SCBA – January 13th, 2022

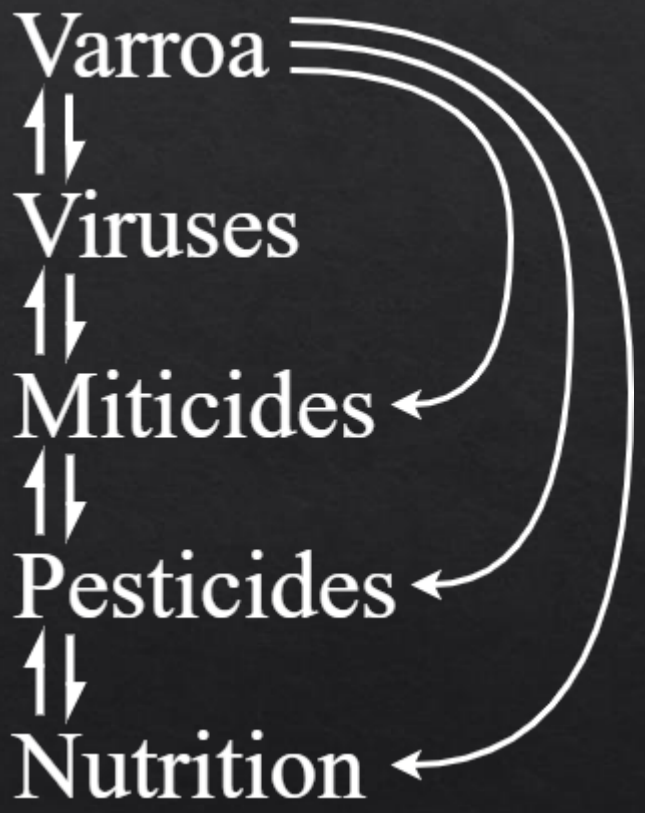
The Saskatraz™ Project

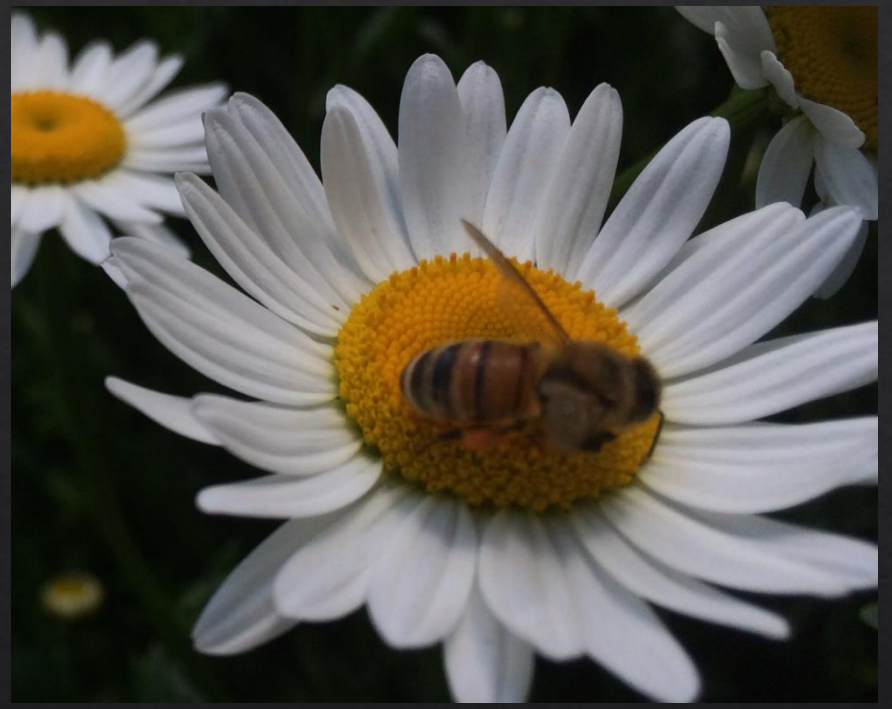
Queens Produce Superior Workers

Objective: To develop productive, gentle honeybees with tolerance to mites and brood diseases

By: Albert J. Robertson
The Saskatchewan Honeybee Breeding and Selection Program

Current Honeybee Health Issues





Outline

- Review of Saskatraz Breeding Program
- Biomarker Development (Micro and Kinome arrays) and screening Saskatraz Families for Virus Susceptibility
- Saskatraz Hybrid Project
  - Olivarez Honey Bees Inc. - Orland, CA www.OHBees.com
- Combined Miticide Treatment Experiments with selected and unselected stock
- Commercial Beekeeping at Meadow Ridge Enterprises with Saskatraz Stock
- Please visit www.saskatraz.com for reviews and publications

Saskatraz Breeding Program

Primary Selection Criteria:

- Honey Production
- Wintering Ability
- Spring Population Growth
- Varroa Resistance and Suppression
- Resistance to Brood Diseases
  (Chalk Brood, AFB, EFB, etc.)
- Viruses and Nosema Susceptibility

Breeding methods used to select and enrich for important traits (natural selection, out crossing, back crossing, recurrent selection, progeny analyses and closed population mating).

The Original Saskatraz Apiary and Crew

Saskatraz natural selection yard site fall 2006 – fenced.
Selection for this Saskatraz yard site is a death sentence.

Saskatraz Team Members

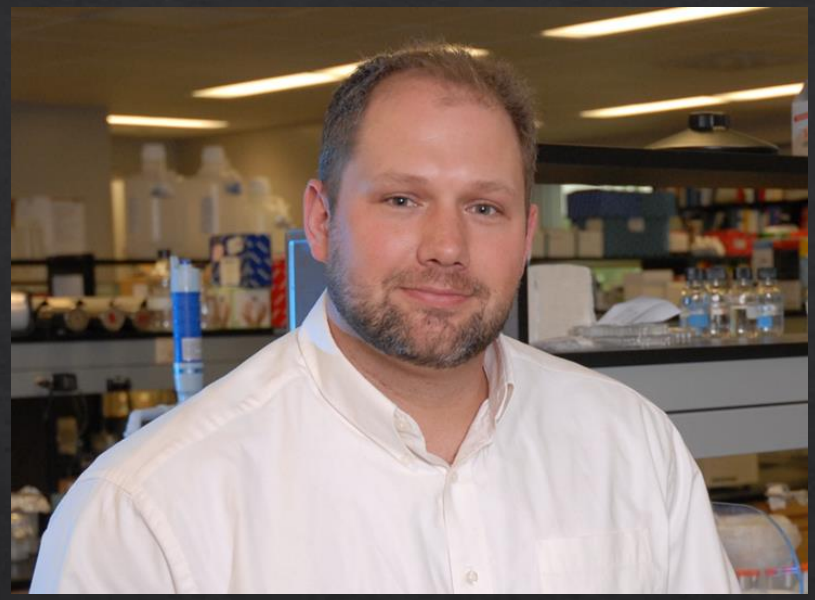
Dr. Syed Shaw

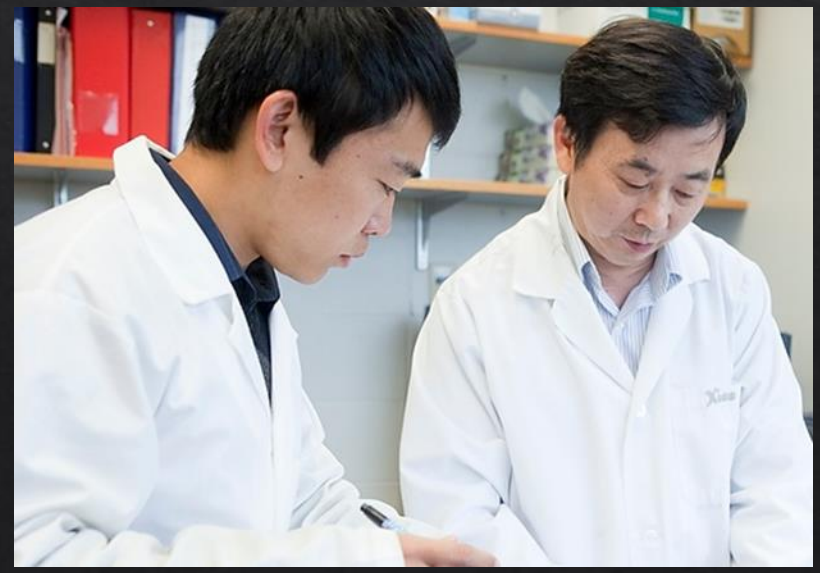
Mohammad Mostajeran

Dr. Philip Griebel

Wayne Connor

Dr. Scott Napper





Sanjie Jiang

Dr. Xiao Qiu

Saskatraz Team Members

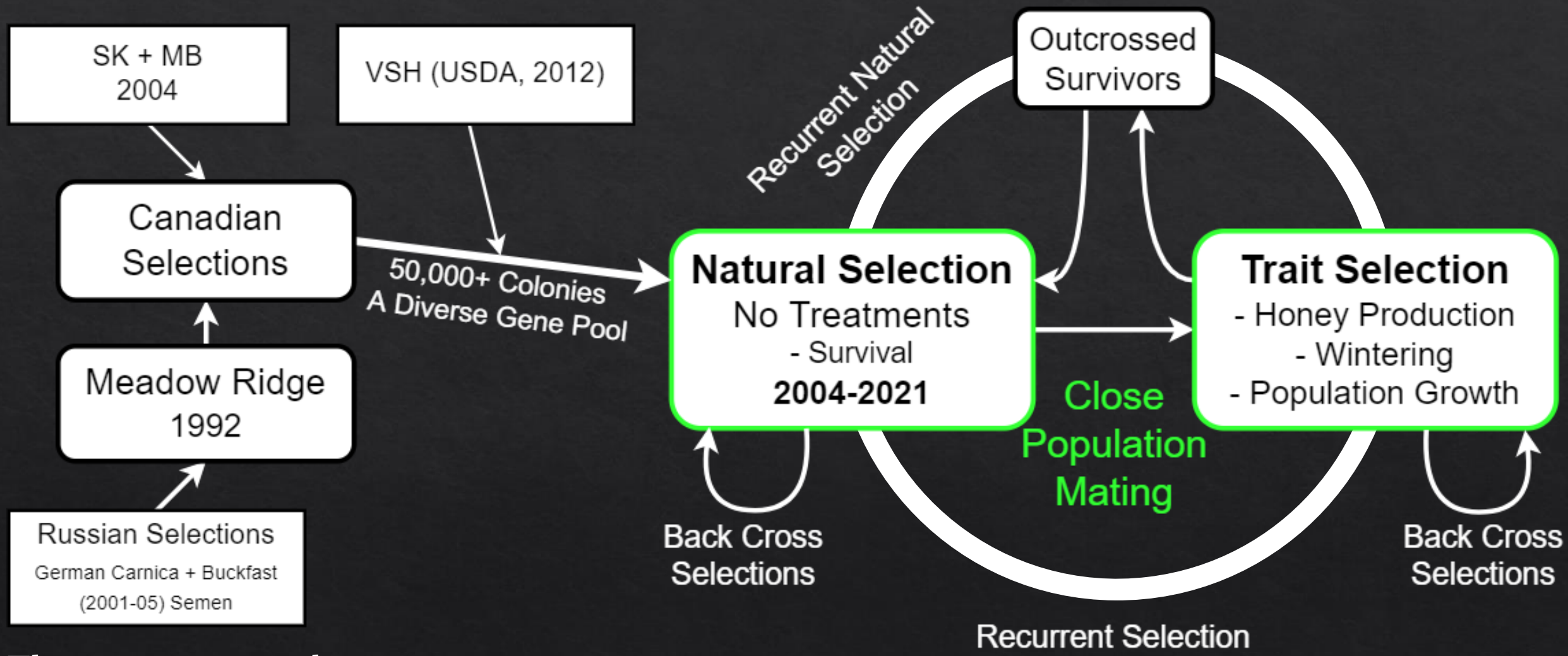
Dr. Abdullah Ibrahim

Antonio Munoz Cerna

Edmundo Munoz Cerna

Colton Rutherford

Saskatraz Breeding Program Logistics



There are currently:

17 Saskatraz Families

Stock Distributed Yearly Since 2006

Saskatraz Breeding Program Logistics

Close Population Mating

Progeny Analysis

To Stabilize Traits

Up to 30 colonies from best breeders

Best daughters crossed between apiaries (SY26 x S96)

Kokay's - SY26

Ben's - S96

Marciniak's - SL25

Trucker - S88

Scott's - S113

Natural Selection

No Treatments

Bainsville

Martins

Economic Trait Selection

Honey Production

+ Winter Survival

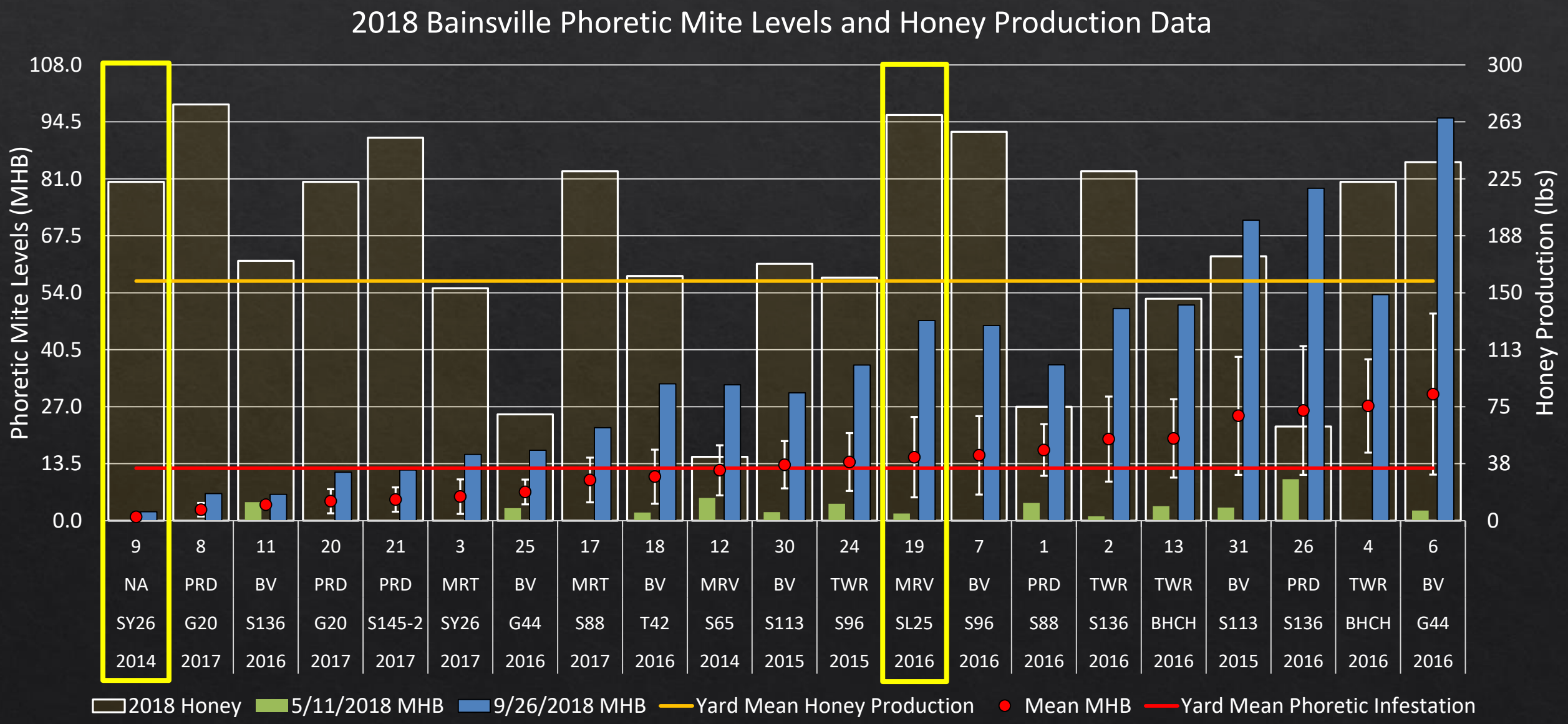
Annual Selection from 50+ Apiaries (1500+ Colonies)

Priddy's

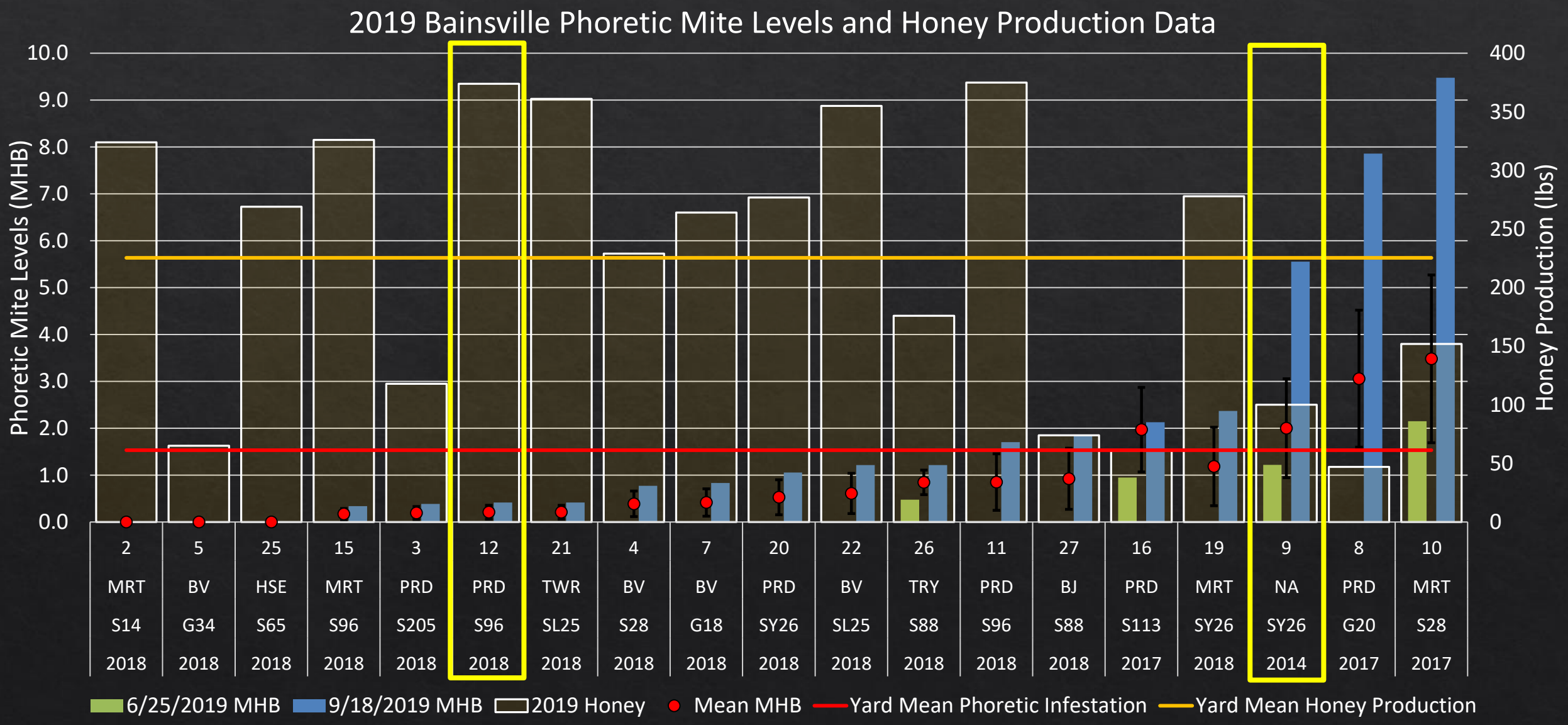
Murphy's

Tower

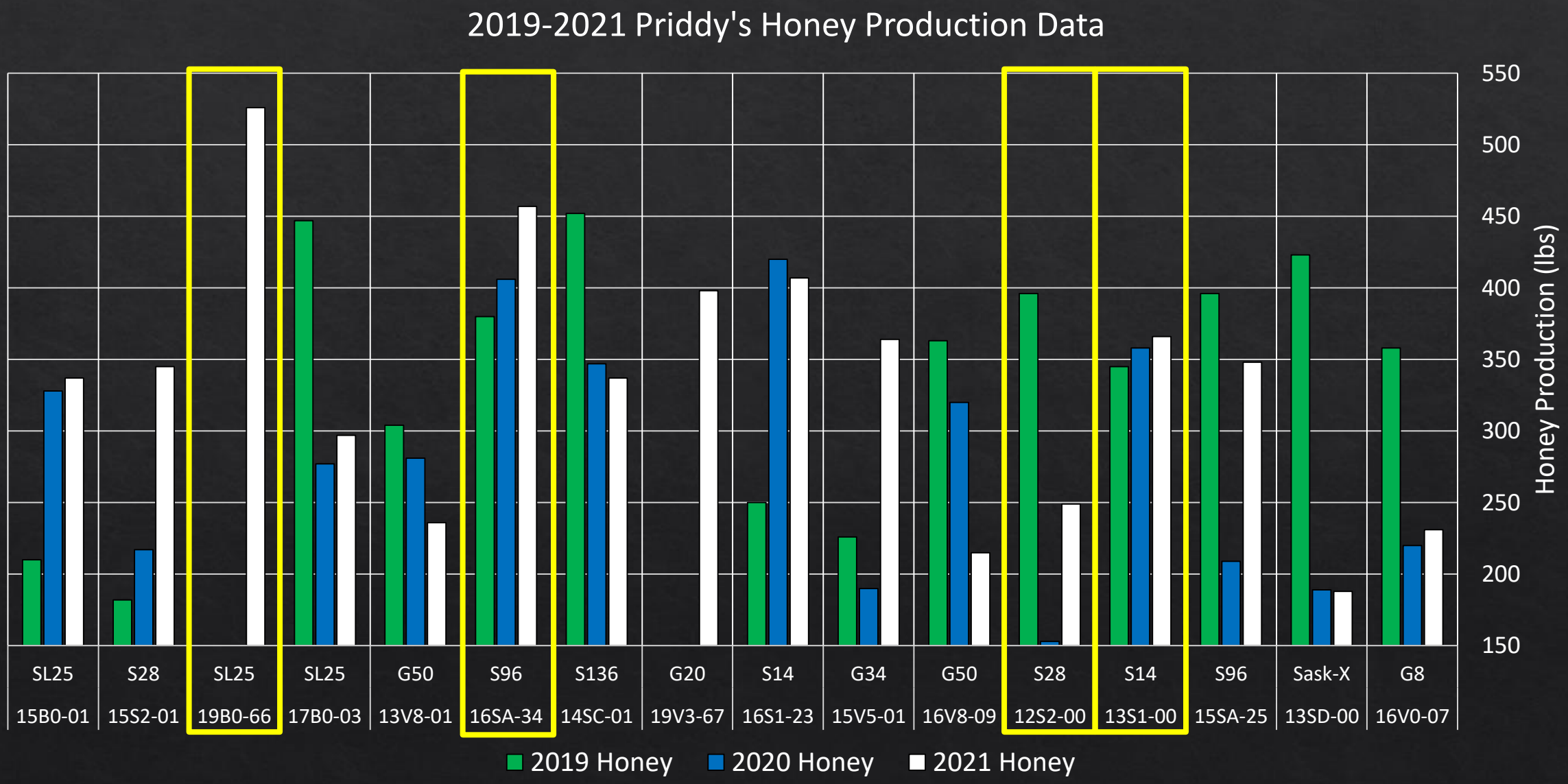
Natural Selection for Varroa Tolerance 2018



Natural Selection for Varroa Tolerance 2019



Selection for Honey Production



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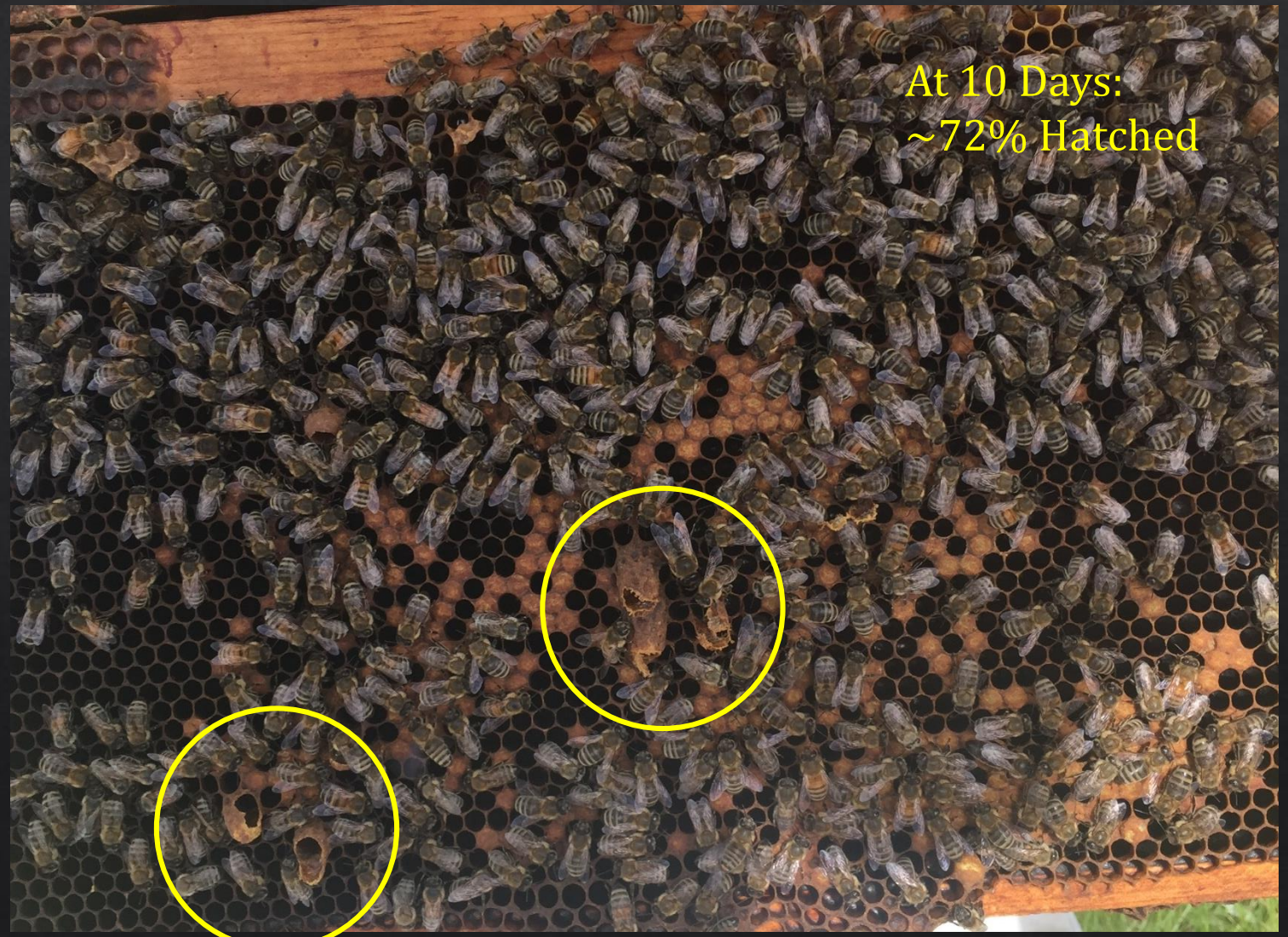
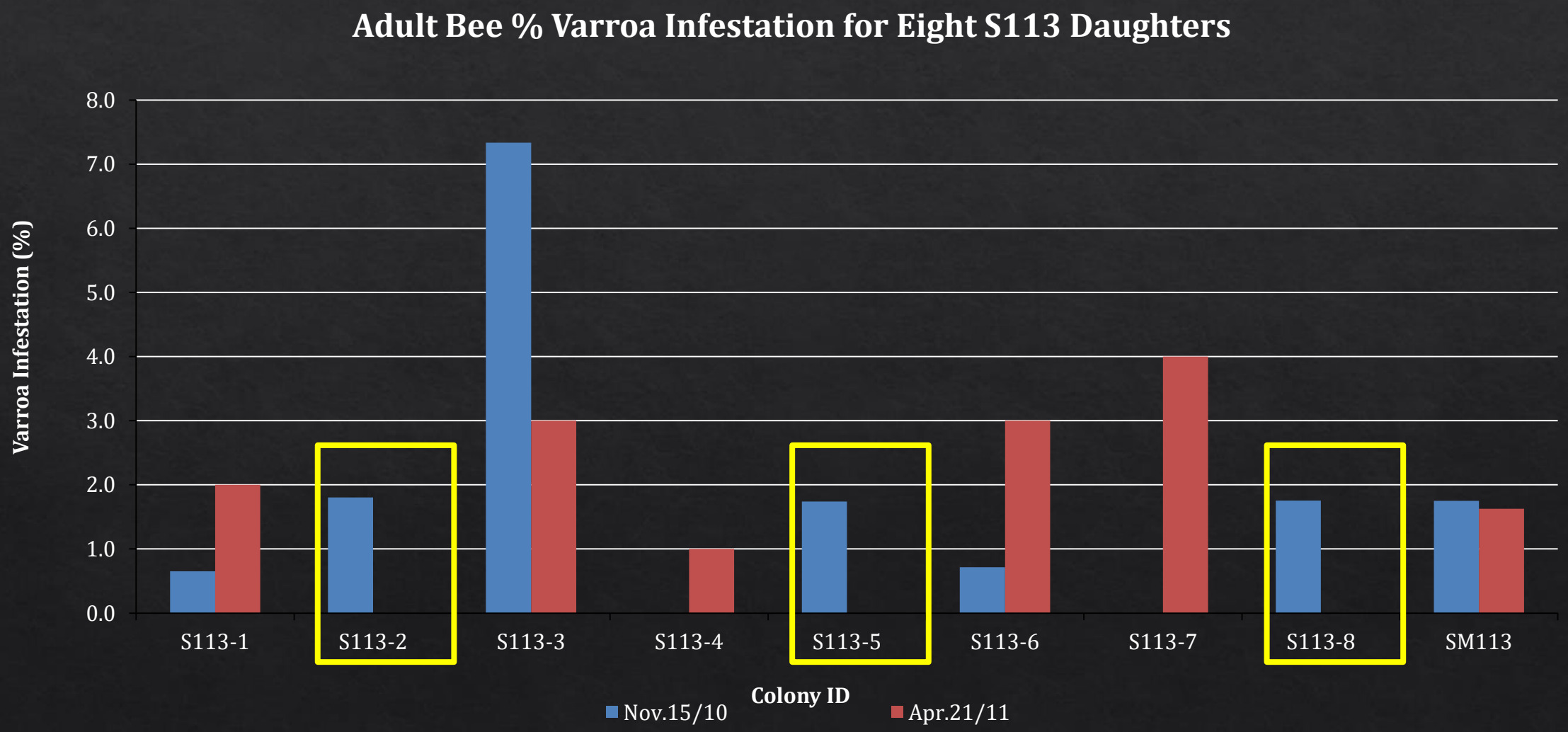
Supersedure Trial

Line	Average Cells per Nuc at 10 Days
G20	1.3
G44	5.7
G44	1.7
G50	2.3
G50	3.7
L25	4.7
L25	4.0
L25	3.3
S136	0.7
S136	1.7
S136	5.7
S14	3.7
S28	2.0
S28	4.0
S28	4.7
S28	4.3
S28	6.3
S28	2.7
S88	2.7
S88	2.7
S96	1.7
S96	3.3
S96	5.3
S96	0.3
S96	5.7
S96	2.0
S96	3.0
Sask-X	3.3
Y26	2.3



Progeny Analyses – S113



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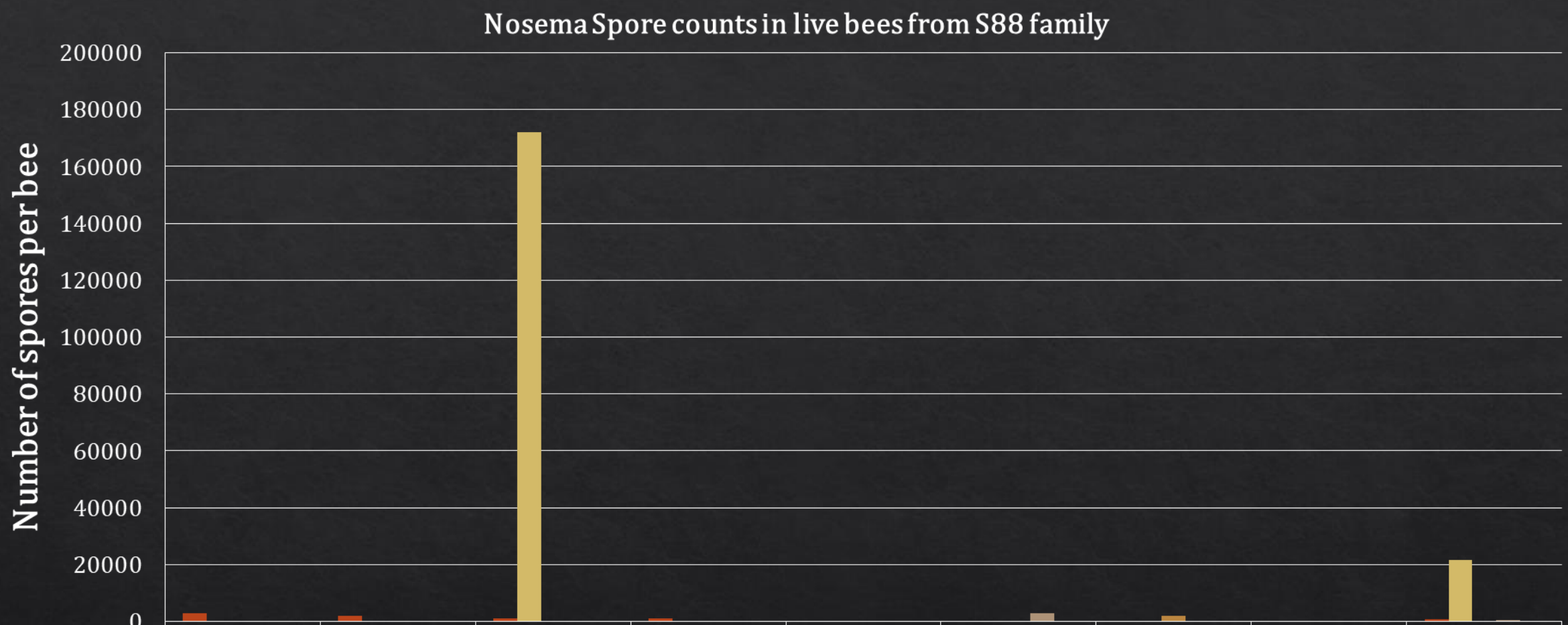
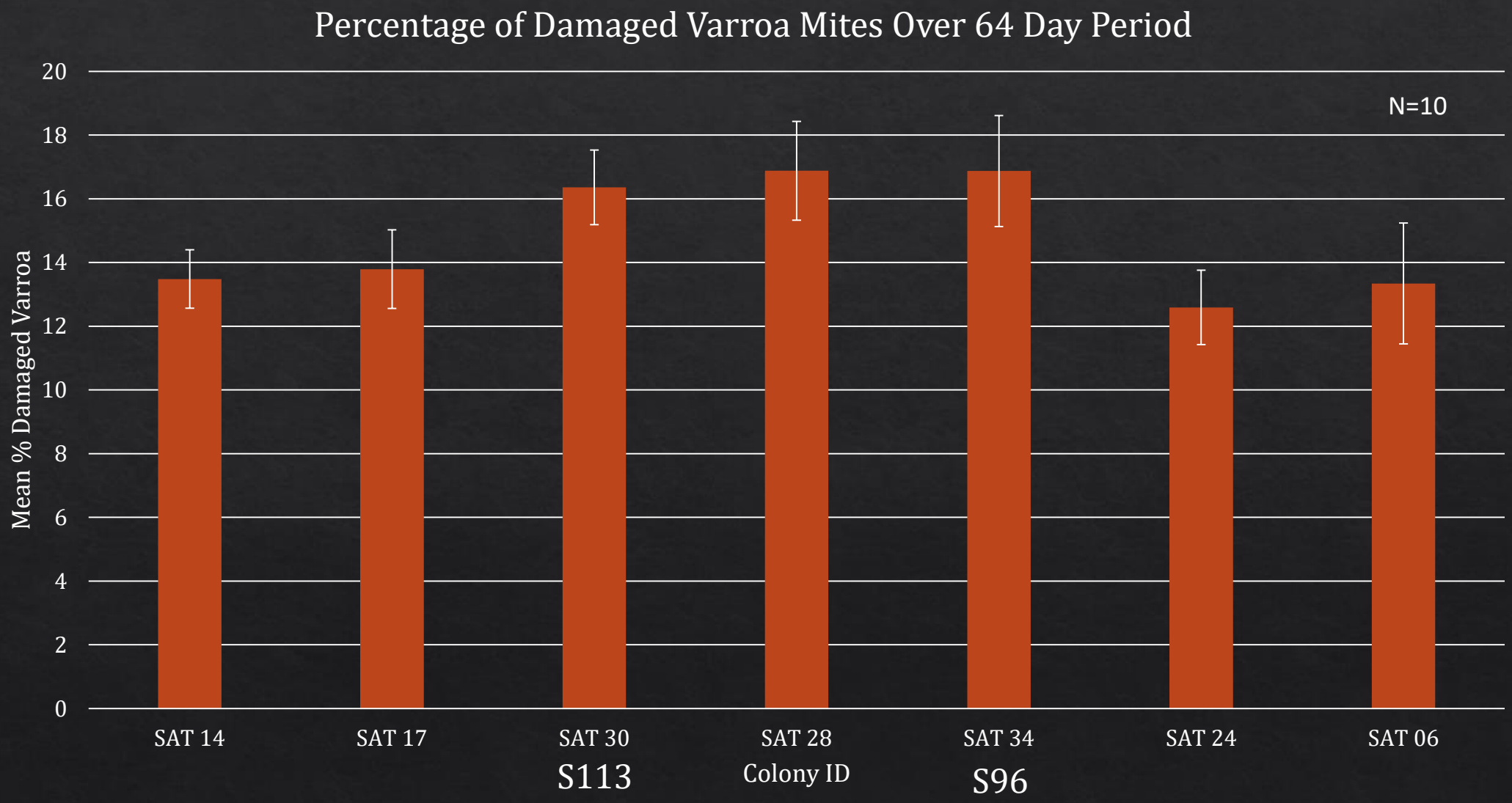


	S88-1	S88-2	S88-3	S88-4	S88-5	S88-6	S88-7	S88-8	Mean
Dec28-10	3000	2000	1000	1000	0	0	0	0	875
Jan-13-11	0	0	172000	0	0	0	0	0	21500
Jan-28-11	0	0	0	0	0	0	2000	0	250
Feb-13-11	0	0	0	0	0	3000	0	0	375
Feb-28-11	0	0	0	0	0	0	0	0	0

SCBA – January 13th, 2022

Mite Biting Analysis



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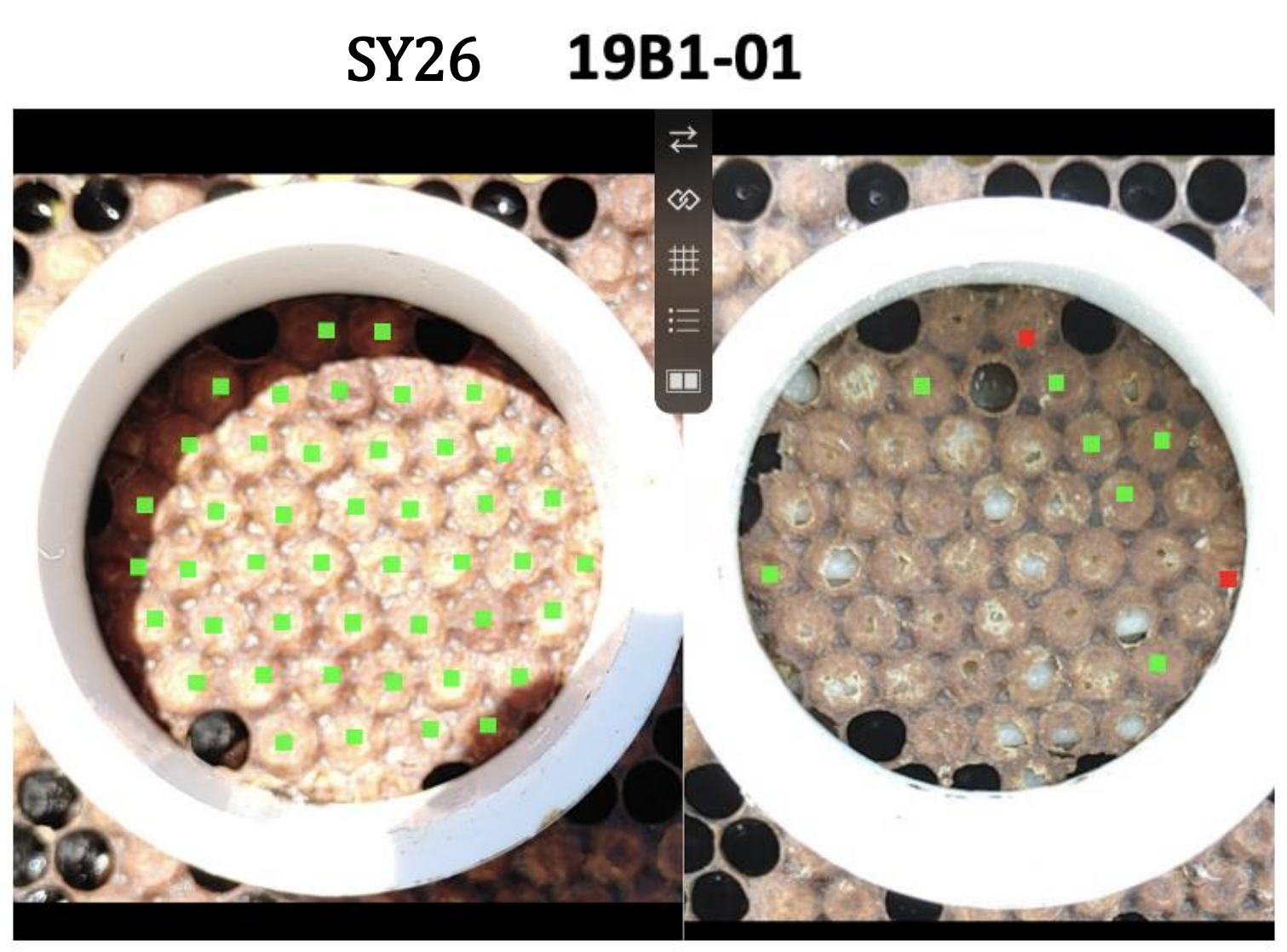
Saskatraz Progeny Analyses



VSH Analysis Using Unhealthy Brood Odor Assay

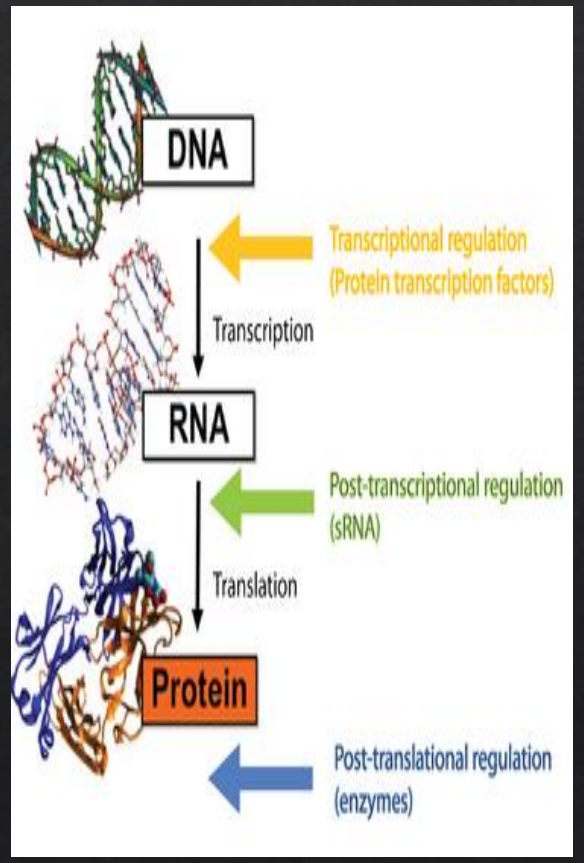
UBO Assay Developed by:
Kaira Wagoner at UNCG

UBO Assay Score:
84.4%

Biomarker Development

- Microsatellites (SNP Discovery)
- Microarrays (transcripts)
- Proteins
- Kinome Arrays (signal transduction)

(DNA) → (RNA) → (Protein) → (Signal Transduction)



You can find our Publications at www.saskatraz.com under the Research heading.

Differentially Expressed Transcripts in G4 and S88 In Varroa Infected and Uninfected Pupa

Category	Gene	S88-/G4-	S88+/G4+	Honey Bee Protein
Signal Transduction (Pupa)	GB17702-RA		2.40	Cadherin-87A-like
	DB777873		2.83	Neurobeachin-like
	GB14355-RA	4.45	2.69	Anosmin-1-like
Lipids (Pupa)	GB11723-RA		6.88	Apolipoprotein D-like isoform 2
	GB18070-RA		2.23	Acyl-CoA Delta(11) desaturase-like
	GB13246-RA		0.47	Phospholipase A1 member A-like isoform 1
	GB16889		3.41	Esterase E4-like
Cytochrome P450 (Pupa)	GB11754-RA		0.31	Cytochrome P450 6a14 isoform 1
	GB12136-RA		4.08	Cytochrome P450 6A1
Immune (Pupa)	GB13473-RA		2.07	Apidaecins type 73

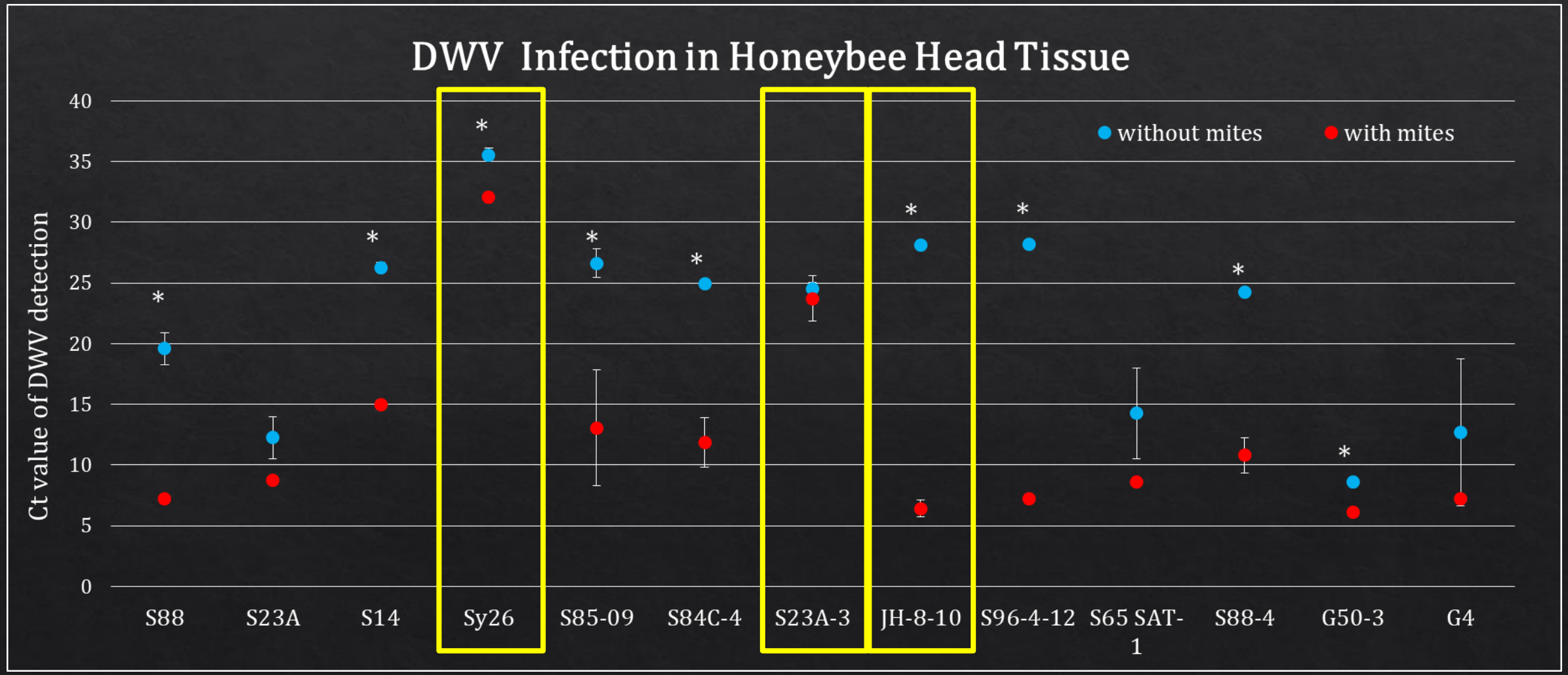
Survivor Colonies

Why do some colonies survive for extended periods in natural selection apiaries?

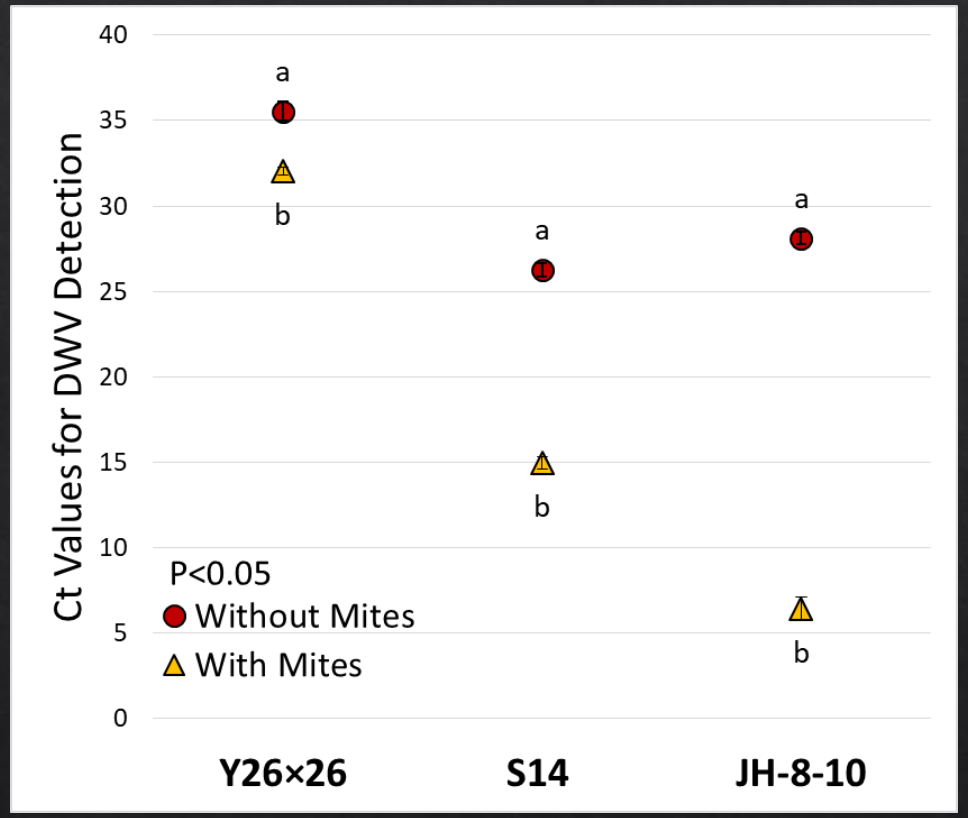
- S88 for 58 Months
- SY26 for 70 Months

- Grooming Behaviour (Mite Biters)
- VSH/Hygienic Activity
- Supersedure Rates
- Stress Resistance – Express higher levels of detoxification factors for pesticide, miticides and environmental stressors - Apolipoprotein D, Esterase E4, Cytochrome P450
- Better Foraging Activity = Better Nutrition
- Virus Immunity (Innate Immunity)

DWV Analysis of Saskatraz Phenotypes with and without Mites

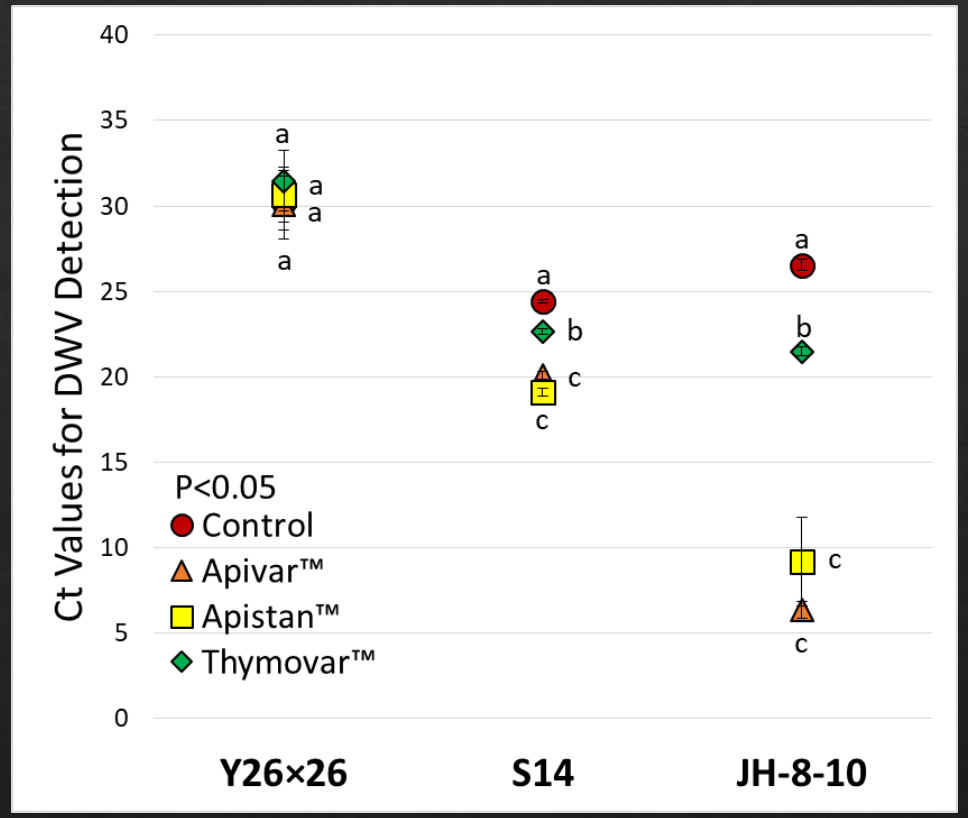


DWV Levels in Response to Varroa Mite Infestation and Miticide Treatments



Tolerant Susceptible

DWV in Head Tissue w/ and w/o Varroa Mites

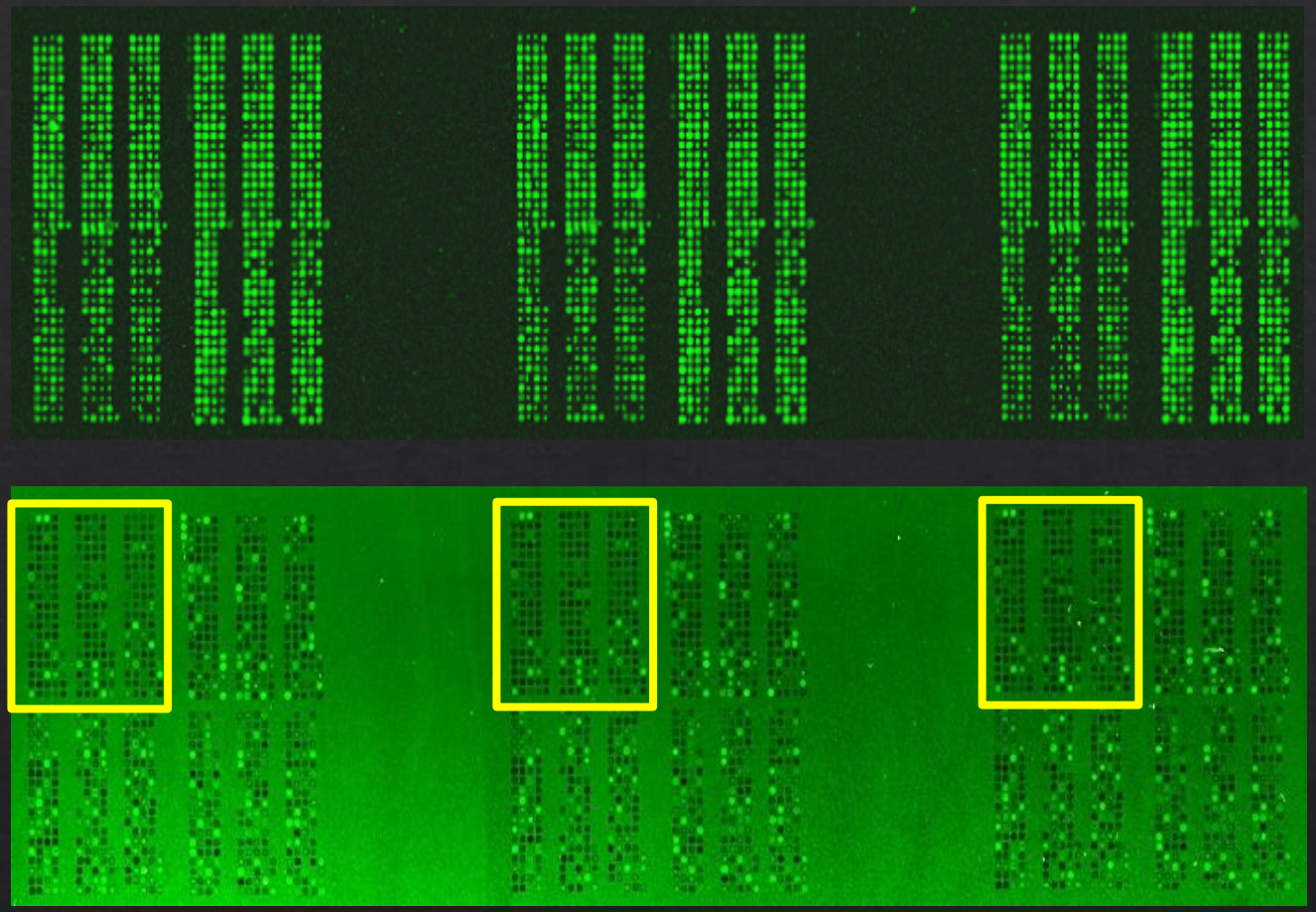


Tolerant Susceptible

DWV in Head Tissue in Miticide Experiment

Quantitative measurements of DWV in two varroa tolerant (SY26 and S14) and one susceptible (JH-8-10) colony in response to varroa mite infestation and miticide treatments. y axis: Ct values for DWV detection (mean ± SEM, N=3); x axis: three colonies (SY26, S14 and JH-8-10). A. DWV in the head with and without varroa mite; B. DWV in the head with and without miticide treatments. The multi-treatment comparisons of Ct values used the LSD (least significant difference) method for difference analysis.

Kinome Analysis of Colony Phenotypes

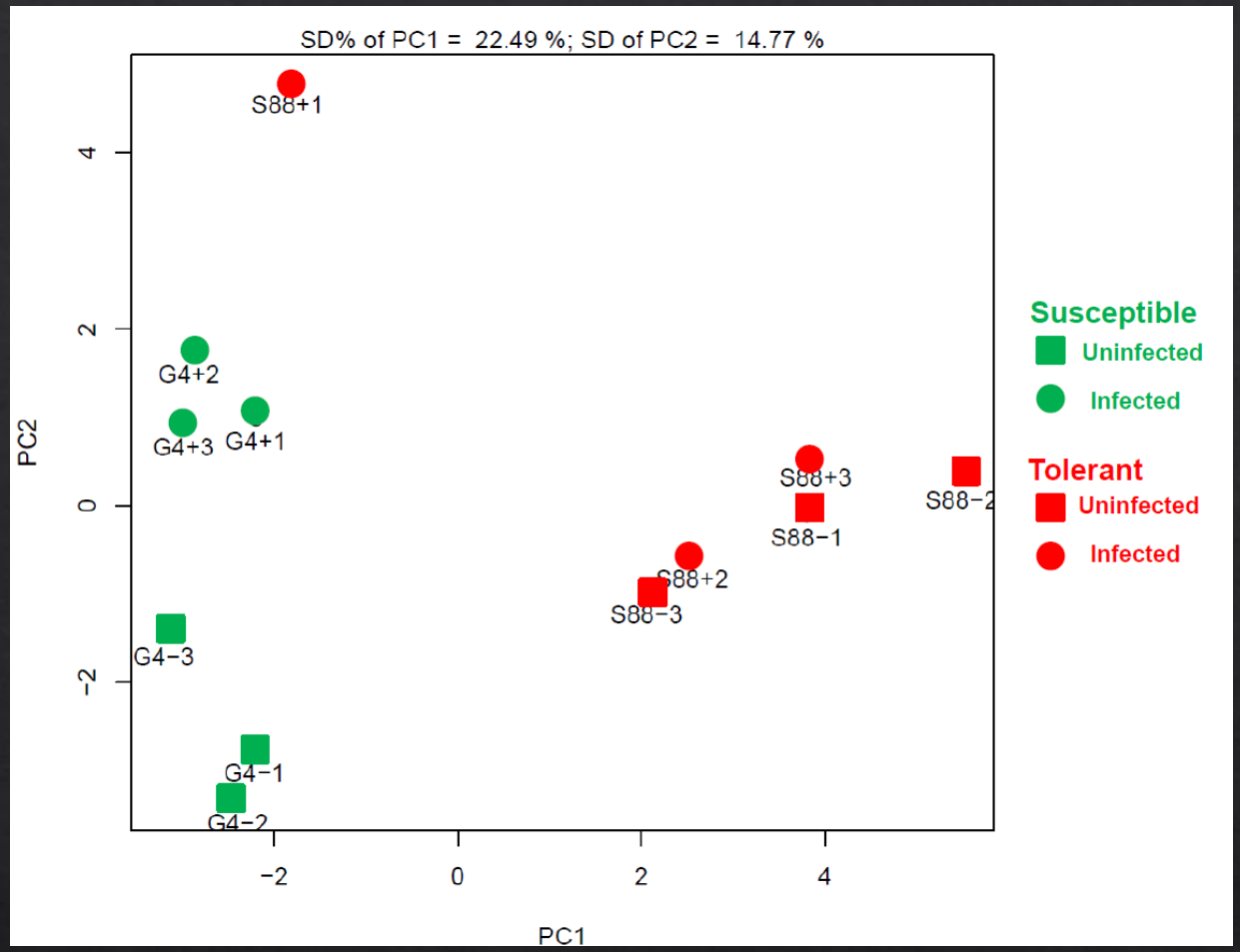


http://www.greendiary.com/hawaii-bees-infested-by-destructive-varroa-mites.html

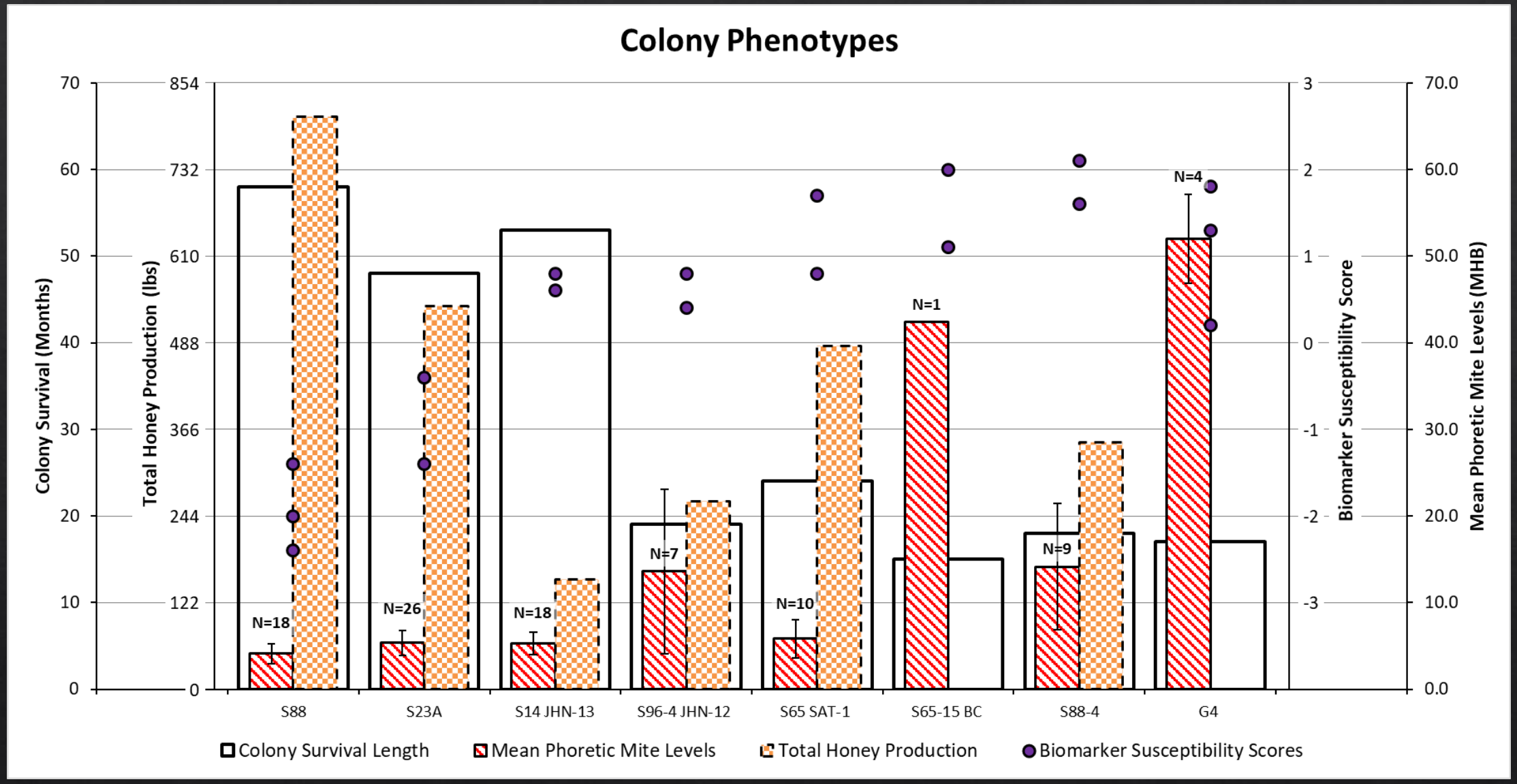
Printing and Validation of the Bee Specific Peptide Array. A) The arrays were printed by a commercial partner (JPT Technologies). For each array each spot is printed in triplicate within each block. Each block is then printed in triplicate for nine technical repeats of each peptide. This image, taken as a quality control step in array production, illustrates the consistency and reproducibility to peptide spotting. B) An image of a data scan of a representative array that had been used for analysis of a whole bee sample. All of the arrays of this work were of comparable quality with respect to the clarity and consistency of peptide phosphorylation. A clear and consistent pattern of extents of peptide phosphorylation is apparent across the three printed blocks.

	Protein	ID	Sequence	P
Innate Immunity	TAK1 kinase	O43318	YMTNNKGSAAWMAPE	0.001
	TAK1 kinase	O43318	CDLNTYMTNNKGSAA	0.003
	Mitogen-activated protein kinase kinase kinase_5	O35099	TETFTGTLQYMAPE	0.009
	Nuclear factor NF-kappa-B p110 subunit Rel-p110	Q94527	YIQLKRPSDGATSEP	0.005
	Transcription_factor p65 Nuclear factor NF-kappa-B	Q04206	IQLKRPSDGALSEP	0.005
	Focal adhesion kinase 1 FADK1	Q05397	IVDEEGDYSTPATRD	0.005
	AP-1 complex subunit beta-1	O35643	VEGQDMLYQSLKLTN	0.008
Metabolism	ATP synthase_subunit_beta	P06576	TSKVALVYGQMNEPP	0.004
	Na-K transporting ATPase subunit alpha1	P05023	ICKTRRNSLFRQGM	0.009
	Glucose-6-phosphate isomerase	P06744	GPRVHFVSNIDGTHI	0.005
	Isocitrate_dehydrogenase subunit_beta,	O43837	TKDLGGQSSTTEF	0.006
Stress Responses	Ribosomal protein S6 kinase alpha	P51812	DSEFTCKTPKDSPGV	0.006
	Elongation factor 2 (EF-2)	P13639	KVMKFSVSPVVRVAV	0.007
	60_kDa_heat_shock_protein	P10809	ILEQSWGSPKITKDG	0.016
	Superoxide dismutase	P07895	SIFWCNLSPNGG	0.008
Other	Ephrin type-A receptor 4 EPH-like kinase 8 (EK8)	P54764	SYVDPHTYEDPNQAV	0.006
	PRKC_apoptosis_WT1 regulator_protein__	Q62627	LREKRRSTGVVHLPS	0.006
	A-Raf Kinase	P10398	QTAQGMDYLHAKNII	0.010
	Intestinal cell kinase (ICK)	Q9UPZ9	CKIRSRPPYTDYVSTRW	0.010

Biomarker Peptides: Differently Phosphorylated Peptides Between Pupae Collected from Varroa Susceptible and Tolerant Colonies.



Clustering of Kinome Data. Kinome datasets were subjected to hierarchical clustering and PCA analysis. Pupae from two colonies (G4 and S88) were selected for either the presence (+) or absence (-) of Varroa mites. Principle Component Analysis: Separation of the samples on the basis of phenotype is clearly observed with further distinction with the susceptible, but not tolerant, samples on the basis of infection status.



The survival time, mean phoretic mite infestation, total honey production, and biomarker susceptibility scores for 8 colony phenotypes are shown here. Error bars are shown as ±SE of the mean phoretic mite level where N is the number of samples tested to calculate the mean where S65-15 BC is represented only by a single sample. The purple dots represent the biomarker susceptibility scores calculated from the kinome array (n=299 peptides) analyses of dark-eyed pupae. Each dot represents a score calculated from one pupa.

Saskatraz Hybrid Project

Objectives

- To commercialize and distribute Saskatraz Breeding Stock to commercial beekeepers.
- Every year colonies are selected for honey production, overwintering ability, temperament, mite resistance and brood diseases.
- This project serves to provide Saskatraz hybrid queens for reasonable prices and results in increasing the frequency of alleles associated with economic traits in commercial populations.

- Saskatraz stock distribution
  - North America
  - Iran
  - Middle East (UAE, Saudi Arabia, etc.)
  - Afghanistan
  - Ukraine
  - Turkey
  - South Korea
  - Virgin Islands, USA
- In progress
  - Australia
  - Hawaii, USA
  - Chile
  - Russia
  - Poland

Olivarez Honey Bees, Ray And Team

www.OHBees.com

Location

Saskatoon, SK

Temperature Range:
-40°C to + 40°C
Mating Season:
~3 Months

Temperature Range:
-1.0°C to + 41°C
Mating Season:
~5-6 Months

Orland, Ca USA

Saskatraz Queen Production

Saskatraz queen production in Saskatchewan is focused on production of Saskatraz breeder queens by recurrent selection and closed population mating procedures.

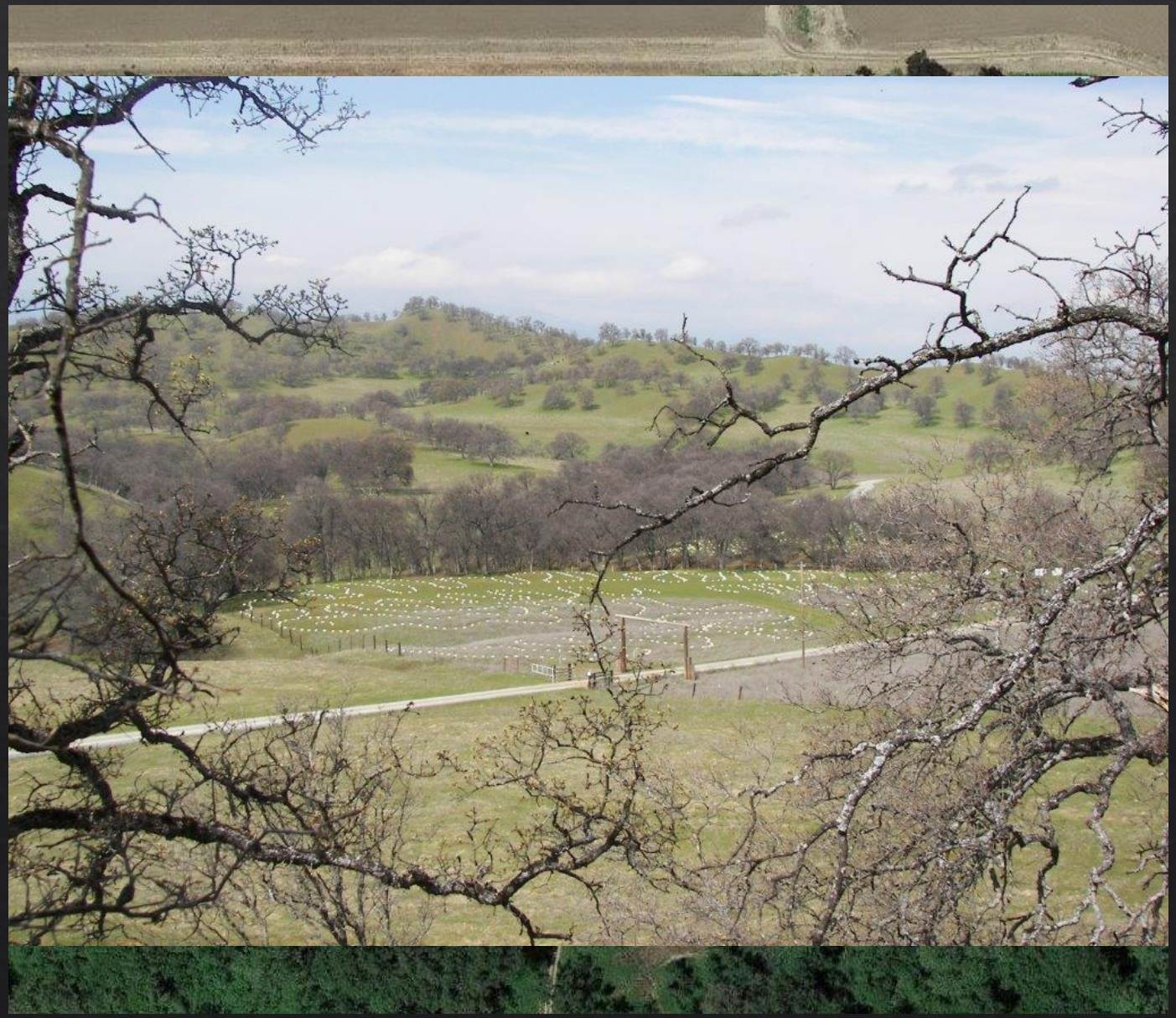
- Short queen production season
- Good location for selection criteria
- Can produce around 2000 queens/season
- Send about 150-200 breeders to be re-selected in California

Saskatraz hybrid queen production in Northern California (Orland) at OHB is focused on large scale commercial production.

- 40-60 Saskatraz breeders used after re-selection
- Ideal area and climate for large scale operations
- High populations of mature drones
- Produce several hundred thousand queens/season

California

Mating Locations



Saskatchewan

Mating Locations

Priddy's

Bainsville

Kokay's

Saskatraz Breeding Program

Behaviour Assays – Orland, CA:

1. Temperament (1 sting, 2 sting, 3 sting)
2. *Behaviour on comb (dancing, calmness, etc.)
3. Low temperature flight
4. *+Queen retinue + mating
5. +Swarming tendency and superceding success
6. +Pollen storage and propolis production
7. +Brood pattern
8. *+Worker uniformity
9. +Queen colour and markings
10. +Varroa Assays

Saskatraz – Orland, USA

The California Tech Transfer Team, Bee informed Partnership has independently evaluated our Saskatraz breeding stock in late February early March in past years. An example is shown below.

Colony Number	Colony ID	Brood Pattern	Chalk-brood Presence (+/-)	Temper-ament	Pollen placement	Queen Presence (+/-)	Queen Mark Presence (+/-)	Phoretic Mite Infestation (MHB)	% Mite Infestation in Worker Brood	% Mite Infestation in Drone Brood	Tech Team Hygienic Behaviour Test	Observation
7	S65 Robin 14	Excellent	-	1	Average	+	+	0	0	0	93%O / 80%R	Green mark on queen
24	SY26 x 26 Martin 14	Good	-	1	Average	+	-	0	0	-	99%O / 99%R	No drone brood; no visible mark on queen
25	SY26x26 Martin 14	Excellent	-	1	Average	+	+	0	0	0	100%O / 100%R	-
37	SG44 JHN 12-9 B.V. 14	Excellent	-	1	Average	+	+	0	0	-	93%O / 75%R	No Drone

SY26x26 Martins (Hygienic Behavior; 100%U+100%R)

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Varroa Assay

SCBA – January 13th, 2022

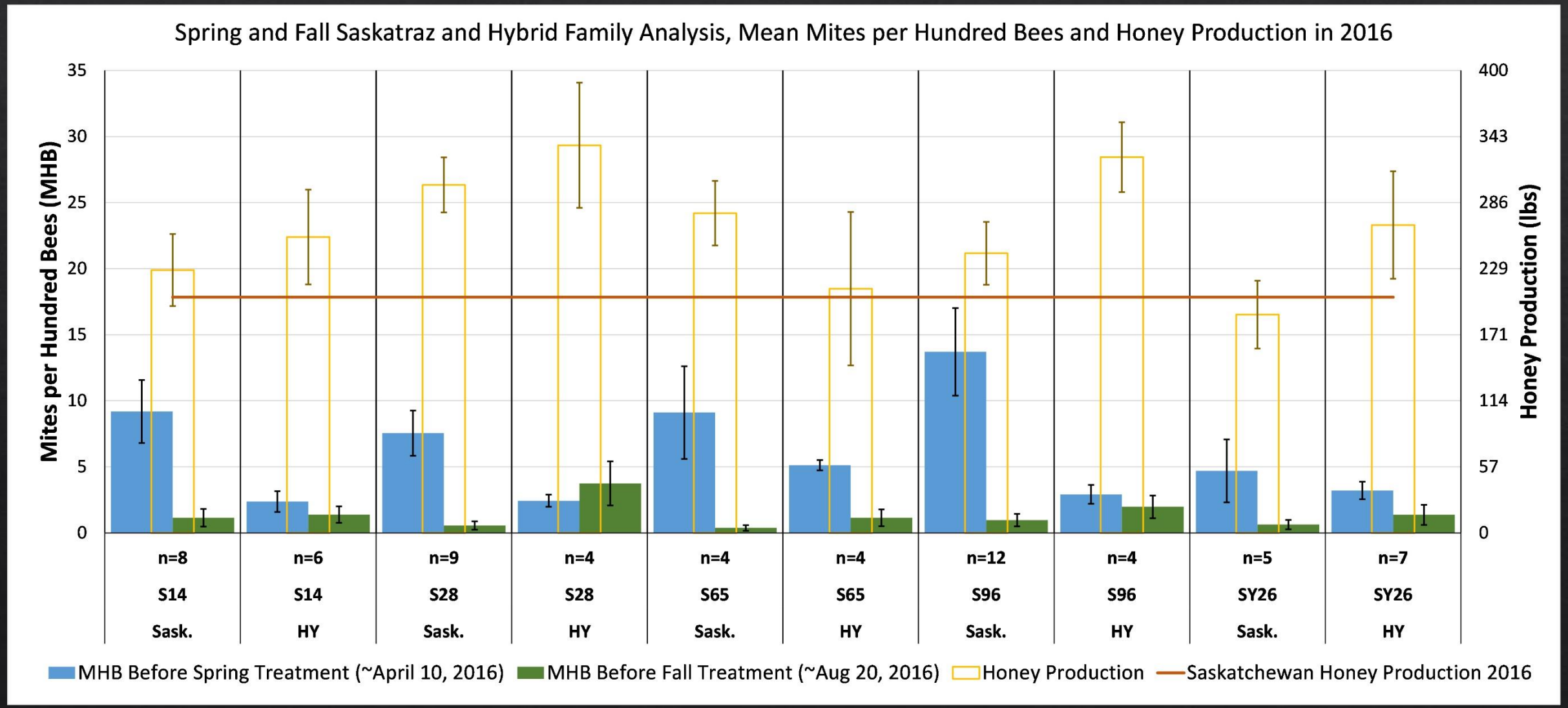
Queen

Retinue

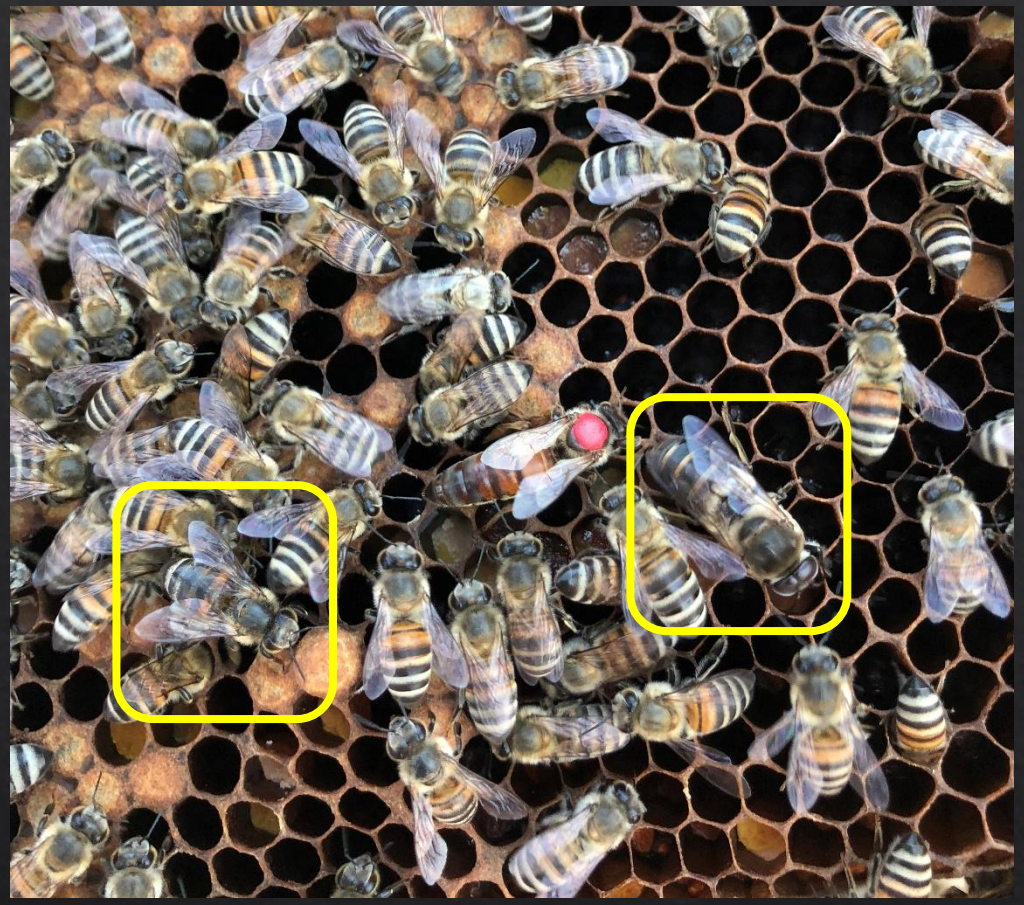
#47-S96 CHR 14


S96 RCD~15 x BV ~18
The Saskatraz Project

Saskatraz Hybrid Performance



Saskatraz – Hatta UAE



A. Mellifera Yemenitica

Miticide Treatments in Stock Selected for Varroa Tolerance

We can select survivor colonies which show excellent tolerance to Varroa, but it is difficult to stabilize the trait in progeny (10-15% heritability). As a result commercial production apiaries still require miticide treatment protocols.

We have found that stock selected for Varroa tolerance improve the efficacy of miticide treatments. In addition, combined miticide treatments show better efficacy than individual miticide applications.

Preliminary experiments show some miticide treatments may increase virus levels in the treated colonies and result in the bees being less able to cope with subsequent Varroa infestations.

Combined Miticide Treatment Experiments

Treatments Experiment in 2011

A comparison of selected, Saskatraz, and unselected colonies when treated with Apivar.

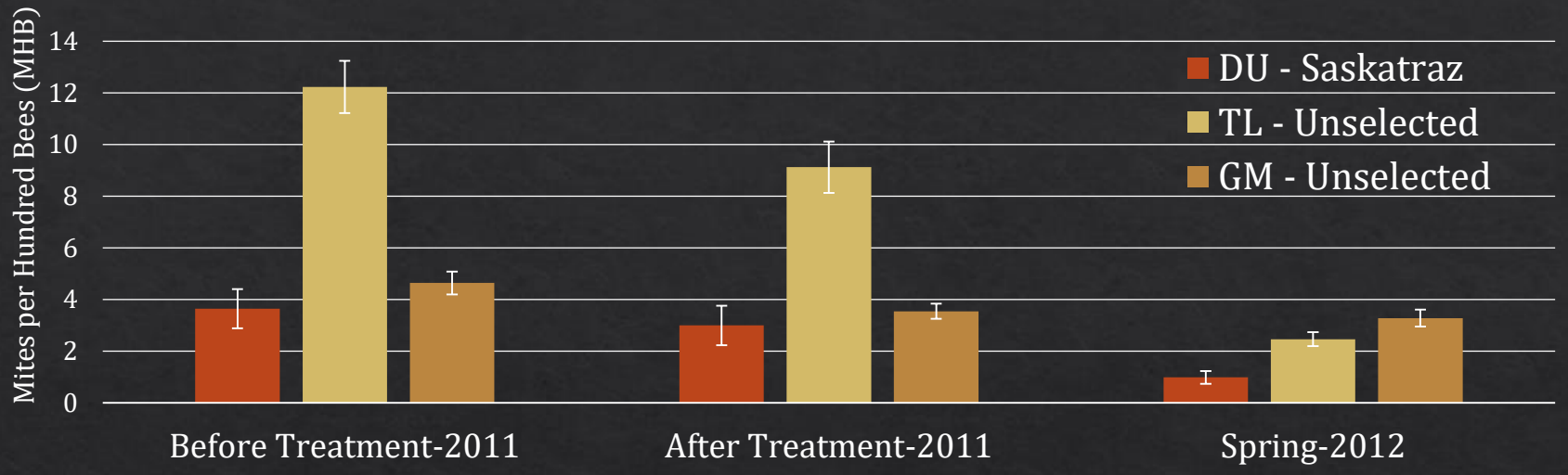
Treatments Experiment 2015

- Apivar® (2 strips)
- Apistan® (2 strips)
- Apivar® (2 strips) + Oxalic Acid (3.2% w/V)
- Apivar® (2 strips) + Thymovar® (1 wafer)
- Apistan® (2 strips) + Thymovar® (1 wafer)
- Apistan® (1 strip) + Thymovar® (1 wafer)
- Apistan® (2 strips) + Oxalic Acid
- Thymovar® (2 wafers) + Oxalic Acid
- Apistan® (1 strip) + Thymovar® (1 wafer) + Oxalic
- Oxalic Acid + Apistan ® (2 strips) after two weeks

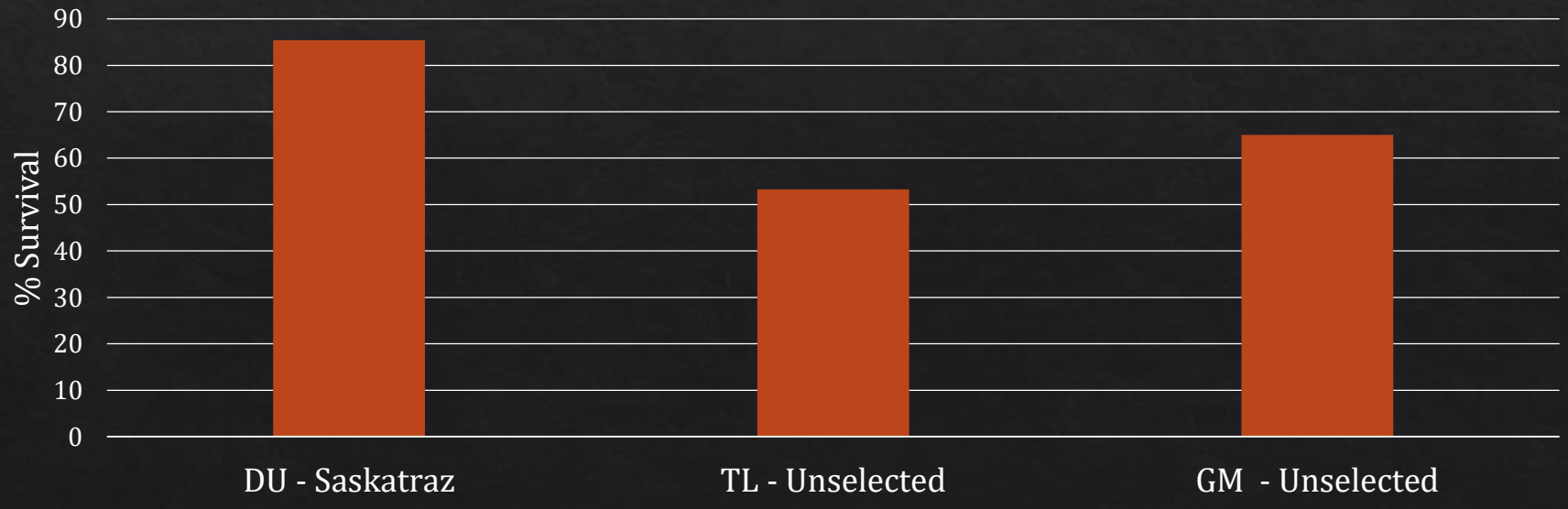
Treatments Experiment 2016-2018

- Apivar® (2 strips)
- Apivar® (2 strips) + Oxalic Acid (3.2% w/V)
- Apivar® (2 strips) + Thymovar® (1 wafer)
- Apivar ® (2 strips) + Thymovar ® (1 wafer) + Oxalic

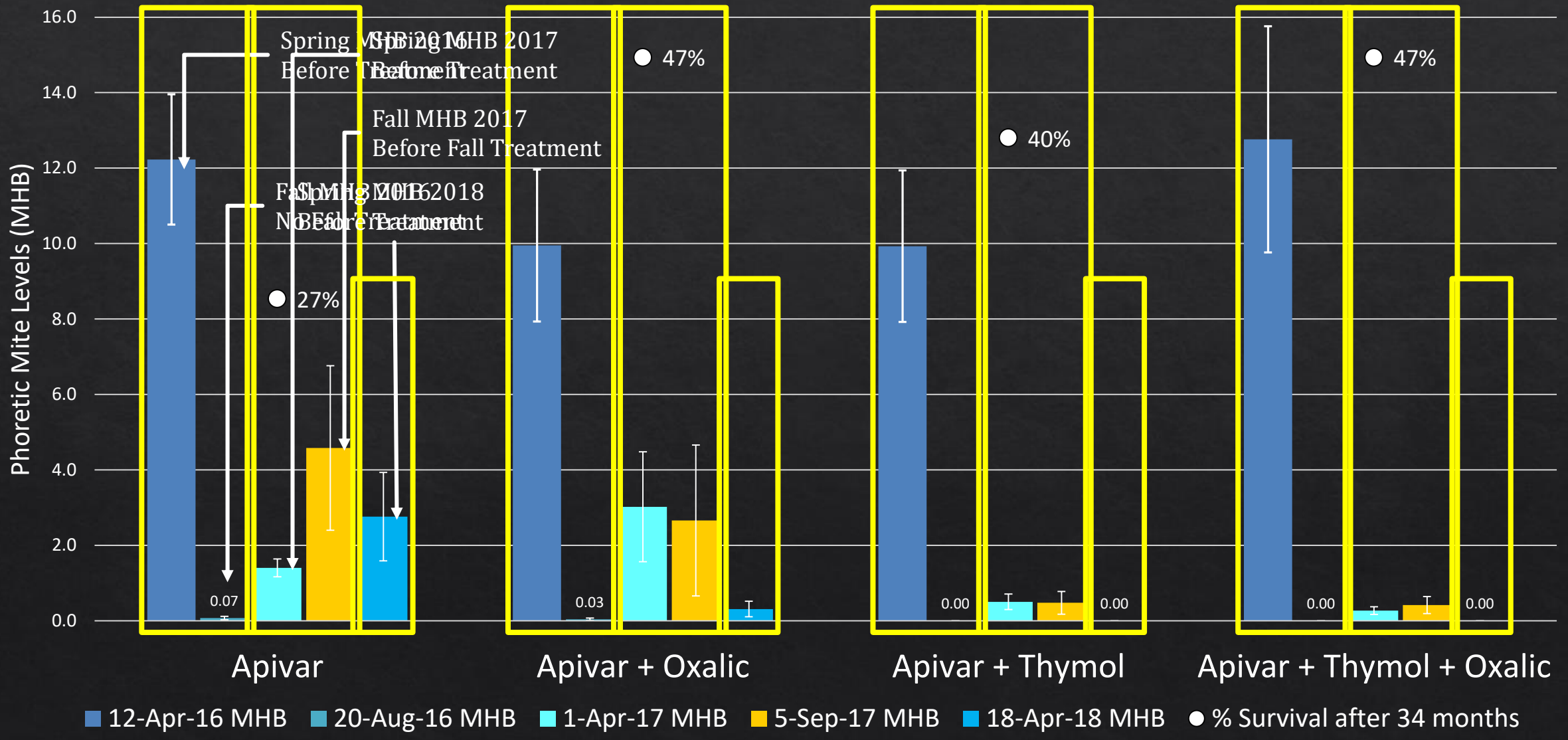
Miticide Treatment Effects in Varroa Susceptible and Tolerant Colonies



Surviving Colonies Spring 2012



Summary of Phoretic Varroa Levels (MHB) and Colony Survival after Four Different Miticide Treatments from April 12th, 2016 to April 18th, 2018



Summary and Work in Progress

We can select Saskatraz families with good honey production, wintering and varroa tolerance, but is difficult to balance the phenotypes. Varroa tolerance is variable in the progeny because of the nature of bee genetics.

Our focus is aimed at stabilizing mite tolerance using extensive progeny analysis with marker assisted selection and the UBO assay to speed up the selection process.

Also looking at the variability in virus susceptibility in our strains in collaboration with USDA Baton Rouge, LA.

Studying the effects of miticide treatments on colony Virus infections and what impact that might have on honeybee health and Varroa population growth (Partially funded by Project Apis M, in collaboration with Dr. Declan Schroeder and Patricia Wolf-Veiga).

Commercial Beekeeping at Meadow Ridge Ent. Ltd.

- Approximately 1500 colonies for honey production
  - Spread across 50+ apiaries and a 70 mile radius
  - Several apiaries are isolated for close population mating and stock maintenance
- Over 2000 wintered each year
- Produce 700-1000 nucs for replacement stock and sale
- Using 3-way nucleus colonies for requeening and double 3-ways for queen banking
- Produce around 2000-2500 Saskatraz Queens each year

Honey Production

Indoor Wintering

Year Mated

Current Loc.

SCBA – January 13th, 2022

Indoor Wintering Treatment - Nucs

Indoor Wintering Treatment - Nucs

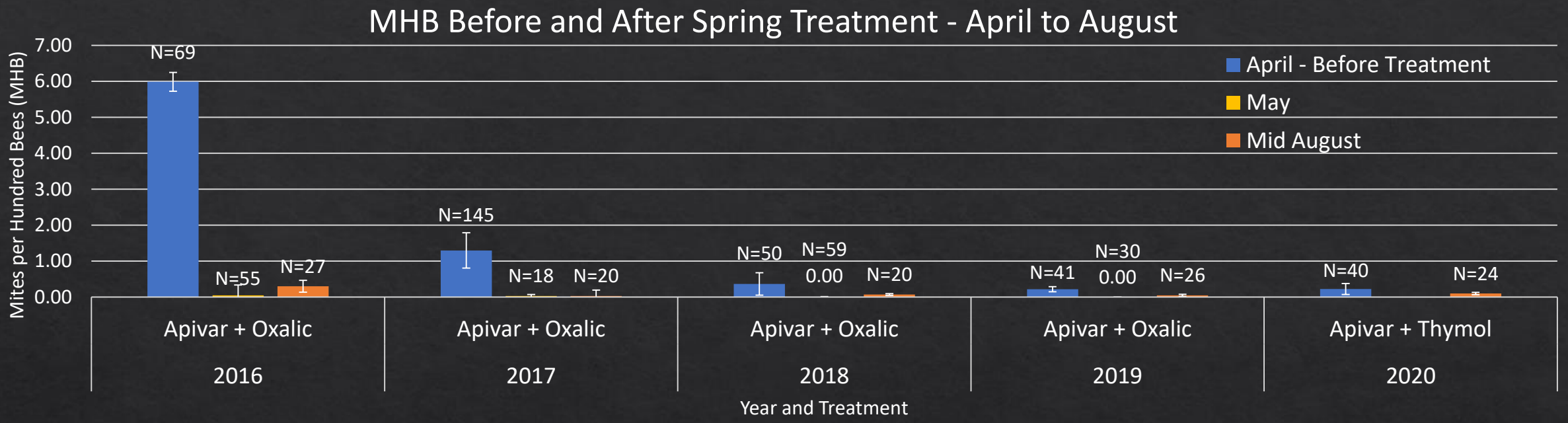
SCBA – January 13th, 2022

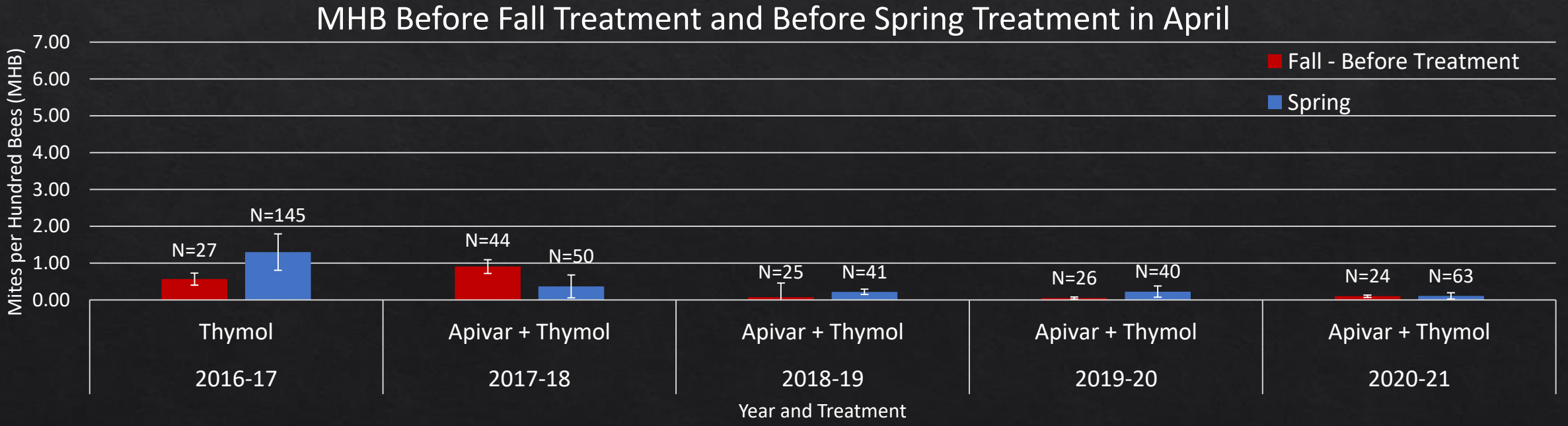
Indoor Wintering
3 Way Transfer

Indoor Wintering Treatment – 3 Ways

Outdoor Wintering Treatment

Spring Treatment 2021:
Apivar (2 Strips) + Oxalic Dribble (3.2 g/100 mL of 50% liquid sucrose, 50mL per colony)


MHB Before and After Spring Treatment - April to August
Mites per Hundred Bees (MHB)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
April - Before Treatment
May
Mid August
N=69
N=55
N=27
N=145
N=18
N=20
N=50
N=59
0.00
N=20
N=41
N=30
0.00
N=26
N=40
N=24
Apivar + Oxalic
2016
Apivar + Oxalic
2017
Apivar + Oxalic
2018
Apivar + Oxalic
2019
Apivar + Thymol
2020
Year and Treatment


MHB Before Fall Treatment and Before Spring Treatment in April
Mites per Hundred Bees (MHB)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Fall - Before Treatment
Spring
N=27
N=145
N=44
N=50
N=25
N=41
N=26
N=40
N=24
N=63
Thymol
2016-17
Apivar + Thymol
2017-18
Apivar + Thymol
2018-19
Apivar + Thymol
2019-20
Apivar + Thymol
2020-21
Year and Treatment

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The Saskatraz™ Project

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Questions?