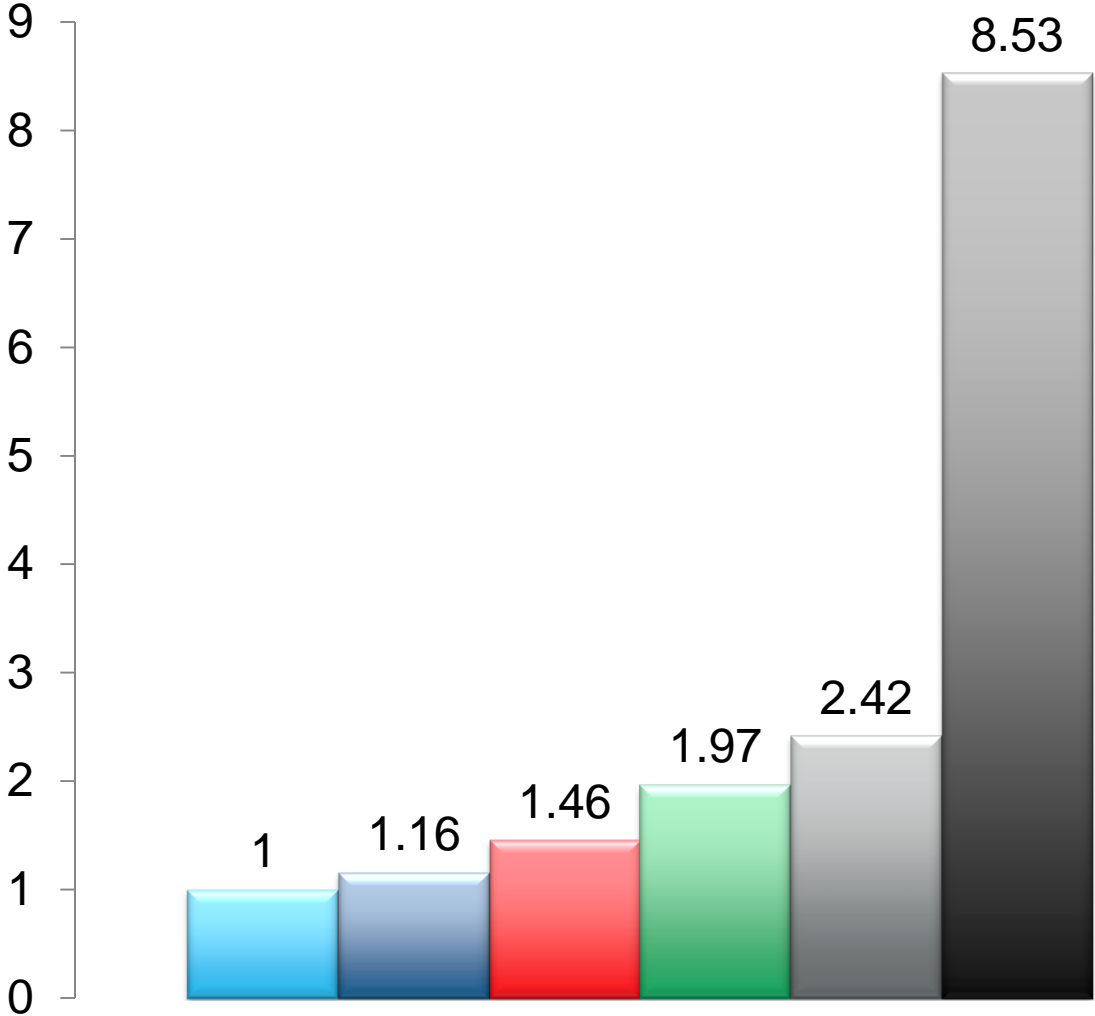
The sequences listed above are from the active sites of both peptides, the linker sequence (not shown) contains lysine



Lysine
(K)

Binding Data

HIHRSTSHTGTP
(HK-18)
SGLGLG SGLGLG
(KG-18)- spider silk peptide

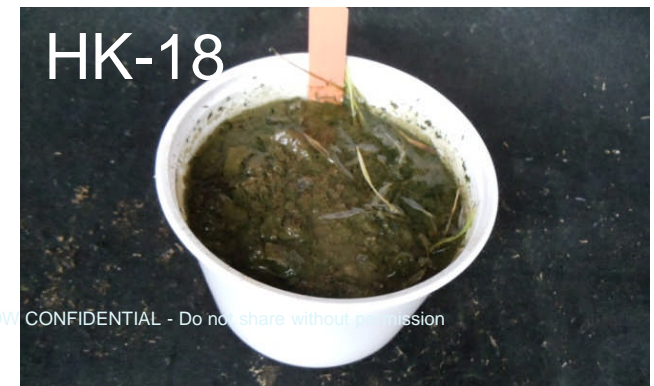
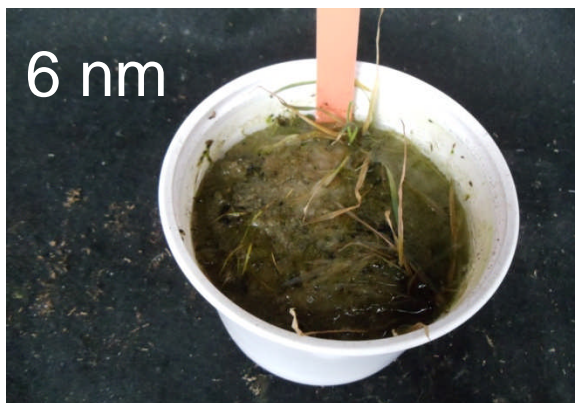


- Peptide free
- SC
- 5X-HK
- HK
- KG
- 5X-KG

SC vs. Functionalized and Peptide Free Capsules

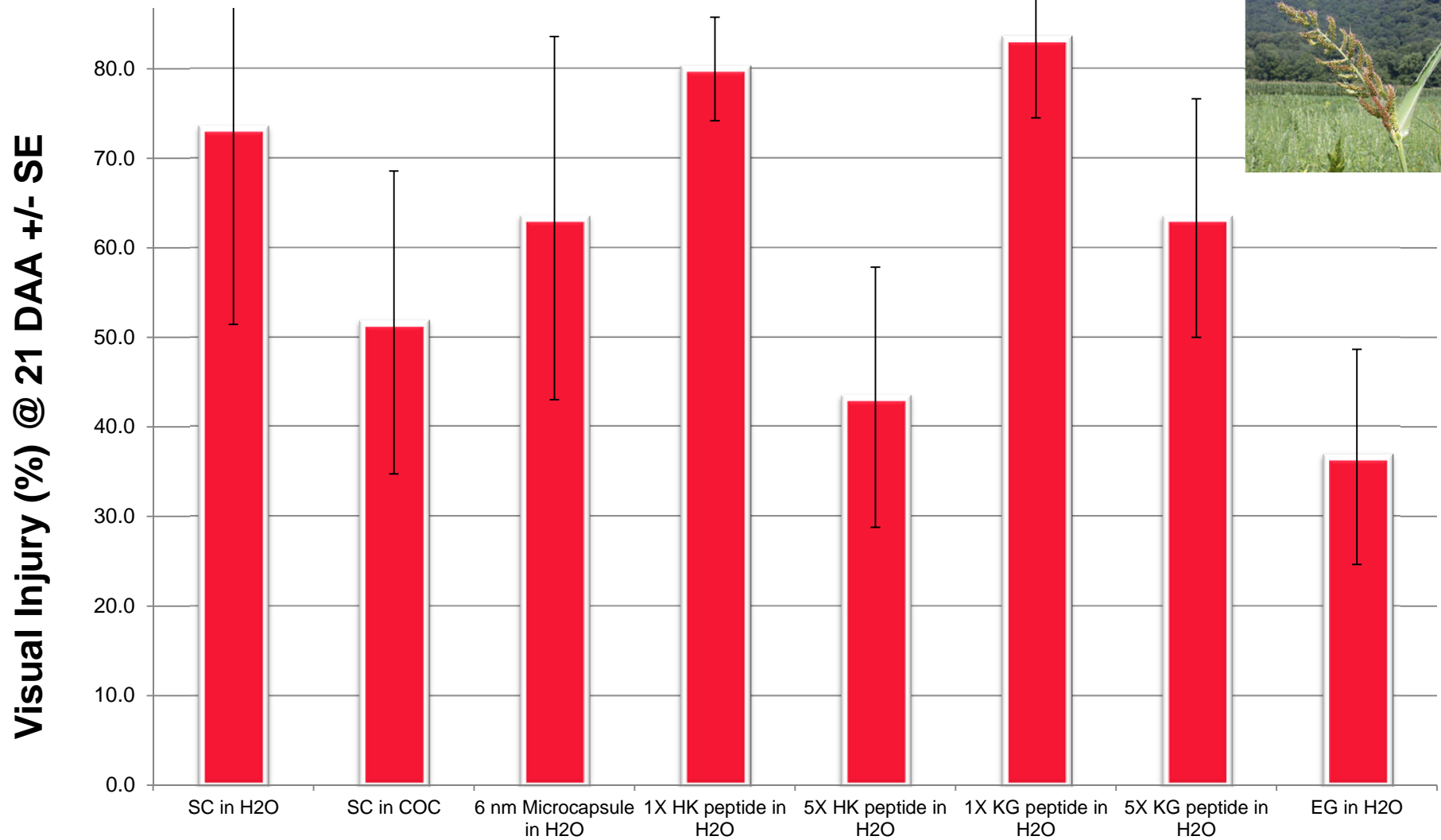
Application rate of 4.38 g ae/ha

Capsule Type	Visual Injury Rating
SC + water	30
SC + COC	60
6 nm Capsules	60
HK Peptide	80
KG Peptide	90



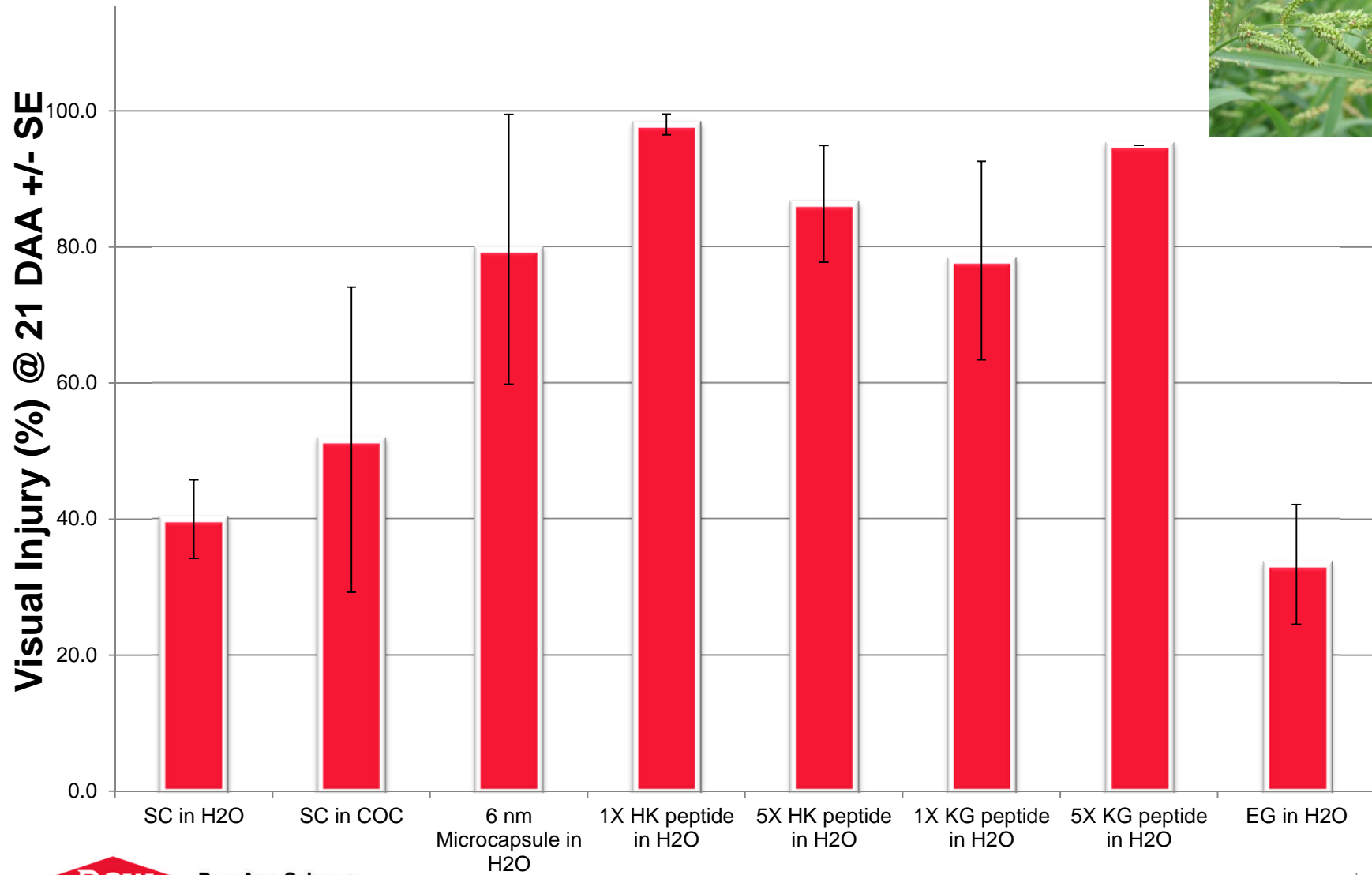
HIHRSTSHTGTP (HK-18)
SGLGLG SGLGLG (KG-18)- spider silk peptide

ECHCG Visual Injury @ 8.75 g ae/ha



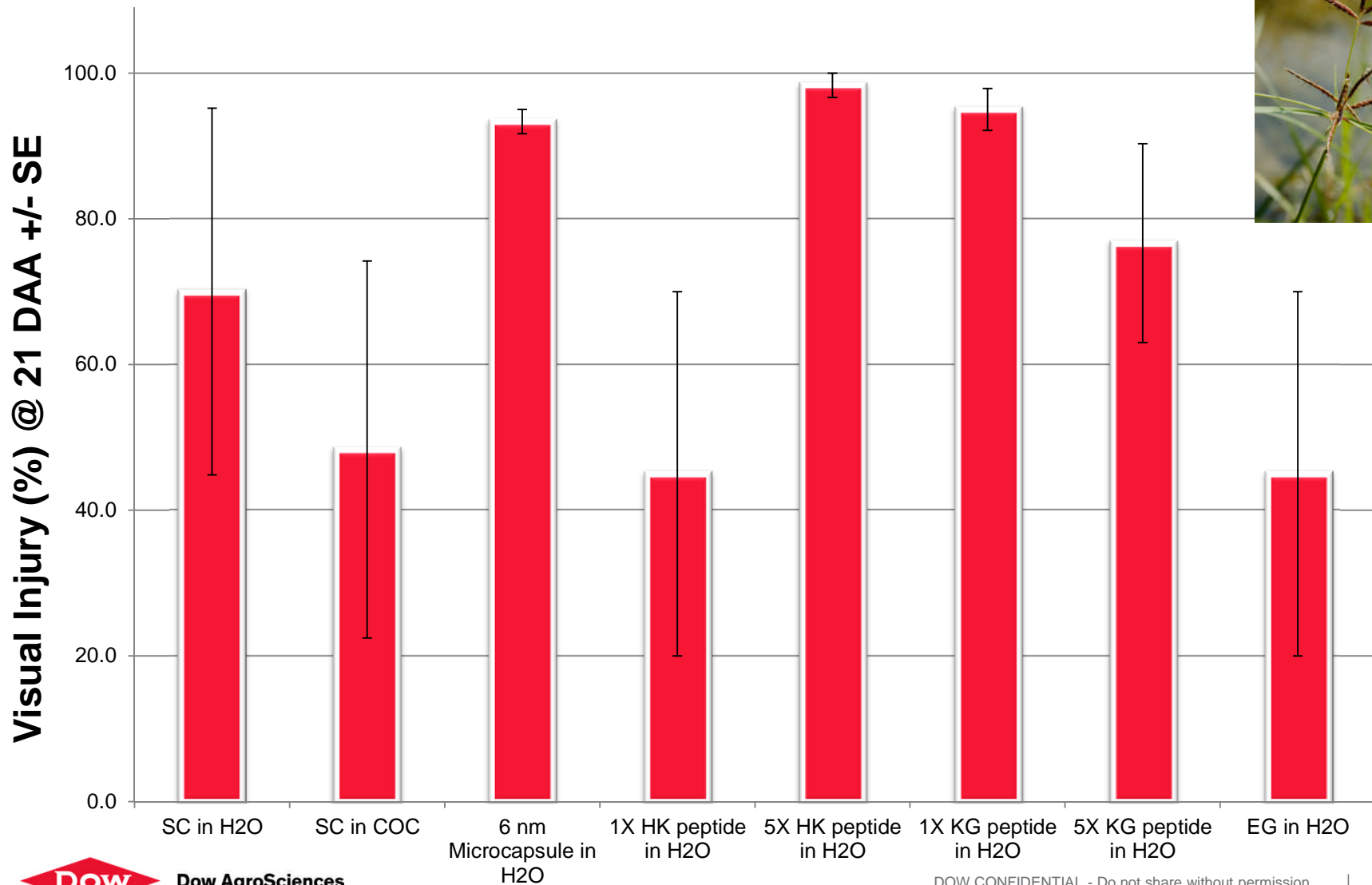
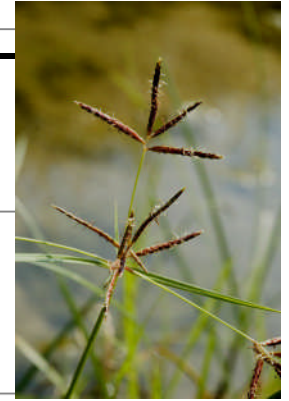
HIHRSTSHTGTP (HK-18)
SGLGLGSLGLG (KG-18)-
spider silk peptide

ECHOR Visual Injury @ 35 g ae/ha



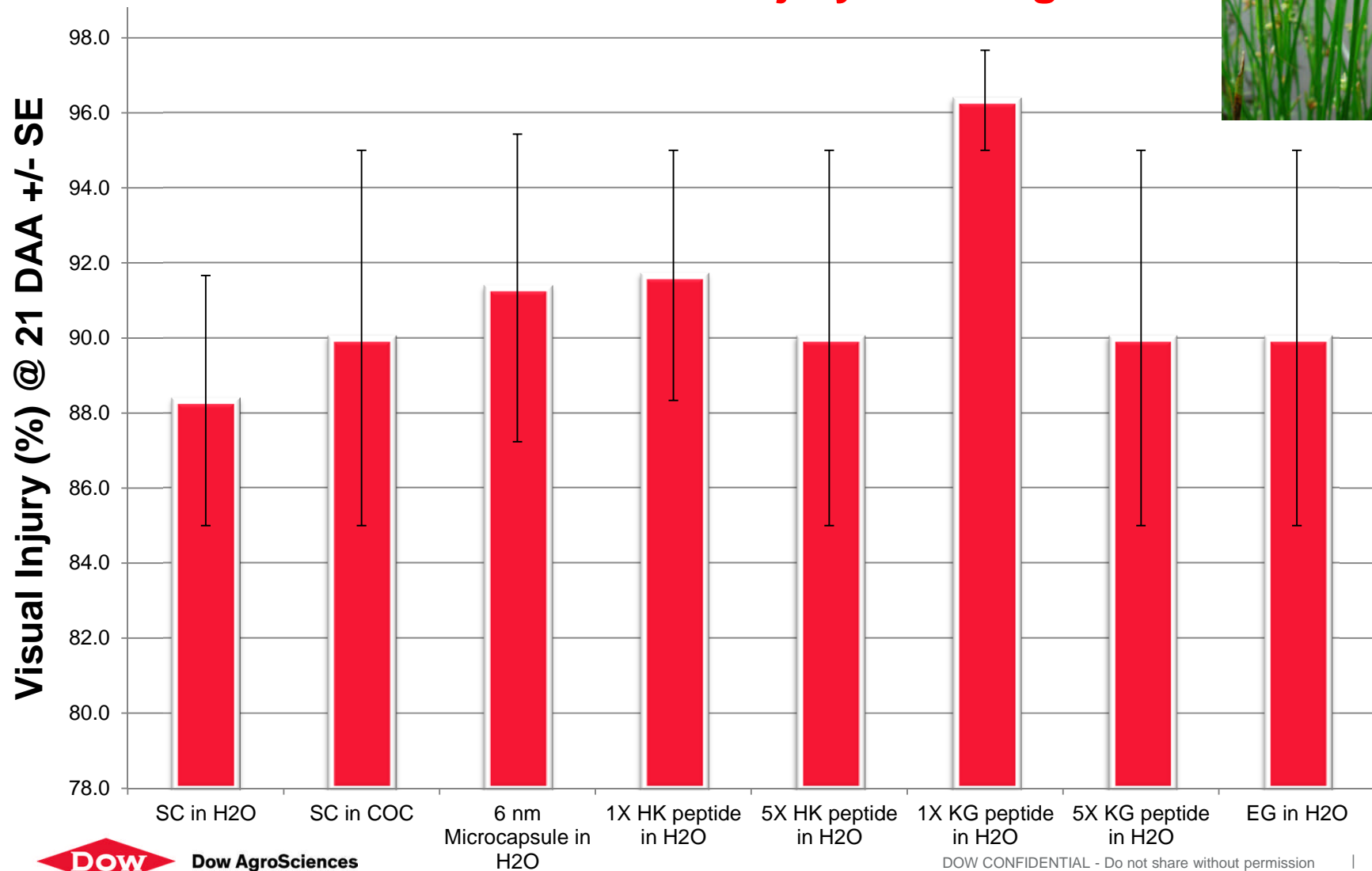
HIHRSTSHTGTP (HK-18)
SGLGLGSLGLG (KG-18)-
spider silk peptide

CYPRO Visual Injury @ 17.5 g ae/ha



HIHRSTSHTGTP (HK-18)
SGLGLG SGLGLG (KG-18)-
spider silk peptide

SCPJU Visual Injury @ 4.38 g ae/ha



Conclusions

- New Rice Herbicide molecule has been encapsulated successfully using a solvent mixture
 - Capsules obtained are very low diameter
 - Surfactant and Cross-linker choice is critical to obtain a stable system
- Capsules functionalized with peptides shown better binding to leaf surface vs non functionalized ones
- Green-house results shown some increase in efficacy even if data are not statistically significant and some inconsistencies are seen