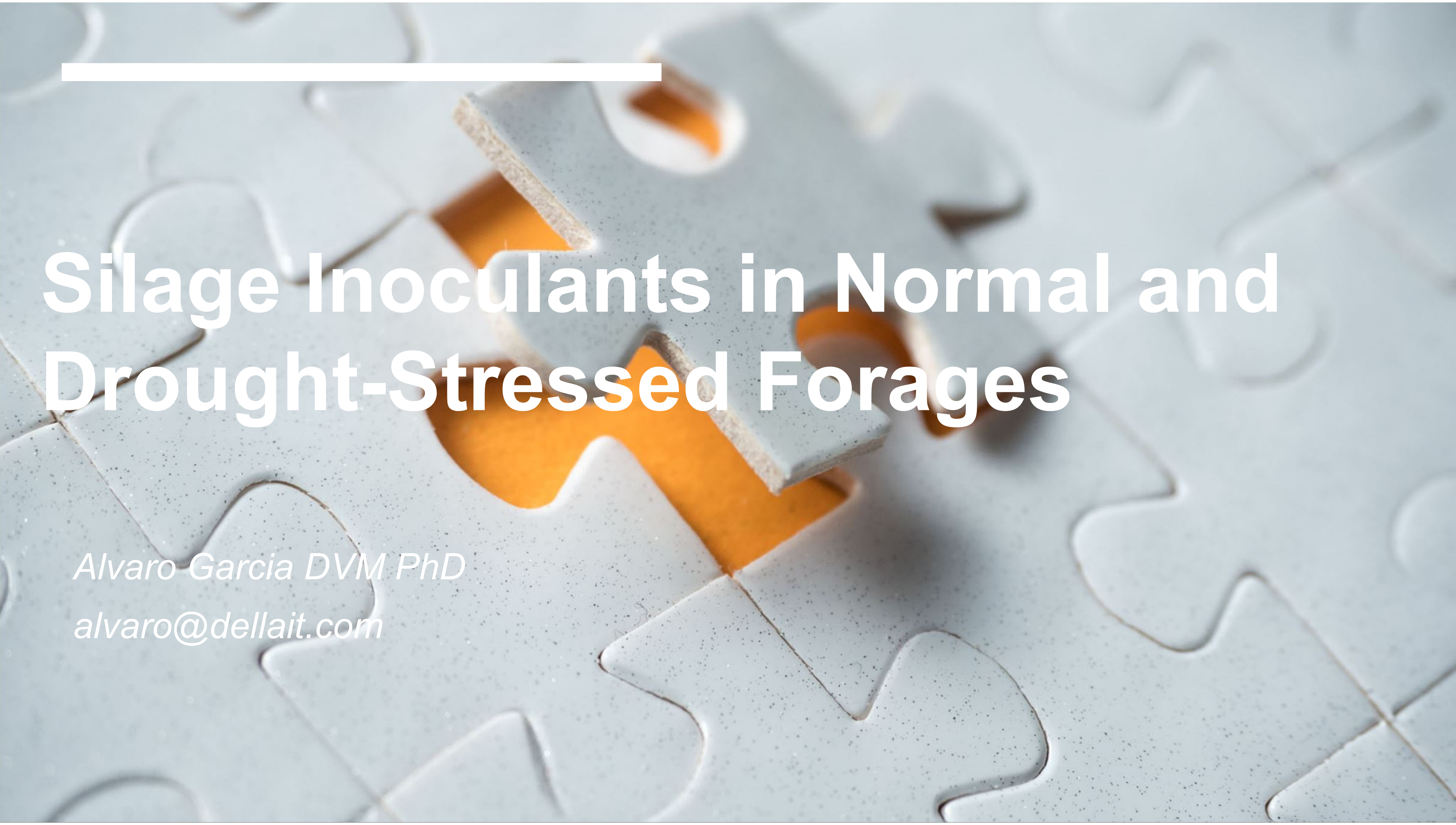
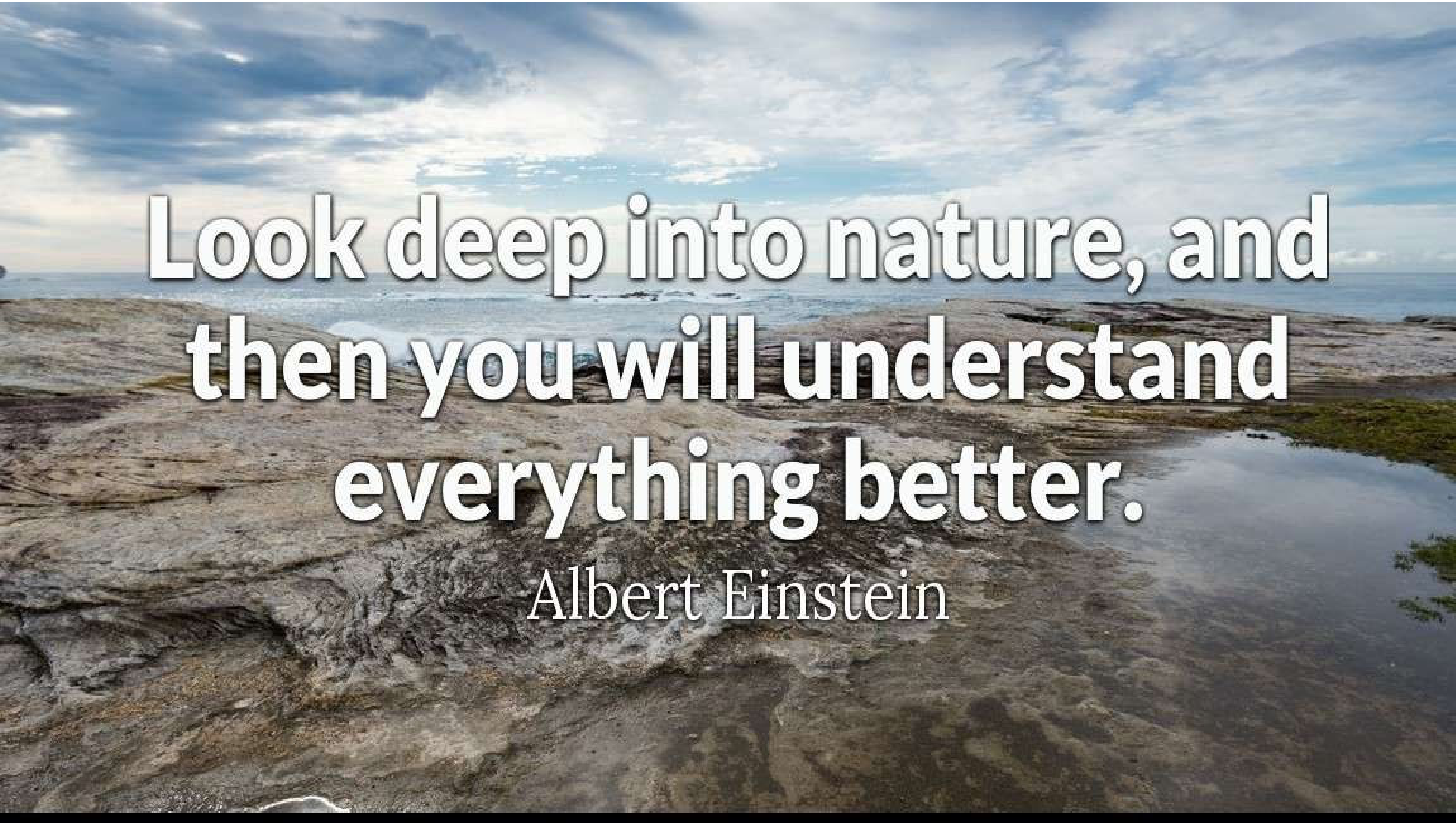
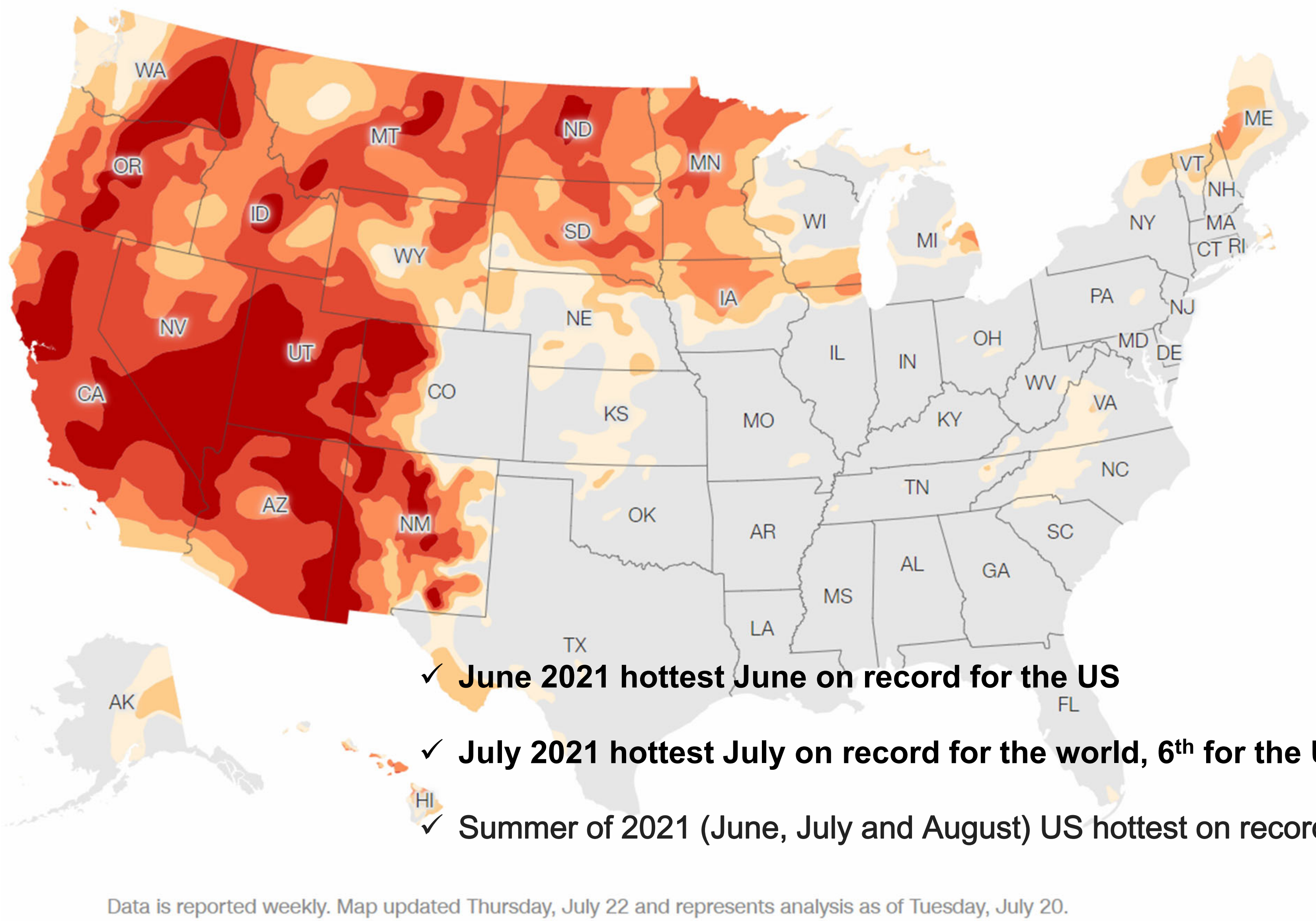


Silage Inoculants in Normal and Drought-Stressed Forages

Alvaro Garcia DVM PhD

alvaro@dellait.com


Look deep into nature, and then you will understand everything better.
Albert Einstein

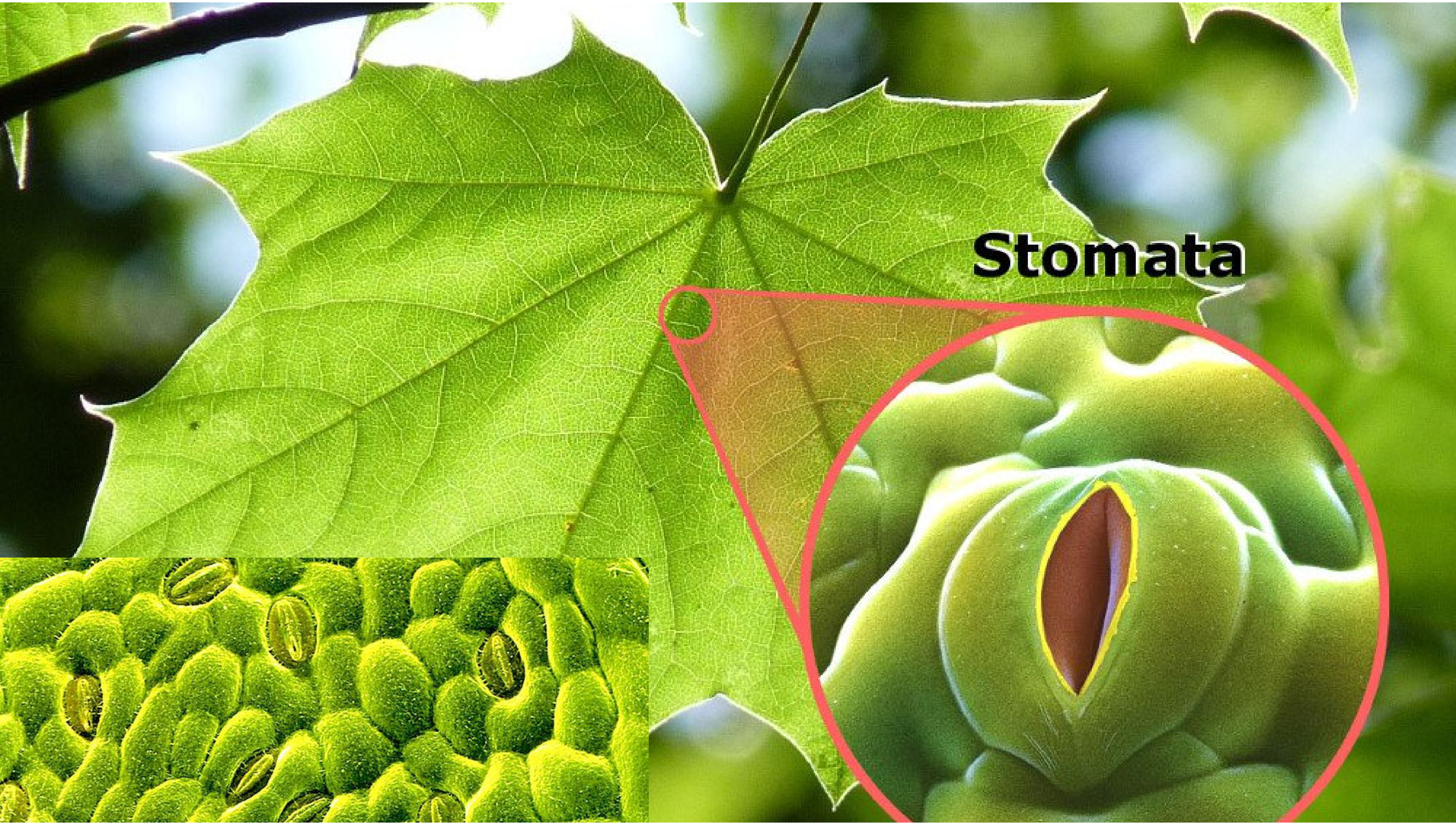


- ✓ **June 2021 hottest June on record for the US**
- ✓ **July 2021 hottest July on record for the world, 6th for the US**
- ✓ Summer of 2021 (June, July and August) US hottest on record

Plant metabolism during a drought

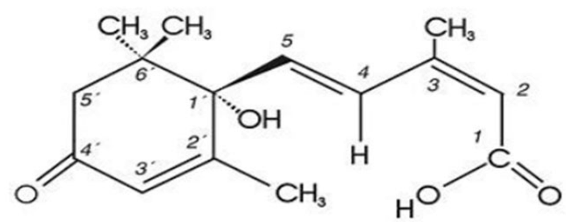
- To increase their chance of survival the plants reduce:
  1. water losses (closing pores or “stomata”).
  2. resources utilization (downregulate photosynthesis)

- Downregulation of photosynthesis reduces growth.

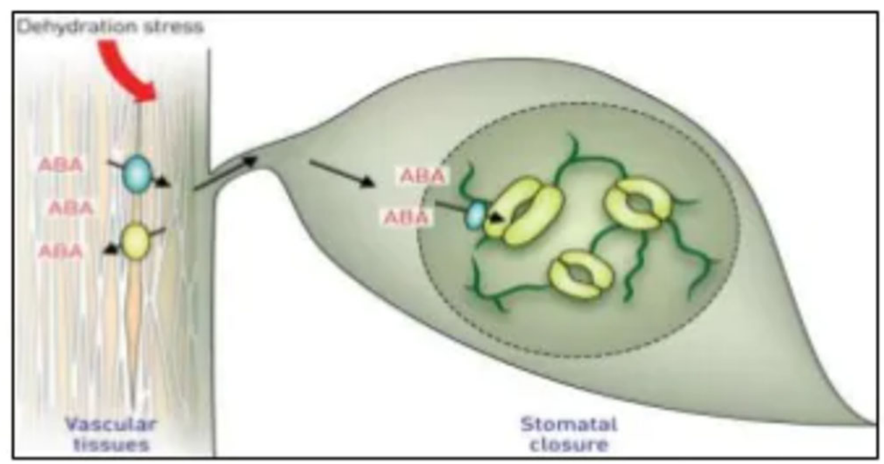

Stomata

Abscisic acid (ABA): “the cortisol of plants”

- Change in ions and water transport stimulates **ABA** synthesis which closes stomata.

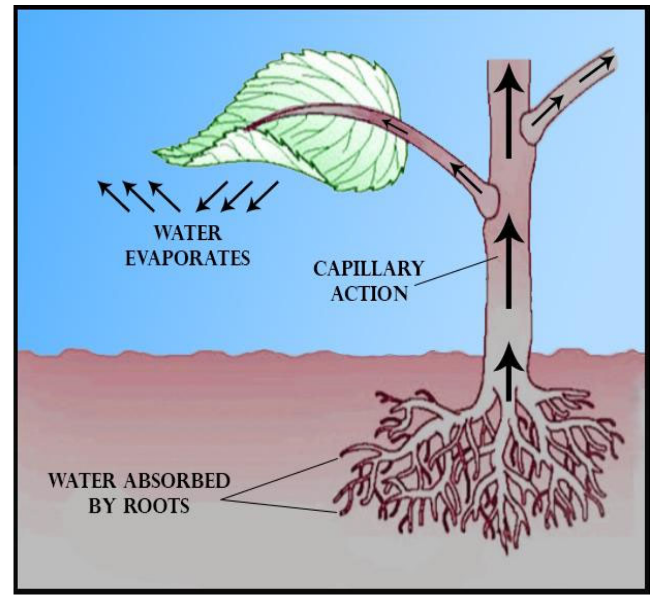


- **ABA is responsible for:**
  - ✓ Seed and bud dormancy
  - ✓ Germination inhibition
  - ✓ Promoting leaf detachment
  - ✓ Determine plant organelle size



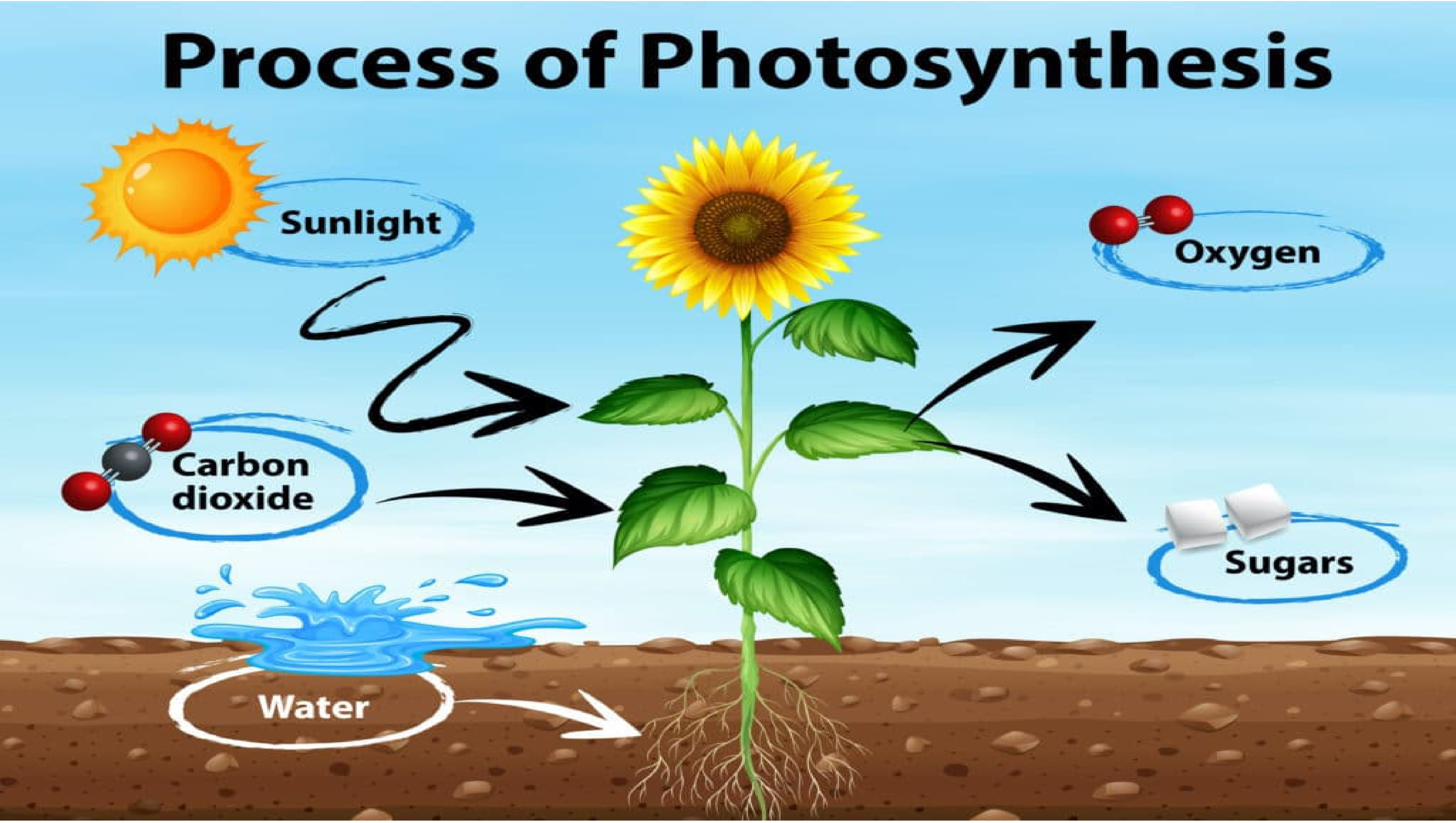
Evaporative cooling the "plant radiator"

❑Water uptake: 1 % for metabolism, 99% for mineral uptake + **evaporative cooling**.



❑Pore closure saves water but **shuts-off evaporative cooling**, foliage temperature ⬆

❑⬆Temperatures slow-down metabolism and save energy and nutrients for regrowth once moisture improves.


Process of Photosynthesis
Sunlight
Carbon
dioxide
Water
Oxygen
Sugars

Soluble sugars “the hormones”

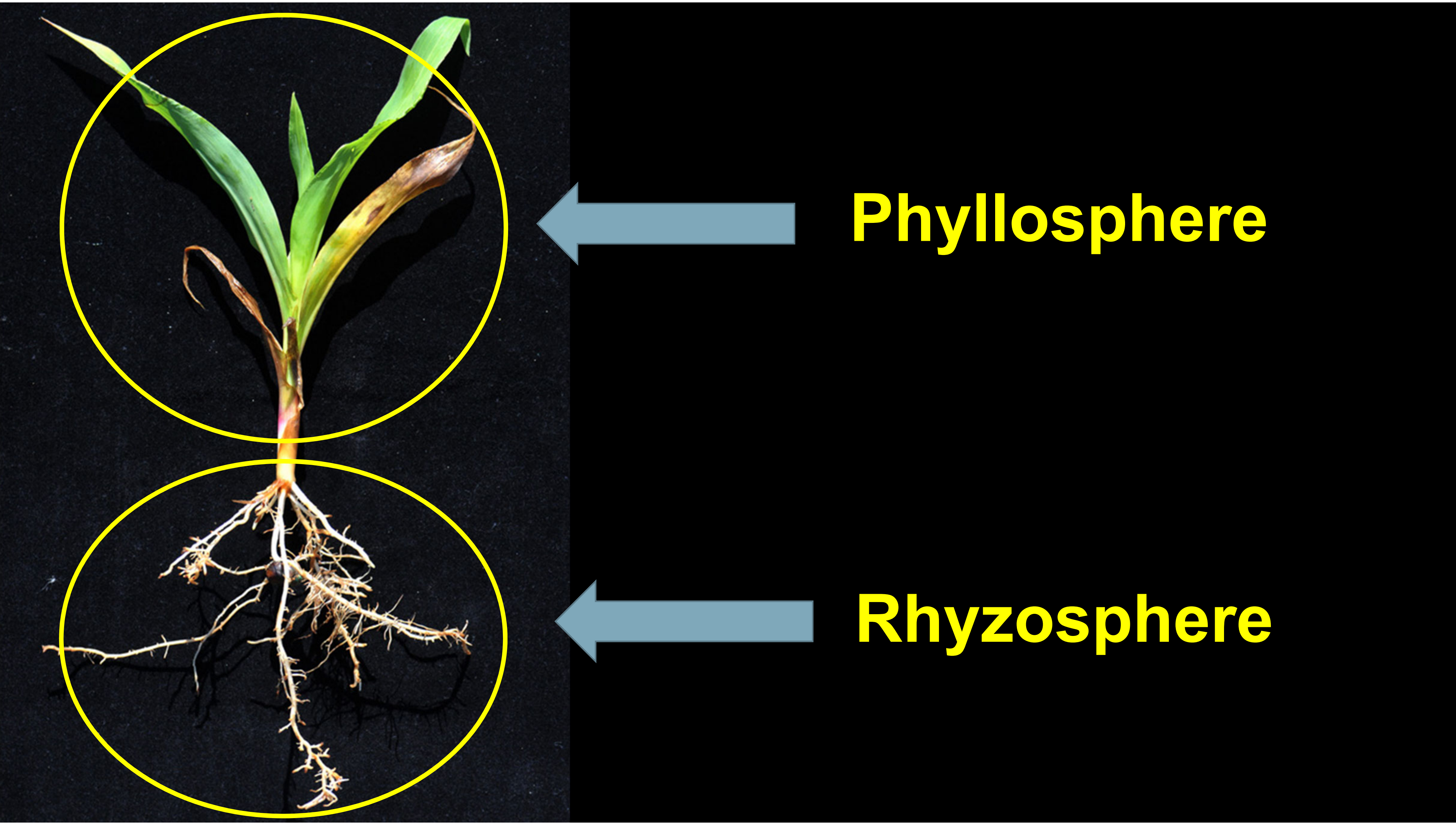
- Regulate signals for genes that promote or inhibit growth.
- **In short supply:** they signal a need for photosynthesis and mobilization of reserves to be used elsewhere.
- **In high supply:** they promote growth and CHO storage.

During a drought

- Photosynthesis less affected than respiration and growth, NFC synthesis increases to facilitate regrowth once conditions improve.

- Restricted stem growth (fiber), improved stem/leaf ratio and reduced starch deposition in kernels, increase fiber digestibility and protein.


The plant microbiota


Phyllosphere
Rhyzosphere

Epiphytic organisms of the phyllosphere

- Bacteria (LAB no spores), yeasts (spores), and molds (spores).
- Dry conditions, microbial cells loose water (increased osmolality).
- Protein synthesis is altered affecting DNA replication, transcription and translation, membrane integrity, and metabolic processes.
- Less water activity leads to oxidative stress and cellular death.

The use of silage additives

- Forage preservation through fermentation used in ancient Egypt (second millennium BC).
- Early 20th century, A. I. Virtanen (Finland), developed a system by adding acids to enhance conservation.
- Other additives have been developed to achieve a desired or inhibit undesirable fermentations.

Bacteria:

- **<u>Homofermentative:</u>** glucose produces two molecules of lactic acid, yielding high DM recovery and little energy loss from the silage. **Lactic acid production.**

- **<u>Heterofermentative:</u>** glucose produces one molecule of **lactic acid**, one of **acetic acid** or **ethanol**, and one of **CO_2.**

- CO_2 production = DM losses. **<u>Acetic acid is not as strong an acid as lactic.</u>**

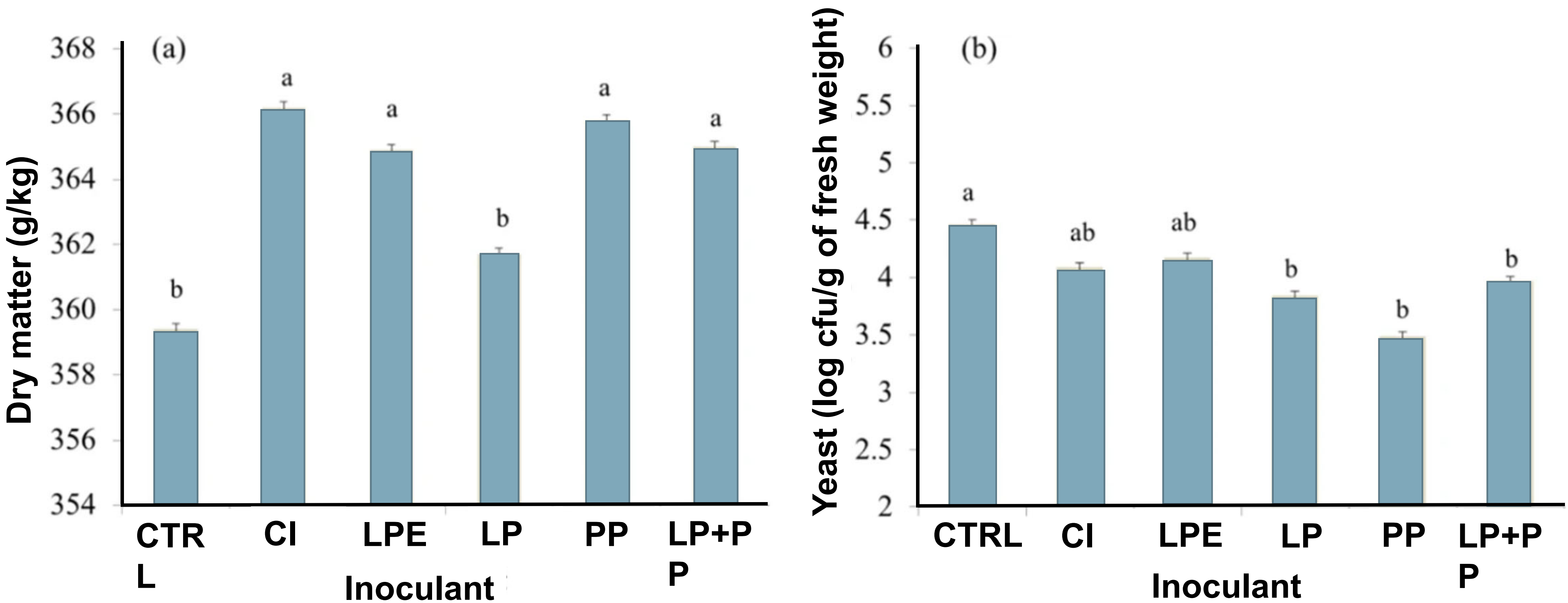
Bacteria:

- *Lactobacillus plantarum* and *acidophilus, Pediococcus acidilactici and pentosaceus,* and *Enterococcus faecium*.

- Synergy: different ideal DM, range of optimal temperatures and pH. E.g. **Growth speed Enterococci > Pediococci > Lactobacilli.**

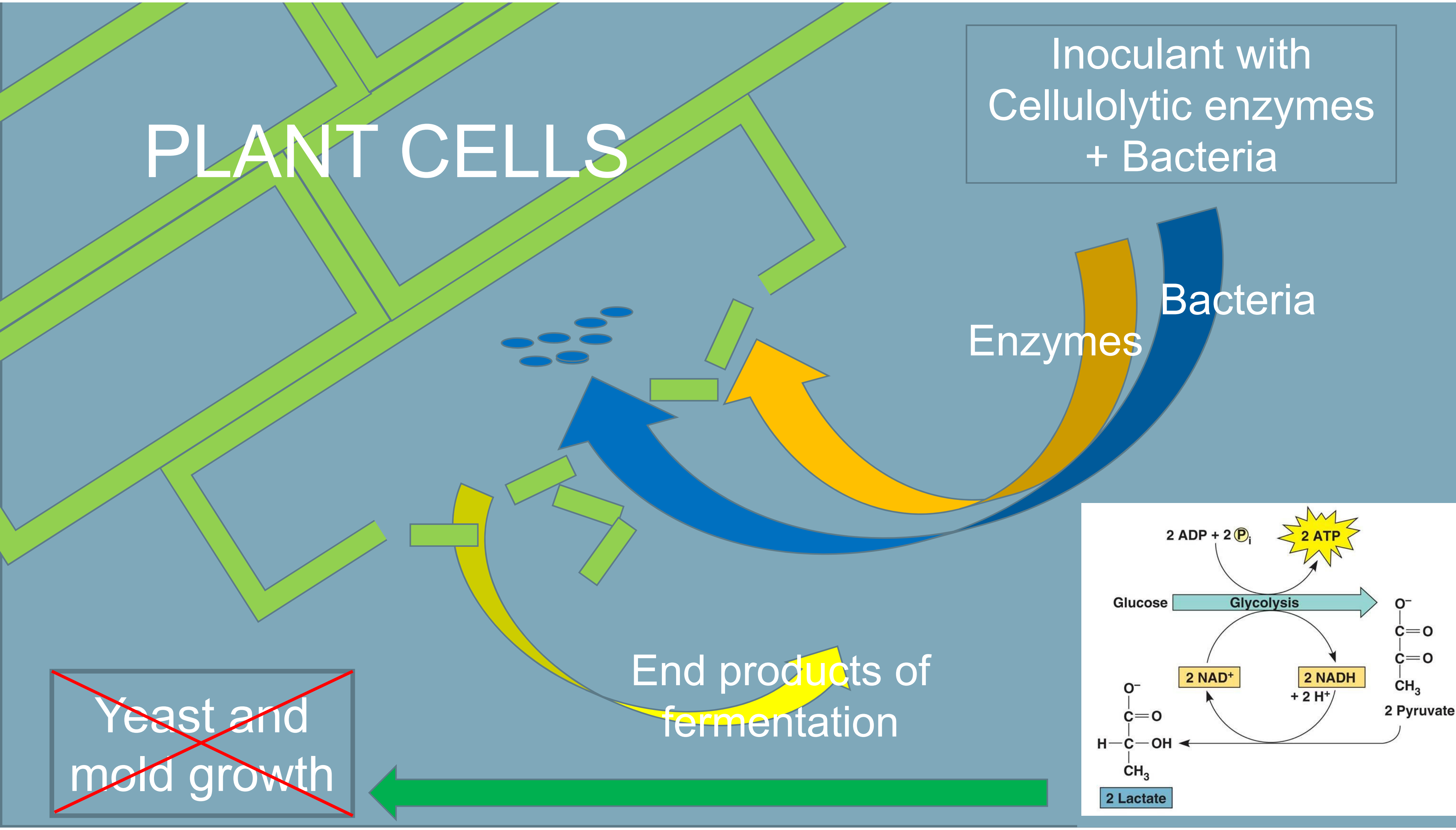
- Recommended inoculation: 100,000 CFU/g of wet forage; no cost-effective response to doubling or tripling this amount.

From: Novel lactic acid bacteria strains as inoculants on alfalfa silage fermentation



Effect of the microbial inoculants on the dry matter content (**a**) and yeast population (**b**) of alfalfa silages. [a,b]Means followed by different letters are significantly different according to Tukey's test ($P < 0.05$). CTRL = Control (without inoculant); CI = Commercial inoculant LPE = *Lactobacillus pentosus*; LP = *Lactobacillus plantarum*; PP = *Pediococcus pentosaceus*; LP + PP = *Lactobacillus plantarum* + *Pediococcus pentosaceus*.

Nascimento Agarussi et al. 2019


PLANT CELLS
Inoculant with Cellulolytic enzymes + Bacteria
Bacteria
Enzymes
End products of fermentation
~~Yeast and mold growth~~
2 ADP + 2 P_i
2 ATP
Glucose
Glycolysis
2 NAD^+
2 NADH + 2 H^+
2 Pyruvate
2 Lactate

Cellulolytic enzymes:

- Most popular: cellulases, hemi-cellulases and xylanases.
- Degrade plant cell walls, ease bacterial access.
- Increase the lactic/acetic ratio (improve efficiency of fermentation)
- Rapid pH reduction inhibits proteolytic enzymes, and ammonia release which would negatively affect palatability and intake.

Cellulolytic enzymes:

- Effect of fibrolytic enzymes sprayed on alfalfa harvested at late maturity and ensiled for 40 and 120 days.

Enzymes included:

1) mixture of cellulase and xylanase (CELL+XYL)
2) xylanase (XYL)
3) ß-glucanase (GLUC)
4) mixture of ß-glucanase and xylanase (GLUC+XYL)

Ordaz UofVermont thesis. 2017

Cellulolytic enzymes:

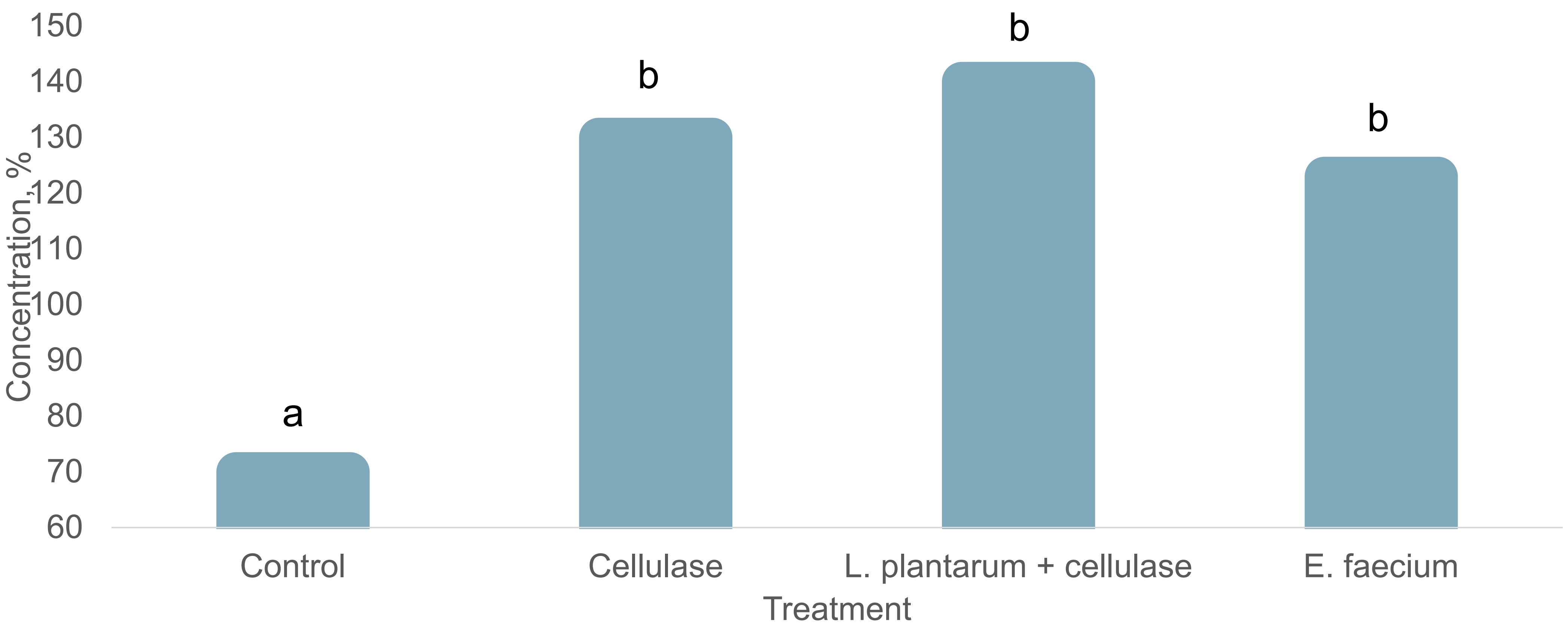
NDF and ADF content at 40 d of ensiling tended to decrease.

VFA production increased at 40 and 120 d of ensiling in silages treated with CELL+XYL.

Fiber digestibility was greater for uNDF at 24 h incubation with CELL+XYL at 40 d of ensiling.

Ordaz UofVermont thesis. 2017

Effect of additives on the conversion of water-soluble carbohydrates into lactic acid in alfalfa silage.



Liu et al. 2020

Fermentation and microbial counts of alfalfa silages (g/kg DM)

	Control	Cellulase + L. plantarum
DM g/100 g	33.5 ± 0.3[a]	35.0 ± 0.20[b]
pH	5.2 ± 0.18[a]	4.7 ± 0.07[b]
WSC g kgDM-1	13.0 ± 0.73[a]	13.5 ± 0.93[b]
Fermentation products		
Ammonia (g kgDM-1)	7.8 ± 0.41[a]	5.3 ± 0.61[b]
Lactic acid (g kgDM-1)	30.7 ± 1.17[a]	51.2 ± 2.41[b]
Acetic acid (g kgDM-1)	12.6 ± 1.48[a]	17.5 ± 0.98[b]
propionic acid (g kgDM-1)	1.8 ± 0.07[a]	0.2 ± 0.06[b]
Butyric acid (g kgDM-1)	3.1 ± 0.23[a]	0.0 ± 0.02[b]
LAB(log cfu g−1 FM)	7.5 ± 0.16[a]	8.5 ± 0.08[b]
Nutrient composition		
Crude protein	21.7 ± 0.09[a]	23.5 ± 0.59[b]
NDF	43.1 ± 1.70[a]	40.2 ± 2.05[b]
ADF	34.9 ± 0.56[a]	31.3 ± 1.59[b]

[a, b, c] **Means with different letters in the same row differ ($P < 0.05$). From Hu et al 2020**

Cattle performance (cellulolytic enzymes)

- **Eun and Beauchemin (2017)** Endo and exoglucanase + xylanase added to alfalfa hay and corn silage at different dosages.

- Endo and exoglucanase improved NDF degradability of corn silage

- Combining polysaccharidases improved fiber degradation.

- Improved fiber degradation of corn silage also decreased acetate/propionate suggesting greater OM conservation.

Dairy cattle performance (enzymes in TMR)

- **Romero et al. (2016)** cows fed enzyme-treated TMR which included 10% bermudagrass.

- Xylanase at 1 mL/kg of TMR DM increased DMI (23.5 vs. 22.6 kg/d), OM (21.9 vs. 20.9 kg/d), and crude protein (3.9 vs. 3.7 kg/d).

- Increased milk yield (kg/d) during weeks 3 (41.2 vs. 39.8 kg/d), 6 (41.9 vs. 40.1 kg/d), and 7 (42.1 vs. 40.4 kg/d).

Beef cattle performance (Lactobacillus in TMR)

- **Vasconcelos et al. (2008)** the effects of feeding live cultures of L. acidophilus + Propionibacterium freudenreichii

- Four treatments with P. freudenreichii combined with L. acidophilus at 4 different concentrations.

- Live cultures of L. acidophilus NP 51 + P. freudenreichii NP 24 increased efficiency of cattle fed steam-flaked corn-based diets by approximately 2%, the effects depending on the dose of Lactobacillus.

When to use inoculants?

- Some farmers use them all the time, some never!
- Research results are also hit and miss; some excellent results, some not so much.
- There are crops that under a good growth year, and excellent management, do very well without inoculants but some...

Cover crops

Table 1. Carbohydrate fractions of small grain silages						
	Barley	Millet	Oats	Rye	Triticale	Wheat
% Acid Detergent Fiber; ADF	35.0	40.2	38.4	37.5	38.1	36.9
% Neutral Detergent Fiber; NDF	54.4	61.3	58.6	57.7	58.5	56.6
% Ethanol sol. carbs (sugars)	5.5	4.3	5.1	6.7	5.1	6.3
% Starch	9.2	3.1	3.5	1.7	2.3	6.3
% TDN	62.5	54.6	60.5	61.8	60.8	60.8
NDFD 30hr, % of NDF	60.0	53.6	59.9	65.2	63.9	60.9

Garcia. 2021. In print

Cover crops' structural carbohydrates

- ❑Millet showed the highest NDF (61.3%) and ADF(40.2%).
- ❑NDFD 30 h: millet the lowest at 53.6%.
- ❑Well-fermented silages very low values of sol. CHO Corn silage approx. 2%, cover crop silages 5-7%, except millet 4.3%.
- ❑Steps to improve accessibility to plant cell contents (e.g., chopping, inoculants) will improve silage fermentation.

Garcia. 2021. In print

Millet for growing beef experiment

- Silage treatments: corn silage (CS), direct-cut millet + inoculant (MS) or inoculant + 0.5% ground corn (MSC).

- Millet silages improved fermentation compared with those in Experiment 1.

- Each silage treatment fed to steers (initial weight 272 kg) in a drylot, for 56 days.

Hill et al. (2013)

Millet for growing beef. Experiment 2

- Steers fed CS had higher ADG and better feed efficiency than steers fed millet (M) or millet + corn grain (M+C).

	M	M+C	CS
ADG, kg/d	0.78	0.78	1.22
Gain:Feed	7.35	7.92	5.05

- Both millet silages had the same ADG showing the inoculant increased the energy supplied by the forage comparable to the supplemental grain.

Hill et al. (2013)

Does it pay to use inoculants?

- When using an inoculant, we want to:

1. Improve silage preservation
2. Prolong aerobic stability at feed-out
3. Obtain greater return on investment!

<u>Greater return on investment:</u> from improved livestock performance and/or reduce the use of expensive concentrates or both!

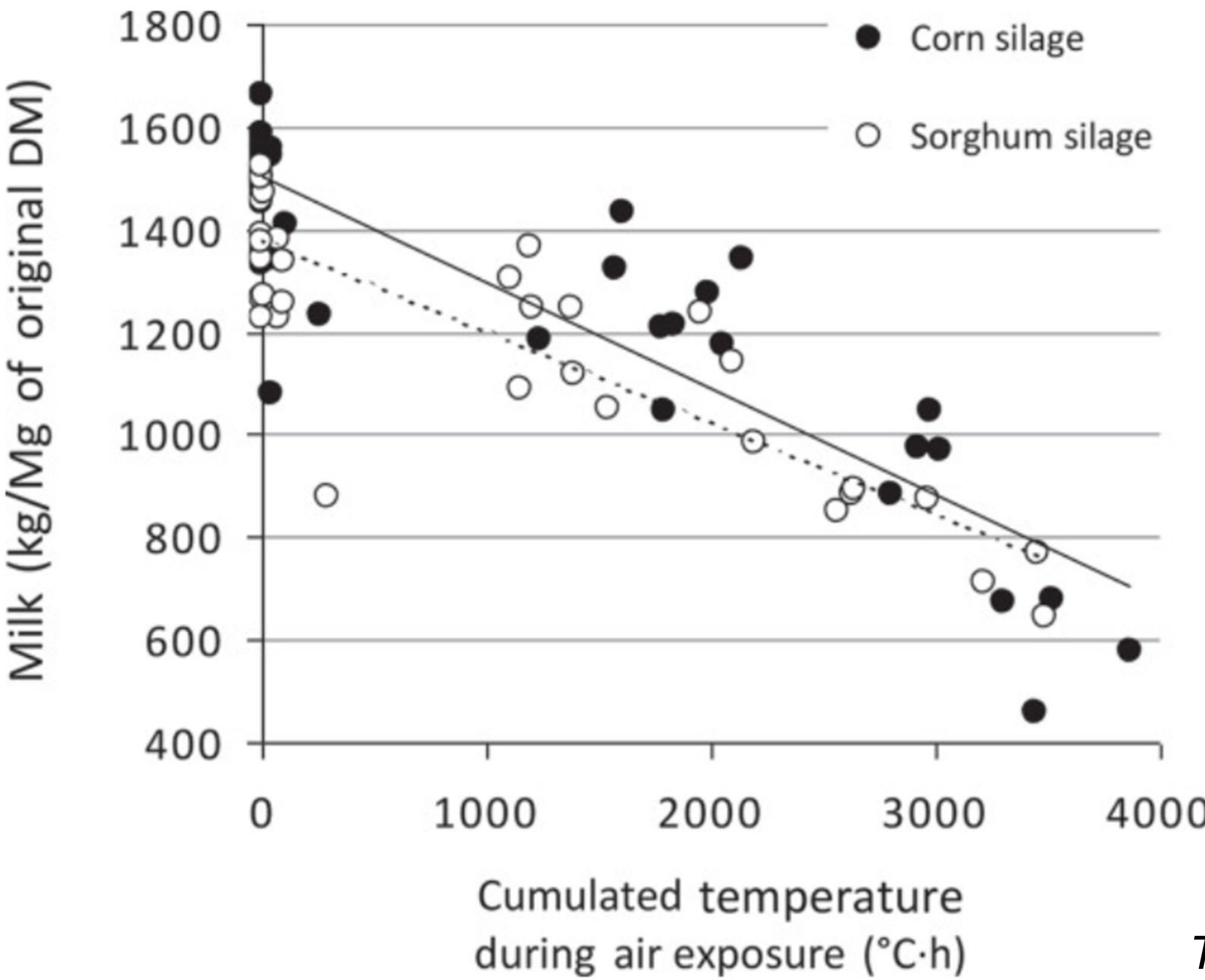
Losses with deteriorated silage

- Each 8.3°C increase in temp in 1 ton of 30% DM silage requires over 6.3 Mcal or around 4 L in milk production/ton of silage

- When aerobic spoilage (sum of hourly differences between silage and air temperature) reached 1,000°C·h the average loss was 10% of expected milk yield.

1,832 ° F

Hoffman and Combs, 2009



Tabacco et al. 2011b

Losses with deteriorated silage

1,832 ° F

- Accumulated temperature-time rise of 1,000°C·h corresponded to 2 to 3 d of silage heating (mold only began to be visible after an accumulated temperature-time rise of at least 1,400°C·h).

2,552 ° F

- When temps begin increasing, nutritive value can decrease up to 16% before molds become visible.

To:	Crosswind Jerseys CHS Inc 21672 482nd Ave Elkton, SD 57026	**Account No.:**	2479 (1)
		Sampled By:	Crosswind Jerseys
		Sampled For:	CROSSWIND JERSEYS
Product:	corn silage 2020 4-5-21	**Test Mode:**	N3
		Feed Type:	Whole plant corn
		Sub Type:	Conventional

Moisture	65.73%
Dry Matter	34.27%
pH	3.29

Corn silage statistics provided for comparison.

		Dry Basis	**Median**	**90% Range**
Crude Protein	%DM	7.28	7.21	5.80 - 9.00
AD-ICP	%DM	0.41	0.72	0.48 - 1.08
ND-ICP w/SS	%DM	0.66	1.02	0.73 - 2.42
Protein Sol.	%CP	73.35	51.05	25.02 - 72.00
Ammonia-CP	%CP	10.30	6.76	2.38 - 10.23
Ammonia-CP	%DM	0.75	0.49	0.17 - 0.72
ADF	%DM	21.96	24.47	19.93 - 30.46
aNDF	%DM	36.68	39.68	33.43 - 49.20
aNDFom	%DM	35.79	38.84	32.21 - 48.10
Lignin (Sulfuric Acid)	%DM	2.64	3.29	2.43 - 4.43

To: Crosswind Jerseys
CHS Inc
21672 482nd Ave
Elkton, SD 57026

Account No.: 2479 (1)
Sampled By: Crosswind Jerseys
Sampled For: CROSSWIND JERSEYS

Product: corn silage 2020 4-5-21

Test Mode: N3

Corn silage statistics provided for comparison.

		Dry Basis	Median	90% Range
NDFD12	%NDFom	39.12	32.80	28.50 - 37.50
NDFD 30	%NDFom	65.07	53.74	47.40 - 62.75
NDFD 120	%NDFom	75.30	68.39	62.68 - 74.88
NDFD240	%NDFom	77.56	70.80	65.58 - 78.20
Sugar (ESC)	%DM	1.18	1.85	0.54 - 5.66
Sugar (WSC)	%DM	2.19	3.58	0.99 - 6.63
Lactic Acid	%DM	5.79	3.86	0.47 - 5.61
Sugar (ESC)	%DM	1.18	1.85	0.54 - 5.66
Sugar (WSC)	%DM	2.19	3.58	0.99 - 6.63

		ADF	OARDC	MLK 2006 NonProc	MLK 2006 Processed	ISU Beef
TDN	%DM	72.47	74.48	77.65	78.00	74.31
Nel 3x	Mcal/cwt	75.33	77.49	75.91	77.48	
Neg	Mcal/cwt	47.09	50.46	56.75	56.75	
Nem	Mcal/cwt	74.66	78.48	85.70	85.70	81.48

Return on Investment

- In 14 lactation studies, when L. plantarum was applied to grass, corn silage or alfalfa, it ***improved DMI by 4.8% and milk yield by 4.6%.***

- Single inoculant used in 5 dairy lactation studies and 4 beef studies.
  - **Dairy:** Milk/cow increased 0.76 L per day.
  - **Beef:** Silage intake increased by 6.14%, and ADG and Gain/Feed improved by 8.0 and 3.4%, respectively.

- Cost of inoculant per ton of silage: $1 as fed or $3 per ton of DM

Return on investment: dairy

- **Research: Improved intake by 4.8% and yield by 4.6%.**
  - ✓ 24 lbs. silage DM/cow/d); inoc. = $0.001.4/lb. of silage DM x 24 lbs. DM = **$0.03/cow/d**
  - ✓ 88 lbs. milk/d @ 4.6% increase = 92 lbs. **(4 lbs. milk/cow/d)**
  - ✓ DMI 4 lbs. milk @ 1.5 effic. = 2.7 lb. feed/d x $0.15 Feed = **$0.40**
  - ✓ $0.64 (4 lbs of $16 CWT milk) – ($0.40 Feed$ + 3 cents inocul.) = **$0.21 per cow/d**

Return on investment: beef

- Cost of inoculant per ton of silage: $1 as fed or $3 per ton of DM
- **Research: Beef improved AVG daily gain by 8%.**
  - ✓ Silage inclusion 45% of diet DM or 6 kg DM silage/steer/d
  - ✓ $0.003/kg of DM inoculated silage x 6 kg DM silage = $0.02
  - ✓ ADG daily gain increase by 8% = 0.12 Kg/d
  - ✓ **ADG 0.12 kg @ $2.7/kg = $0.32 - $0.02 inoculation = $0.30/kg**

In summary

- Solid and extensive body of research suggests inoculants both as enzymes and/or bacteria should improve silage fermentation.
- Despite this research results are often variable an inconsistent likely due to differences in silage fermentation and crop year.
- When conducting inoculants' research, it would be advisable to use a range of potential rates rather than just the "standard".

Questions?