MHBA Meeting – March 12th, 2024

Meadow Ridge Enterprises Ltd

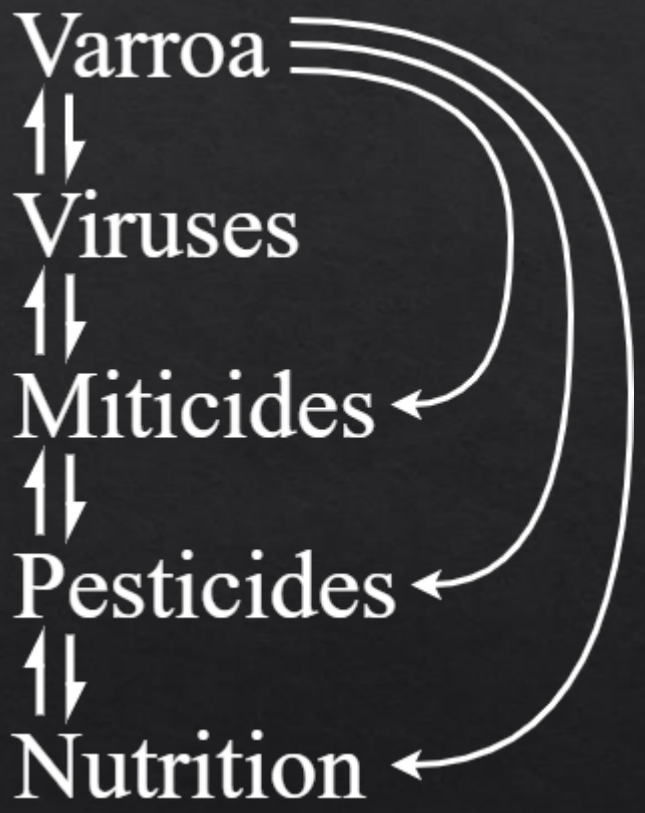
The Saskatraz™ Project

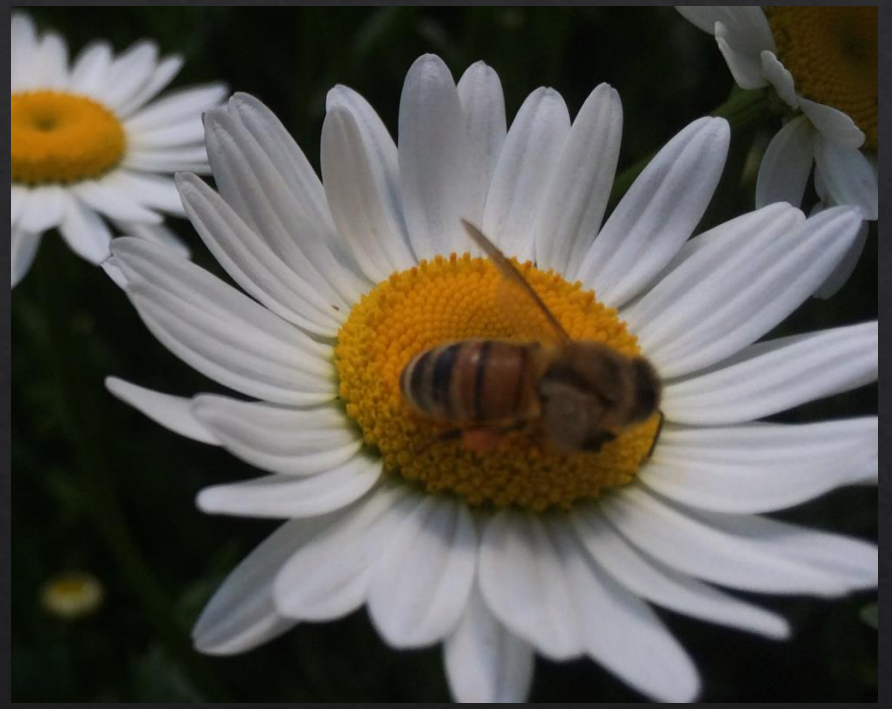
Queens Produce Superior Workers

Objective: To breed productive, gentle honeybees with tolerance/resistance to mites and brood diseases

By: Albert J. Robertson
The Saskatchewan Honeybee Breeding and Selection Program since 2004

Current Honey Bee Health Issues





Outline

- Saskatraz Breeding and Selection Program, www.Saskatraz.com
- Selection for Economic Traits
- Selection for Varroa resistance and hygienic traits
  - Natural selection, VSH phenotyping, and UbeeO assay
  - Screening for grooming behaviour, mite biting, virus and nosema susceptibility, and brood diseases
- Biomarker Development (Microsatellites, Proteomics, Micro and Kinome Arrays)
- Combined Miticide Treatment Experiments with selected and unselected stock
- Saskatraz Hybrid Project to distribute genetics
  - Olivarez Honey Bees Inc. - Orland, CA www.OHBees.com

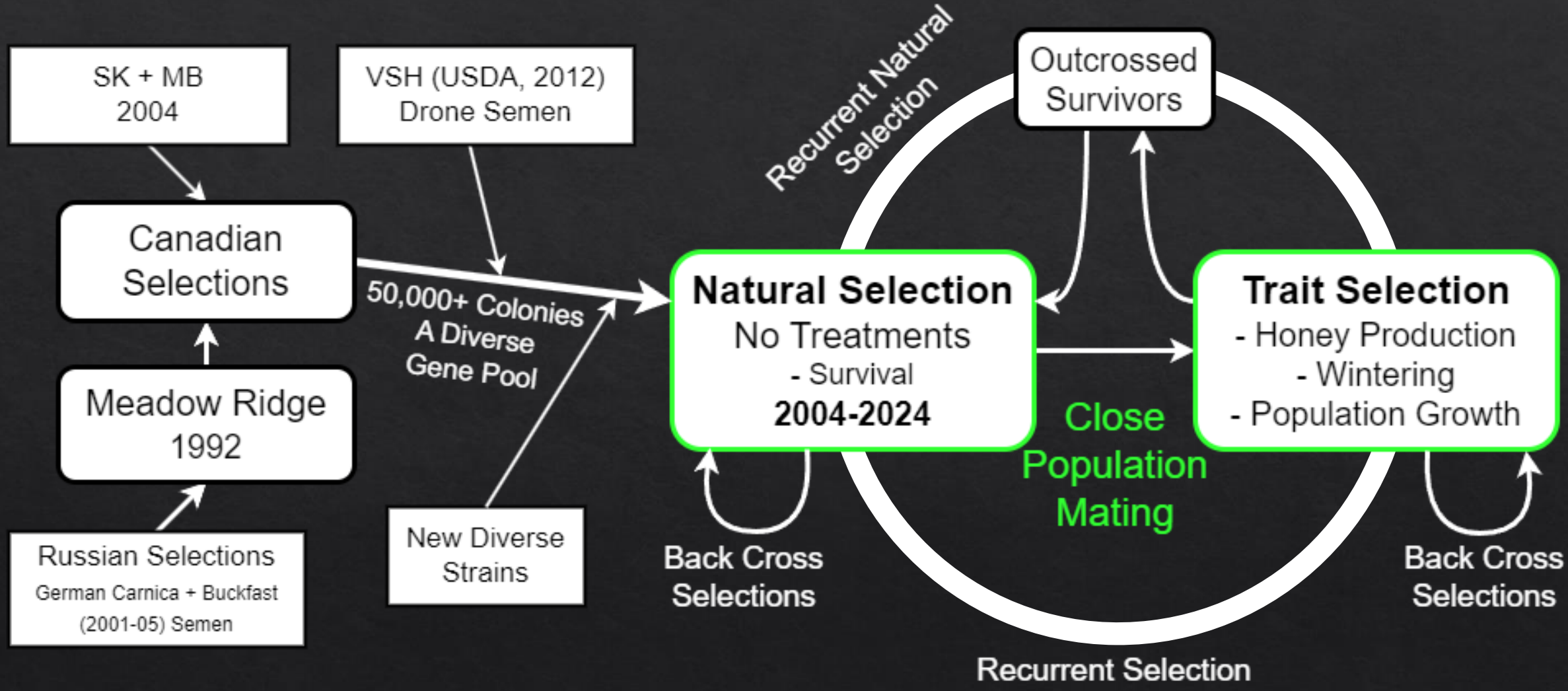
Saskatraz Breeding Program

Primary Selection Criteria:

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

Saskatraz Breeding Program Logistics

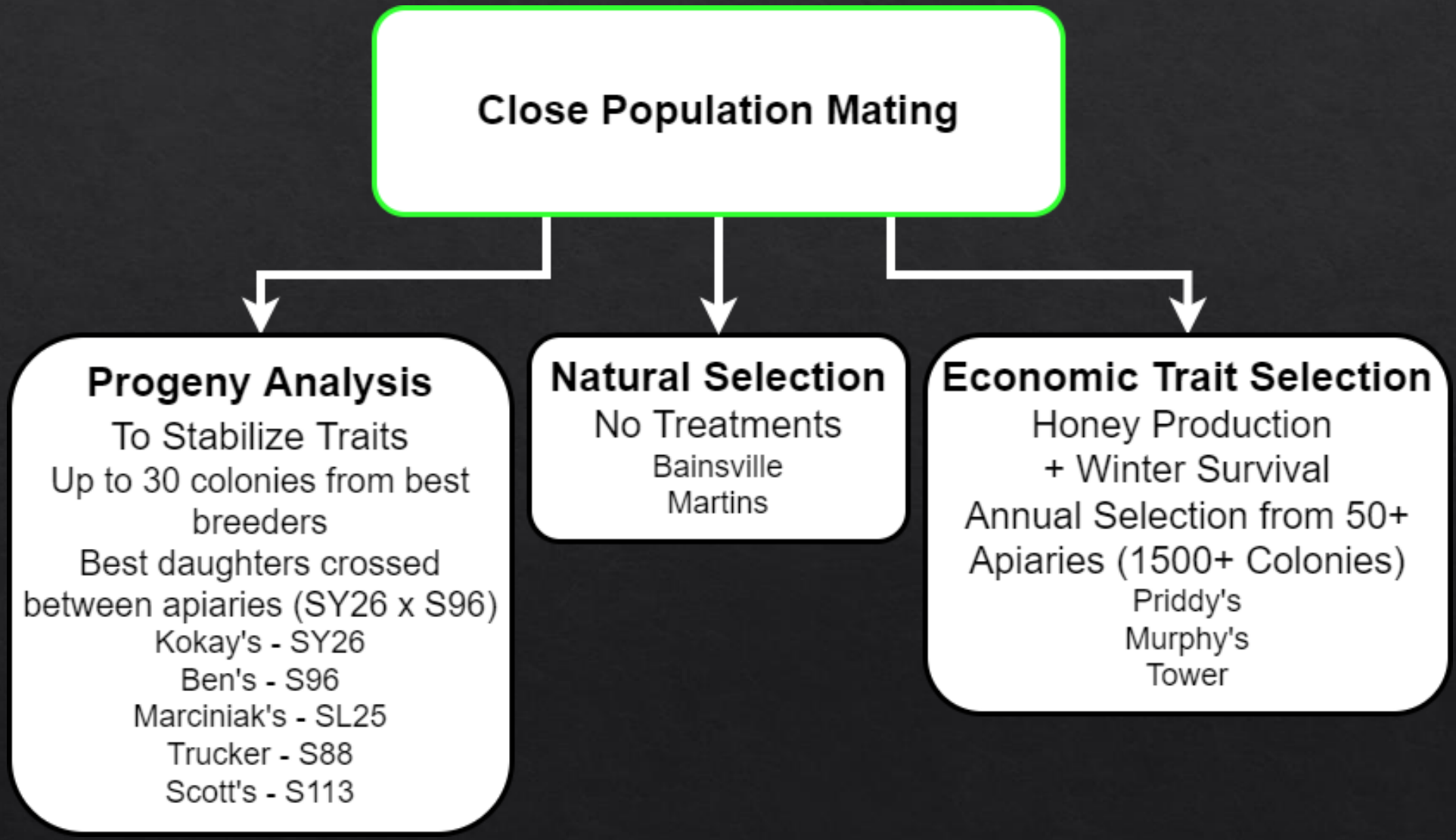


There are currently:

17 Saskatraz Families

Stock Distributed Yearly Since 2006

Saskatraz Breeding Program Logistics



Saskatraz Breeding Program Logistics

Year Mated

Current Location

2019 Unique ID 19B1-38

Priddy's Sampling ID P27

Family Y26

Mating Yard Bainsville Mother's ID

Mother SY26 2014 14B1-00

Honey Production and Wintering Selection

Annual selection from all apiaries (~50) – Recurrent Selection

Wintering Survival (Spring)

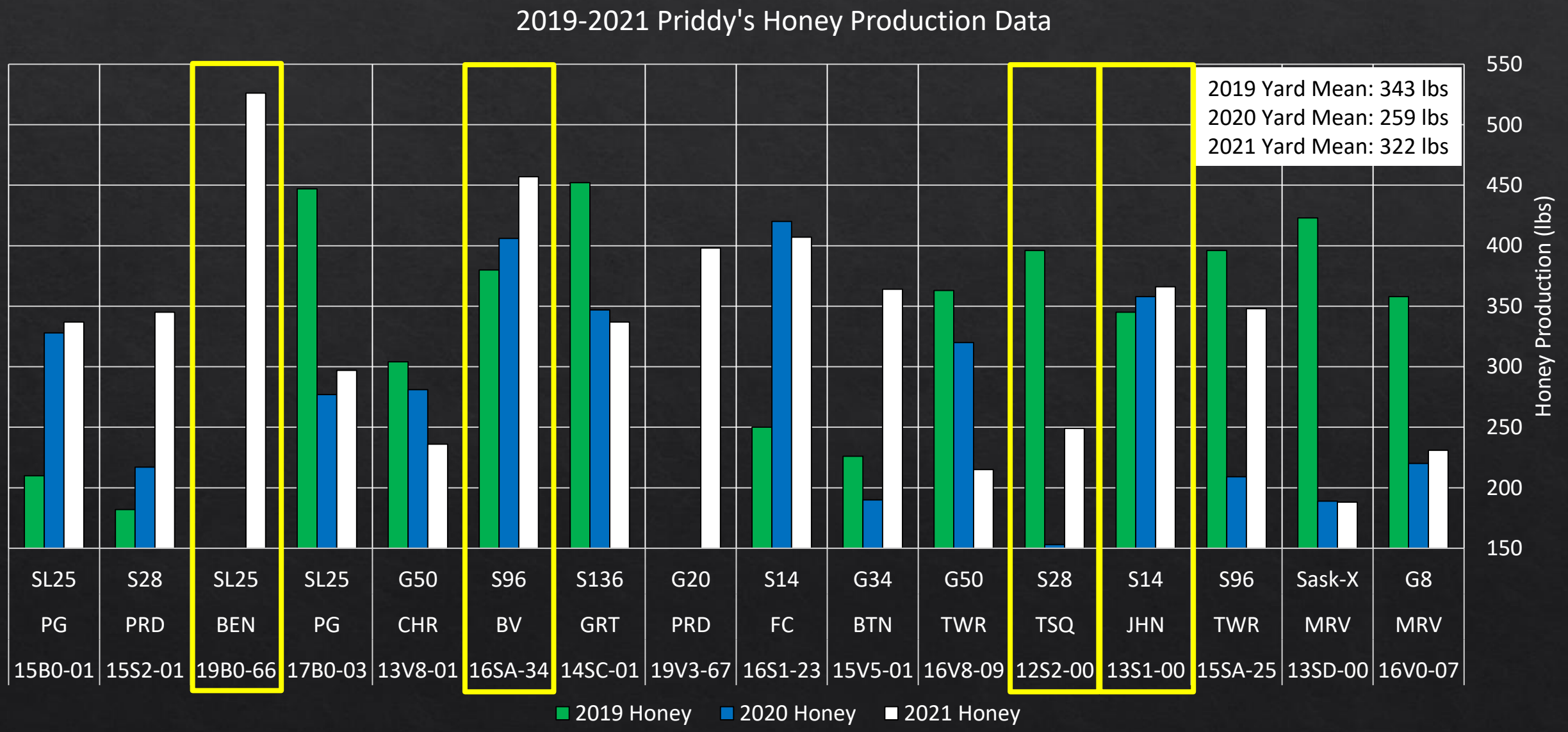
- Colony strength, food stores, clean bottom boards, absence of brood disease (chalkbrood, etc.), gentleness, and brood pattern are assessed.
- Mite washes are performed on the two best colonies, the weakest colony, and a composite yard sample.

Honey Production (Summer)

- Two top honey producing colonies are marked at each apiary during each round of honey harvest.
- Final selection are made on or after a colonies second year.

Final selections are moved to an isolated area for close population mating, for reselection, and use as breeders and drone mothers.

Selection for Honey Production



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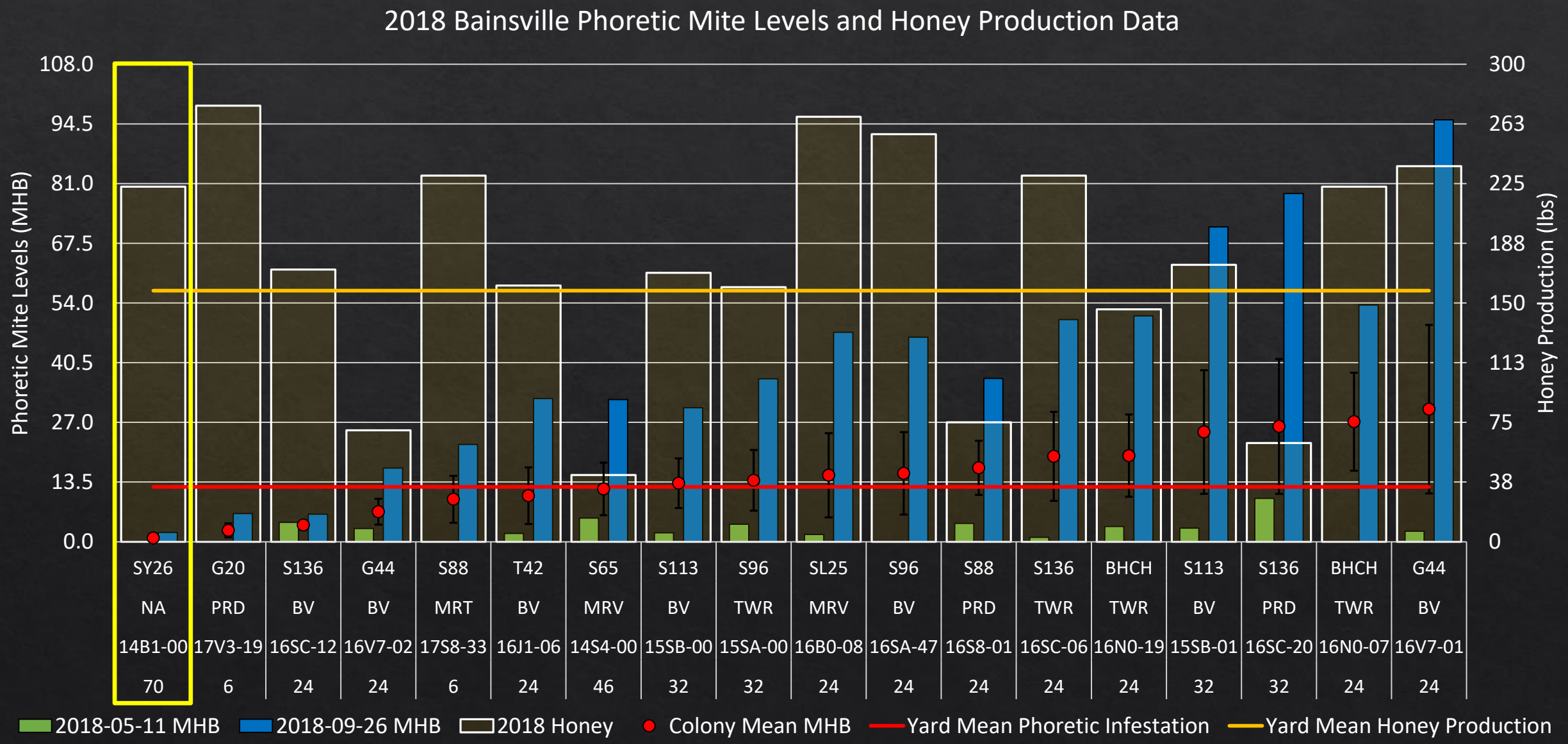
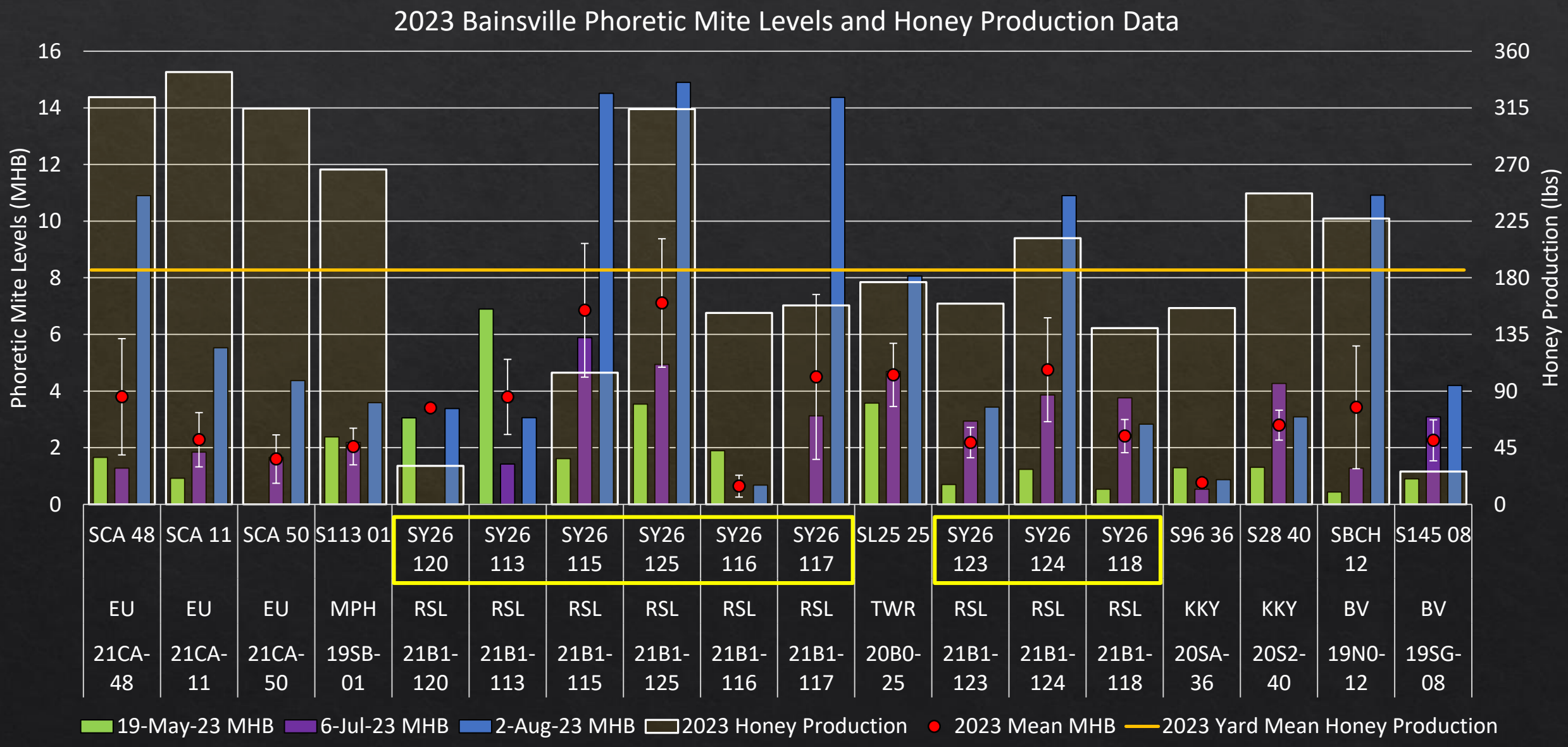
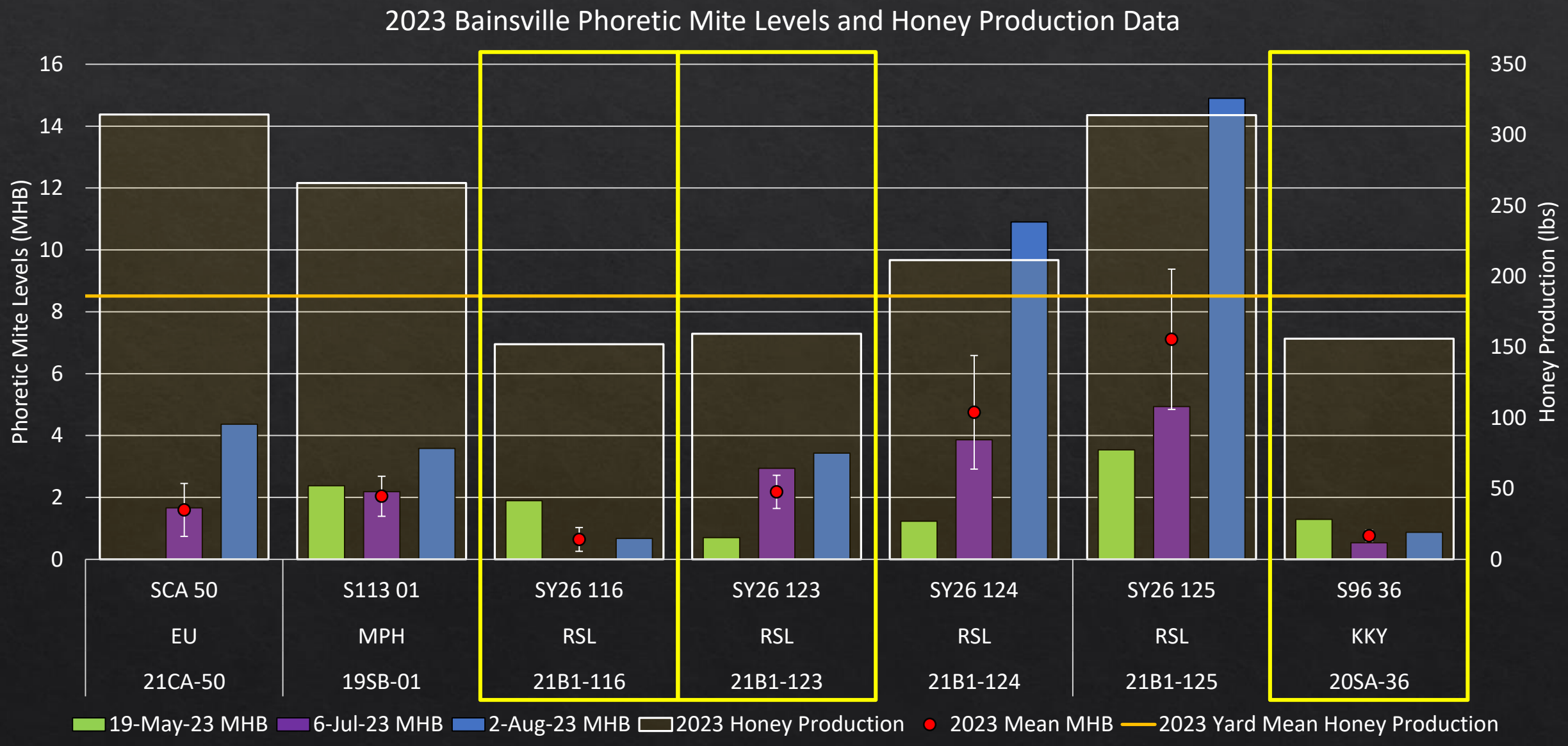
Natural Selection for Varroa Tolerance 2018



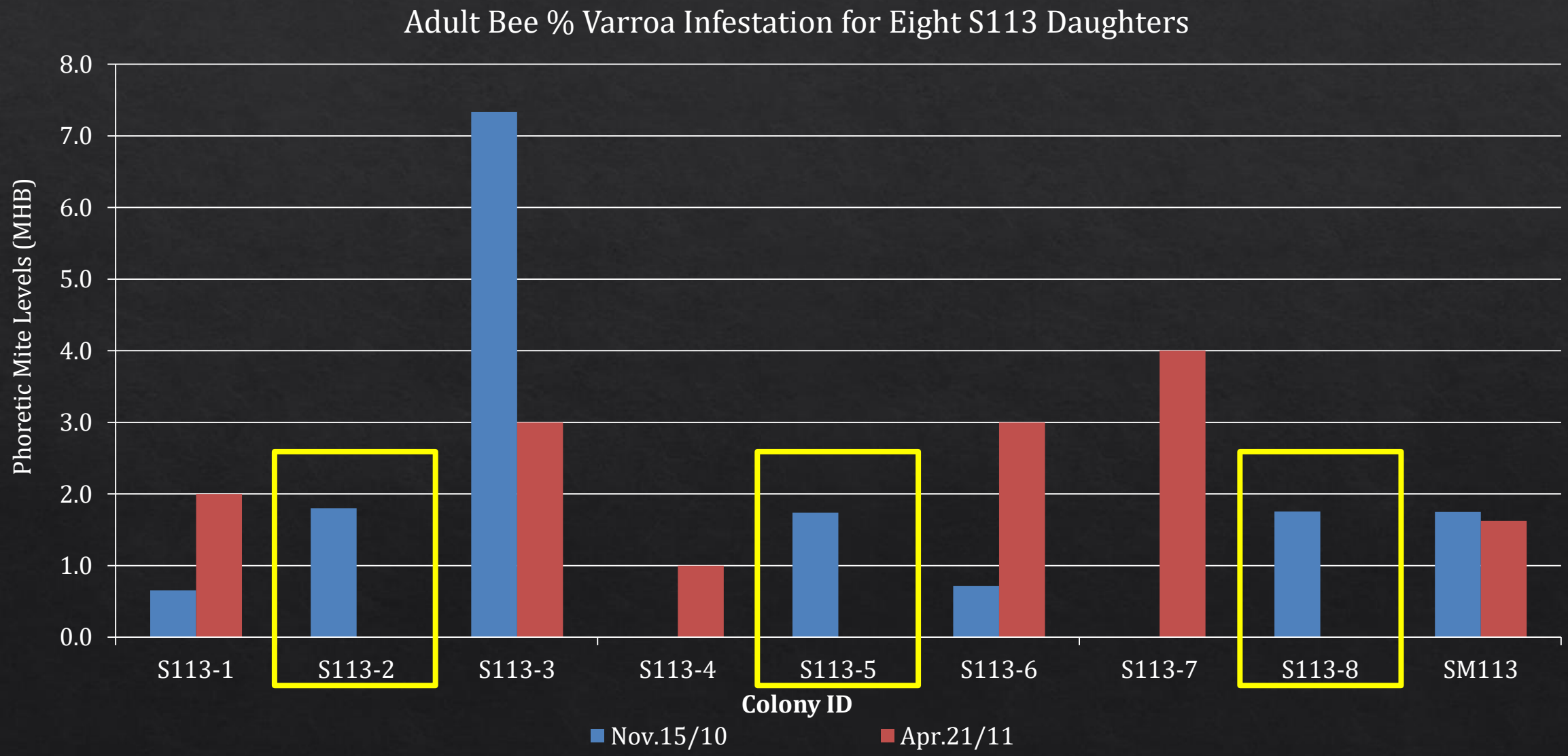
Natural Selection for Varroa Tolerance 2023



Natural Selection for Varroa Tolerance 2023



Progeny Analyses – S113




SL25 x GNS -18
The Saskatraz Project

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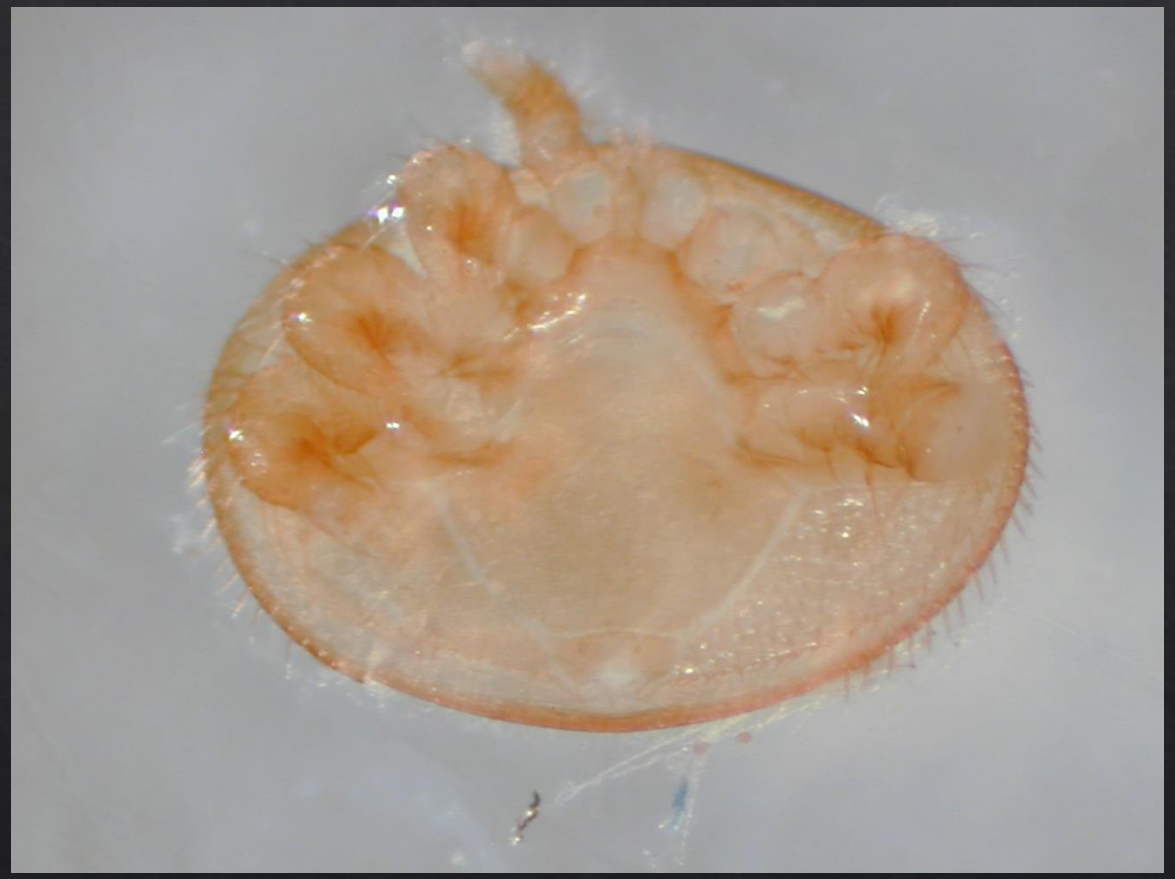
Mite Biting

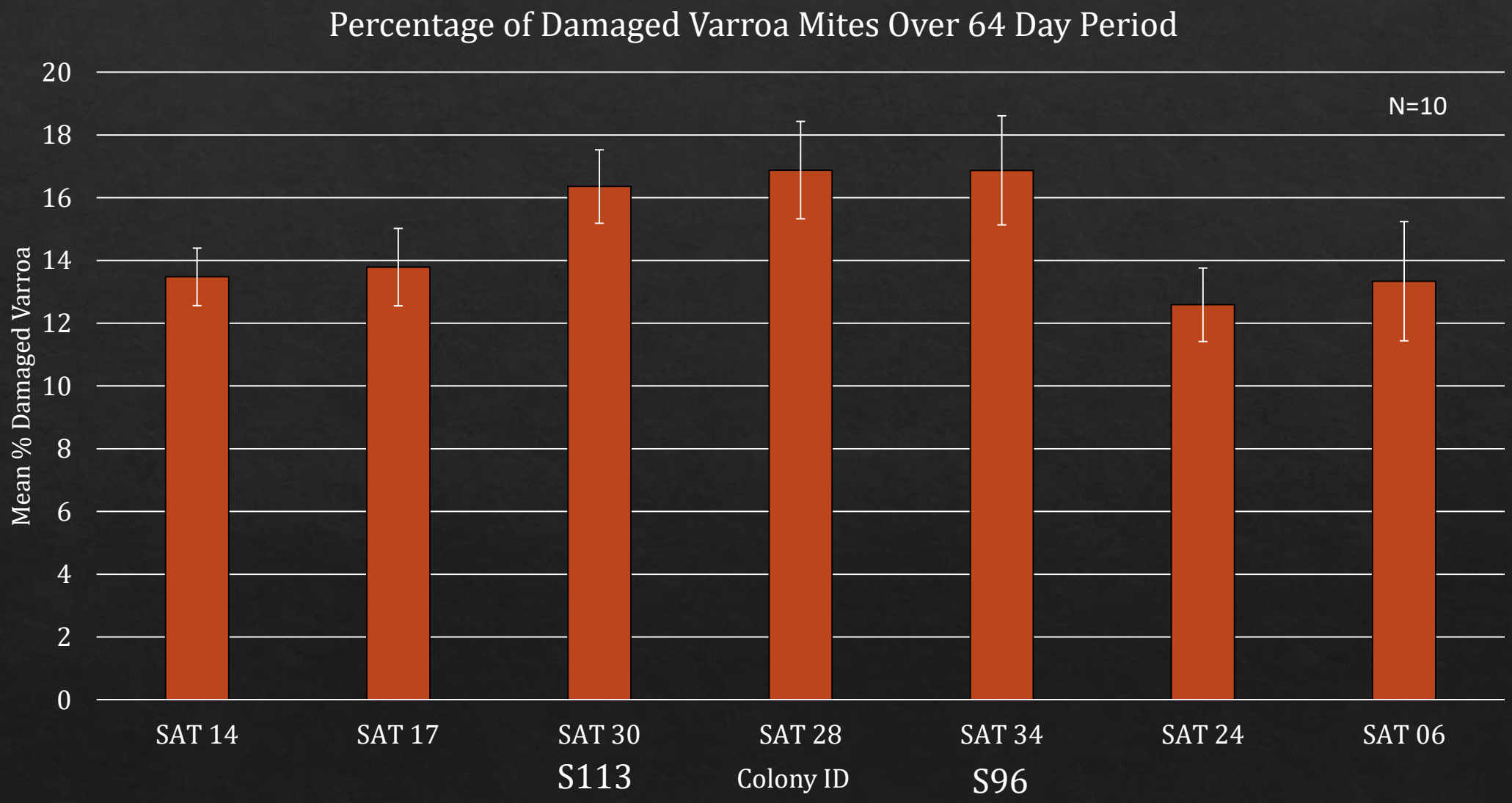
Intact Varroa Mite



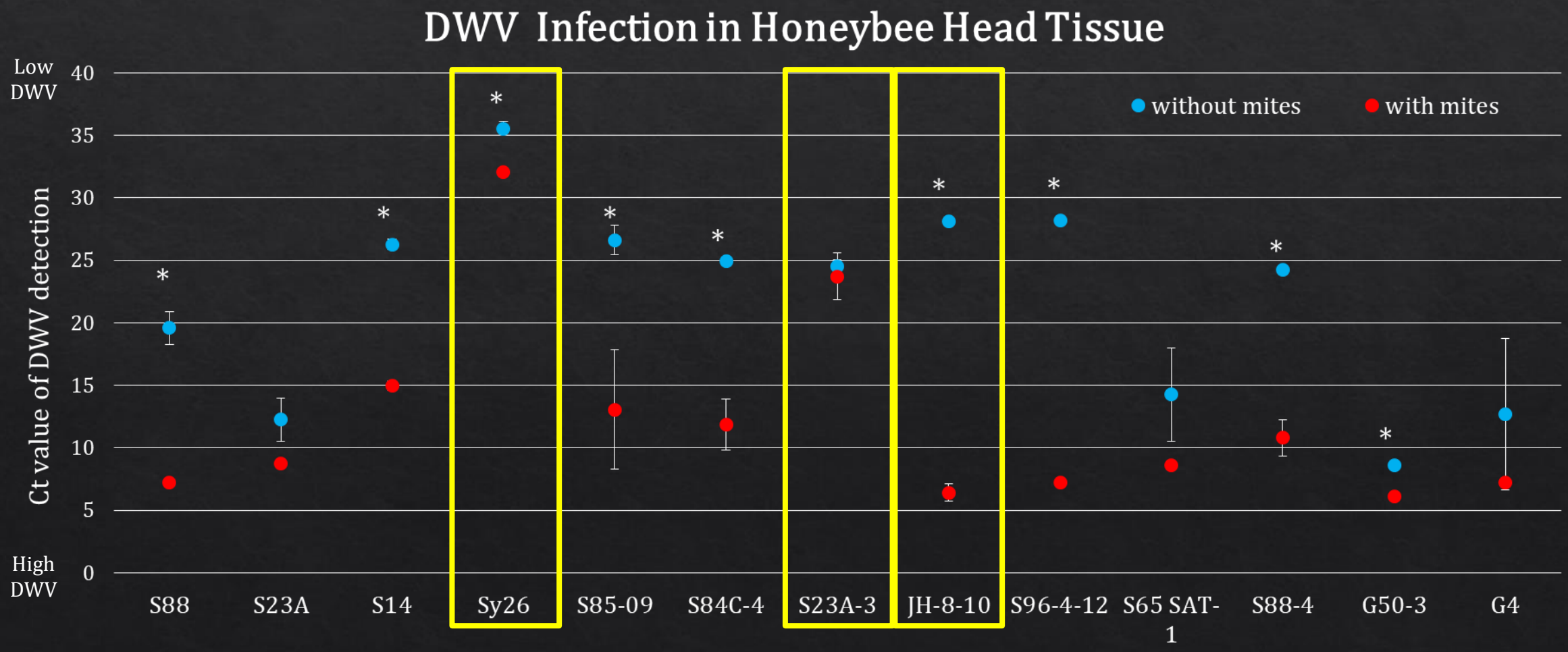
Mutilated Varroa Mite

Molecular assay available – Neurexin transcript expression levels correlated with mite biting activity.
Morfin, Nuria, et al. "Grooming behavior and gene expression of the Indiana “mite-biter” honey bee stock." *Apidologie* 51 (2020): 267-275.

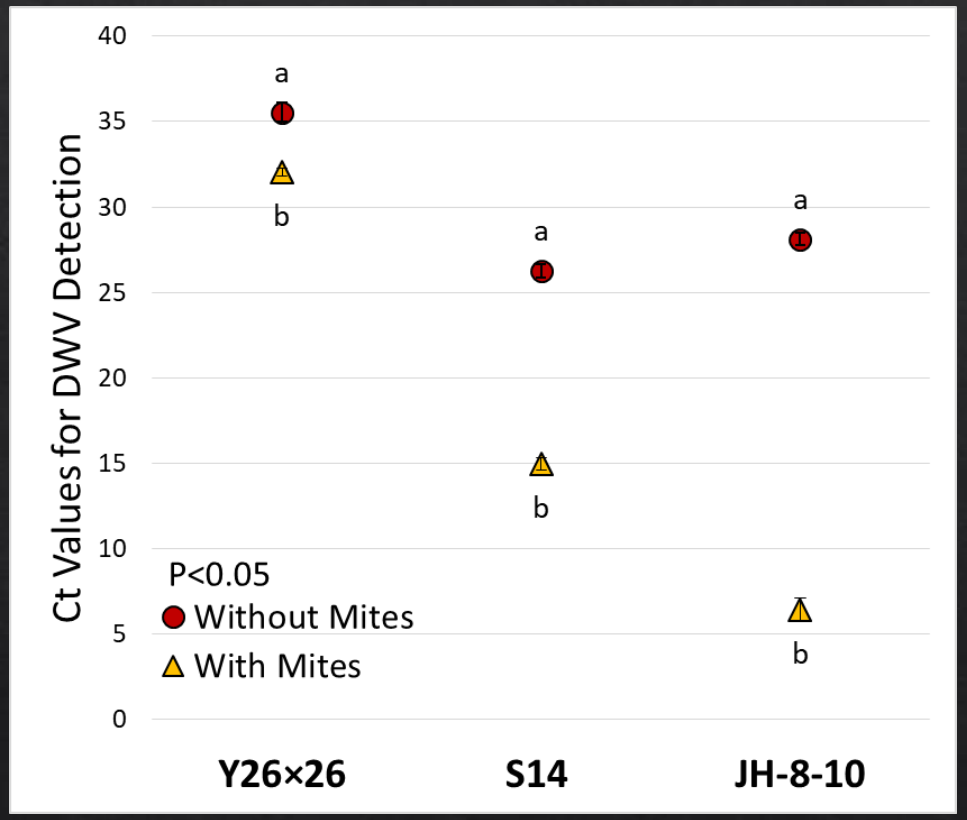
Mite Biting


Percentage of Damaged Varroa Mites Over 64 Day Period
Mean % Damaged Varroa
20
18
16
14
12
10
8
6
4
2
0
N=10
SAT 14
SAT 17
SAT 30
SAT 28
SAT 34
SAT 24
SAT 06
S113
Colony ID
S96

DWV Analysis of Saskatraz Phenotypes with and without Mites

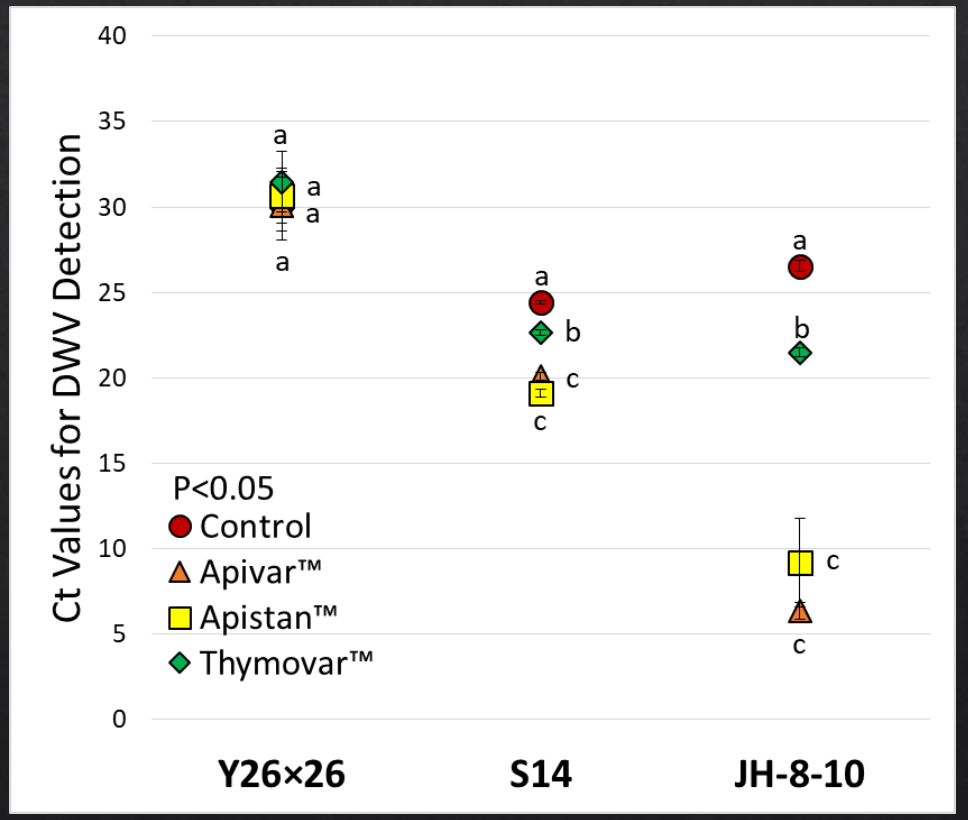


DWV Levels in Response to Varroa Mite Infestation and Miticide Treatments



Tolerant Susceptible

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



Tolerant Susceptible

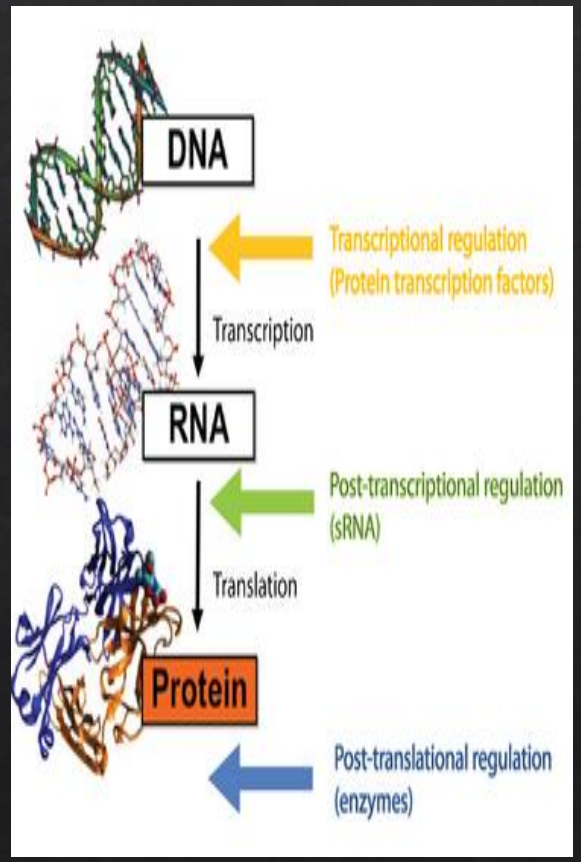
DWV in Head Tissue in Miticide Experiment

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.

Biomarker Development

- Microsatellites (SNP Discovery)
- Microarrays + RNA seq (transcripts)
- Proteomics
- Kinome Arrays (signal transduction)

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

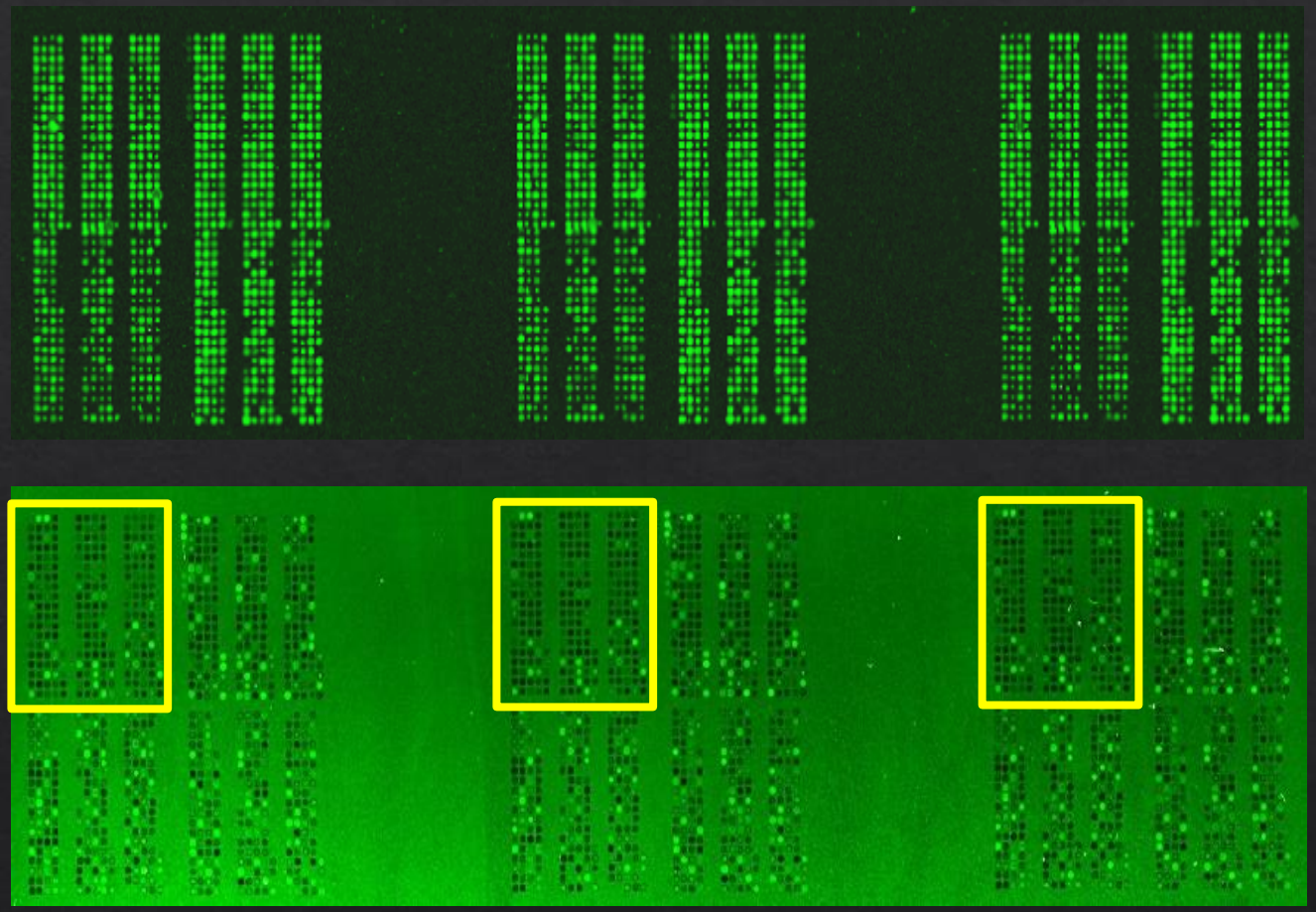


You can find our Publications at www.saskatraz.com under the Research heading.

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

Kinome Analysis of Colony Phenotypes

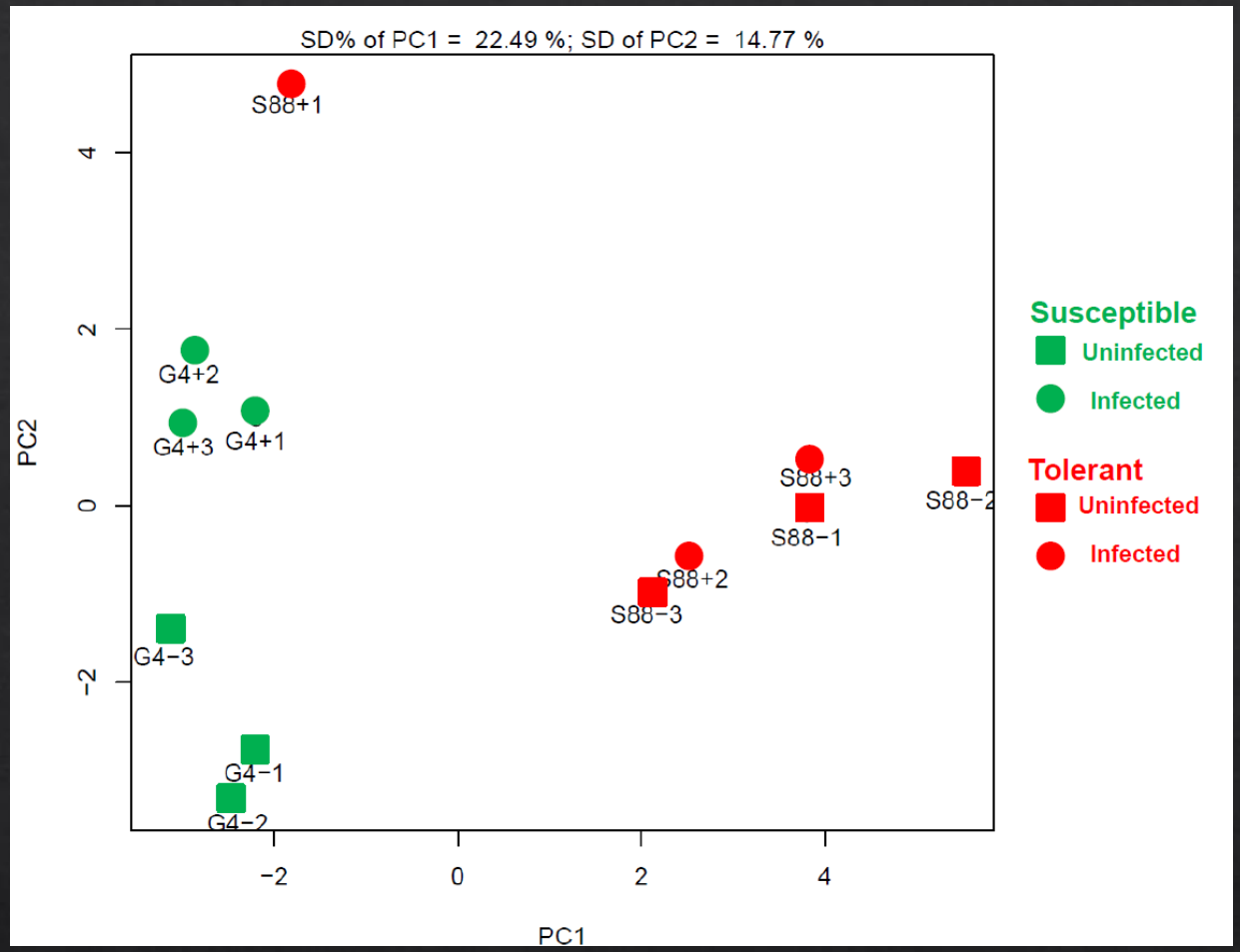


http://www.greendiary.com/hawaii-bees-infested-by-destructive-varroa-mites.html

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.

Selecting for Hygienic Behaviour Traits

Spring Evaluations

- Evaluate the cleanliness of bottom boards and presence of brood diseases
- Quick and simple selection method with minimal manipulation

Freeze Kill Test with Liquid Nitrogen or Pin Prick Test

- Tests require 24 – 48 hours
- Brood is killed using liquid nitrogen or pin pricks in a selected area of ~100 cells
- Hygienic bees detect death pheromone (oleic acid) and are scored based on percentage of brood removed
- Not well correlated with Varroa resistance

Host Brood suppression of Varroa reproduction

- Brood is caged immediately after caping to prevent worker intervention and brood assessed after 9 days
- Evaluation of non-reproductive mites found in brood without the intervention of worker bees
- Scaramella, Nicholas, et al. "Host brood traits, independent of adult behaviours, reduce Varroa destructor mite reproduction in resistant honeybee populations." *International journal for parasitology* (2023).

Selecting for Hygienic Behaviour Traits

Natural Selection

- Minimum evaluation time is 15 months
- Uncapping/recapping behaviour and presence of removed pupae
- Colony Varroa populations, honey production, and virus levels are measured regularly
- Provides detailed information on which colonies survive without treatment with increasing Varroa pressure

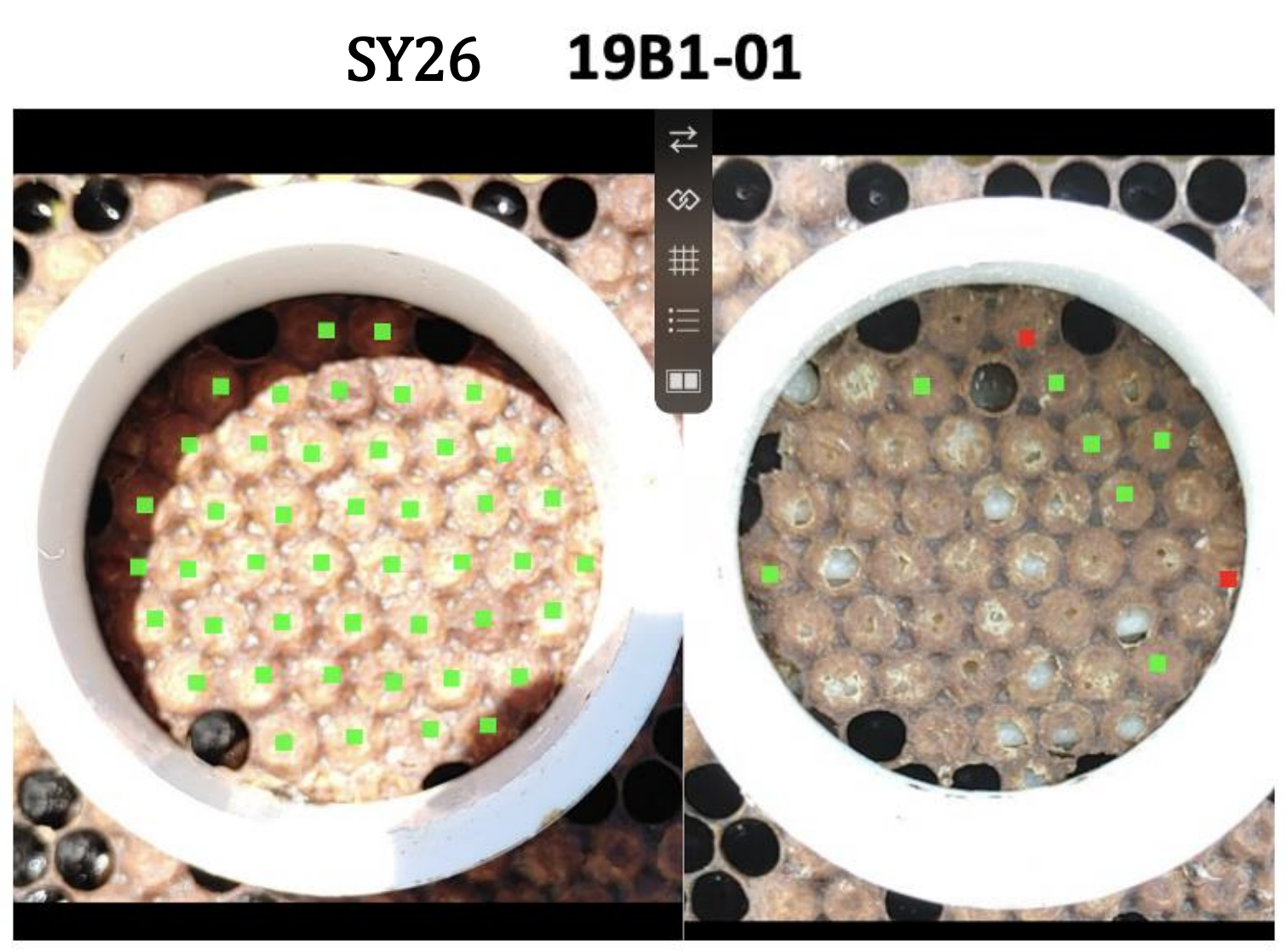
Harbo Assay for colony VSH score

- Single colony assays require approximately 45 minutes
- Score 200 brood cells on a rubric devised by John Harbo which are 7-11 days post capping (purple-eyed tan joints to purple-eyed grey wings) for non-reproductive mites
- VSH bees detect and remove reproductive mites

UBeeO Assay (Unhealthy brood odour)

- ~50 colonies can be assayed in 4 hours
- Based on the observation that Varroa infected brood produce increased levels of cuticular hydrocarbon pheromones
- 0.5ml of a hexane solution (C33, C31, C17, C15 monoalkenes) is sprayed on ~50 cells
- Scored based on the number of cells that have been opened 2 hours after application of UBeeO compound
- Potential rapid selection method for VSH activity

UBeeO Assay



Using Unhealthy Brood Odor Assay to Assess VSH Activity

UBO Assay Developed by:
Kaira Wagoner at UNCG

UBO Assay Score:
84.4%

Available Now
opterabees.com

Project *Apis m.*

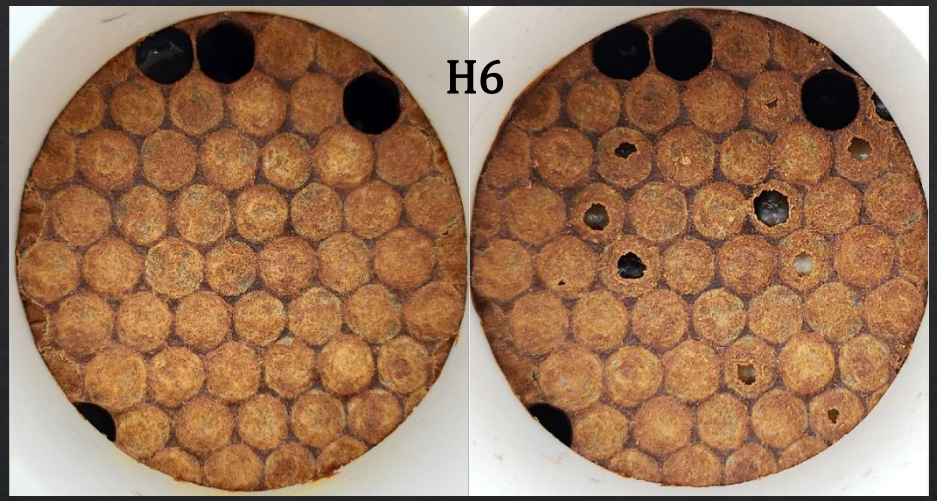
UBeeO Application Method

UBeeO Application Errors

UBO Assays of Unselected Colonies

Australian Colonies

Before After 2 Hours



Before After 2 Hours

UBeeO Progeny Analysis 2022 – SY26 Daughters

SY26 113

SY26 113
Honey Production: 103%
UBO Assay Score: 55.6%

SY26 124

SY26 124
Honey Production: 68%
UBO Assay Score: 79.5%

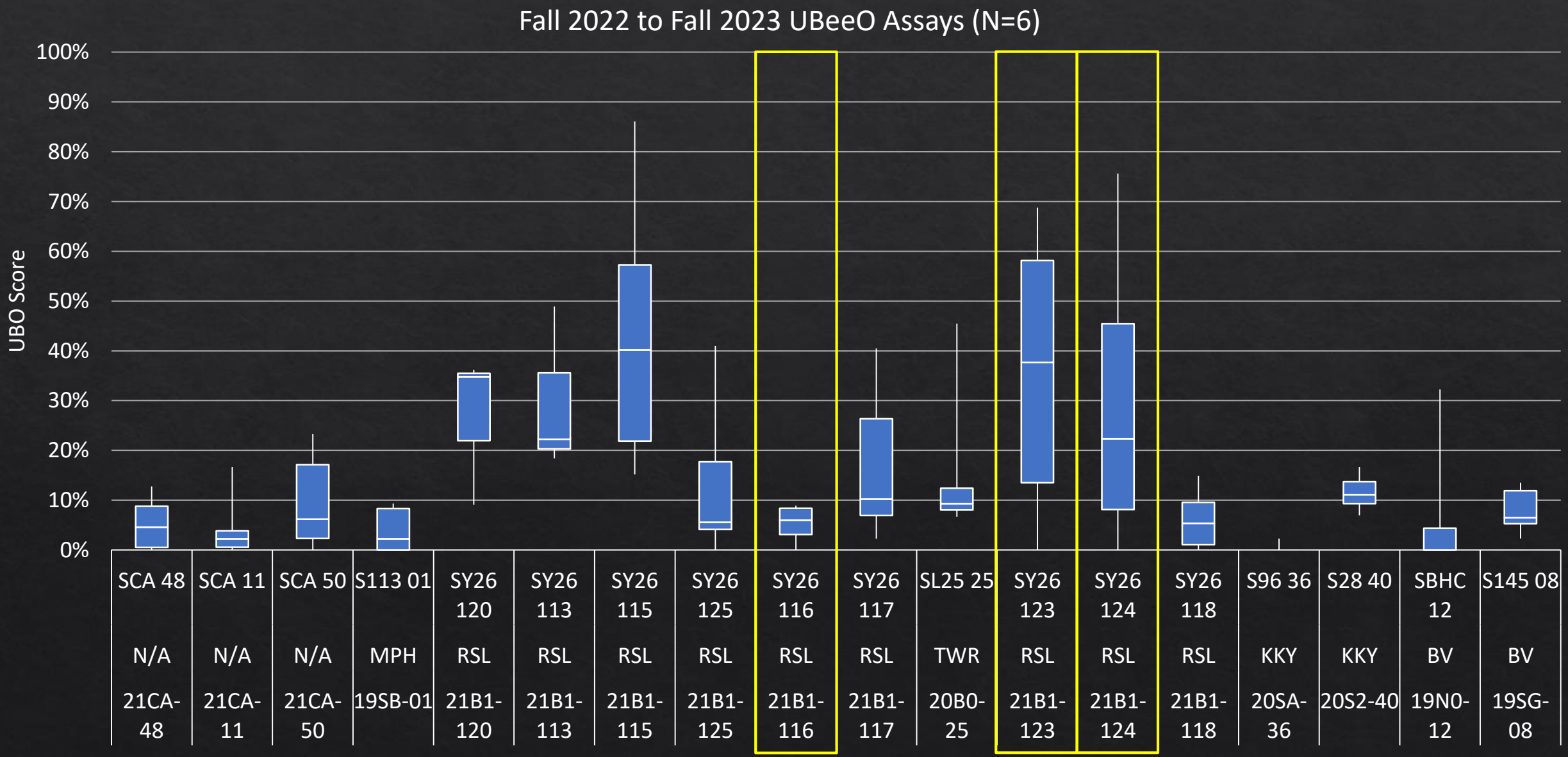
SY26 123

SY26 123
Honey Production: 163%
UBO Assay Score: 64.3%

SY26 118

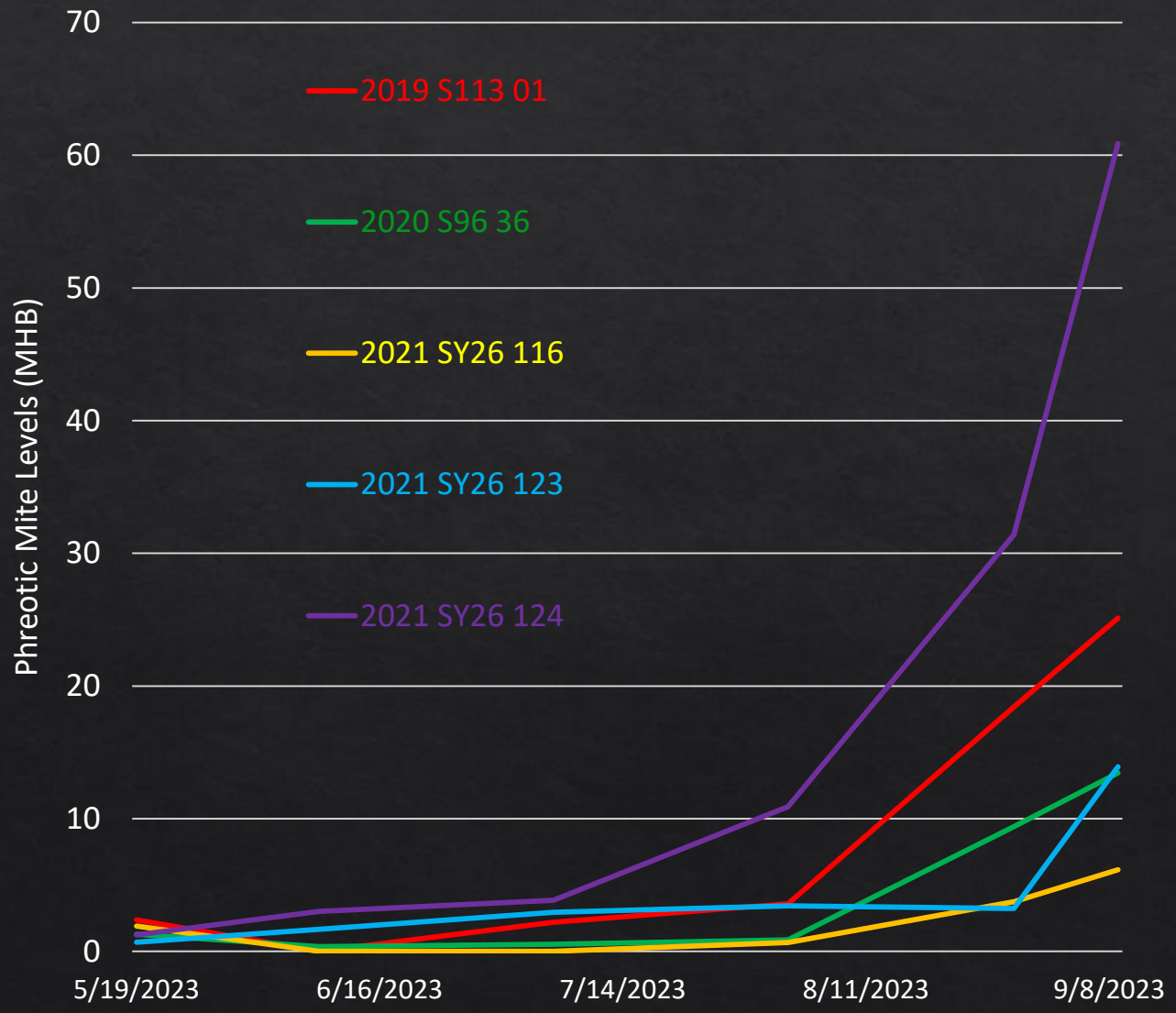
SY26 118
Honey Production: 155%
UBO Assay Score: 7.0%

UBeeO – Natural Selection Apiary

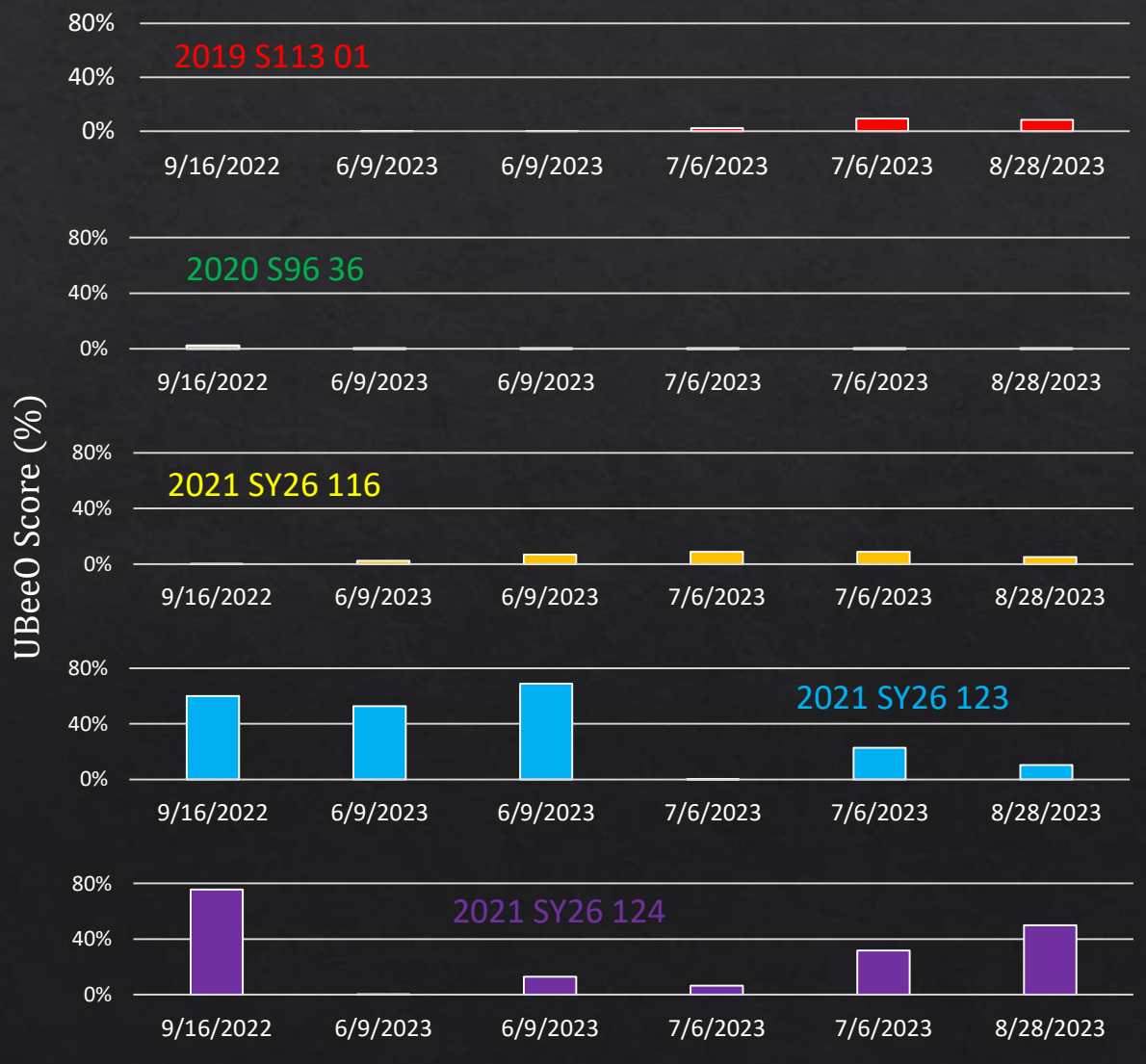


UBeeO – Natural Selection Apiary

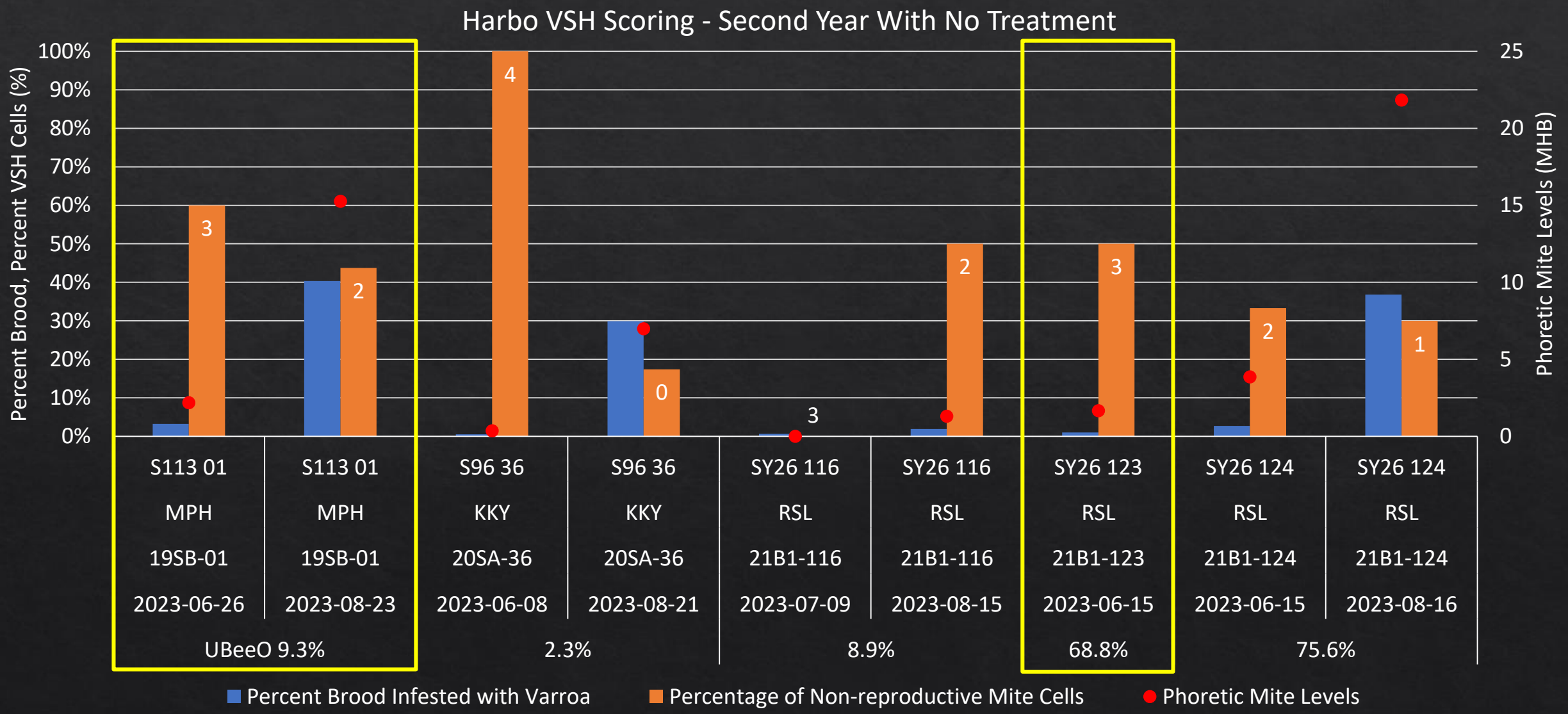
Phoretic Mite Levels



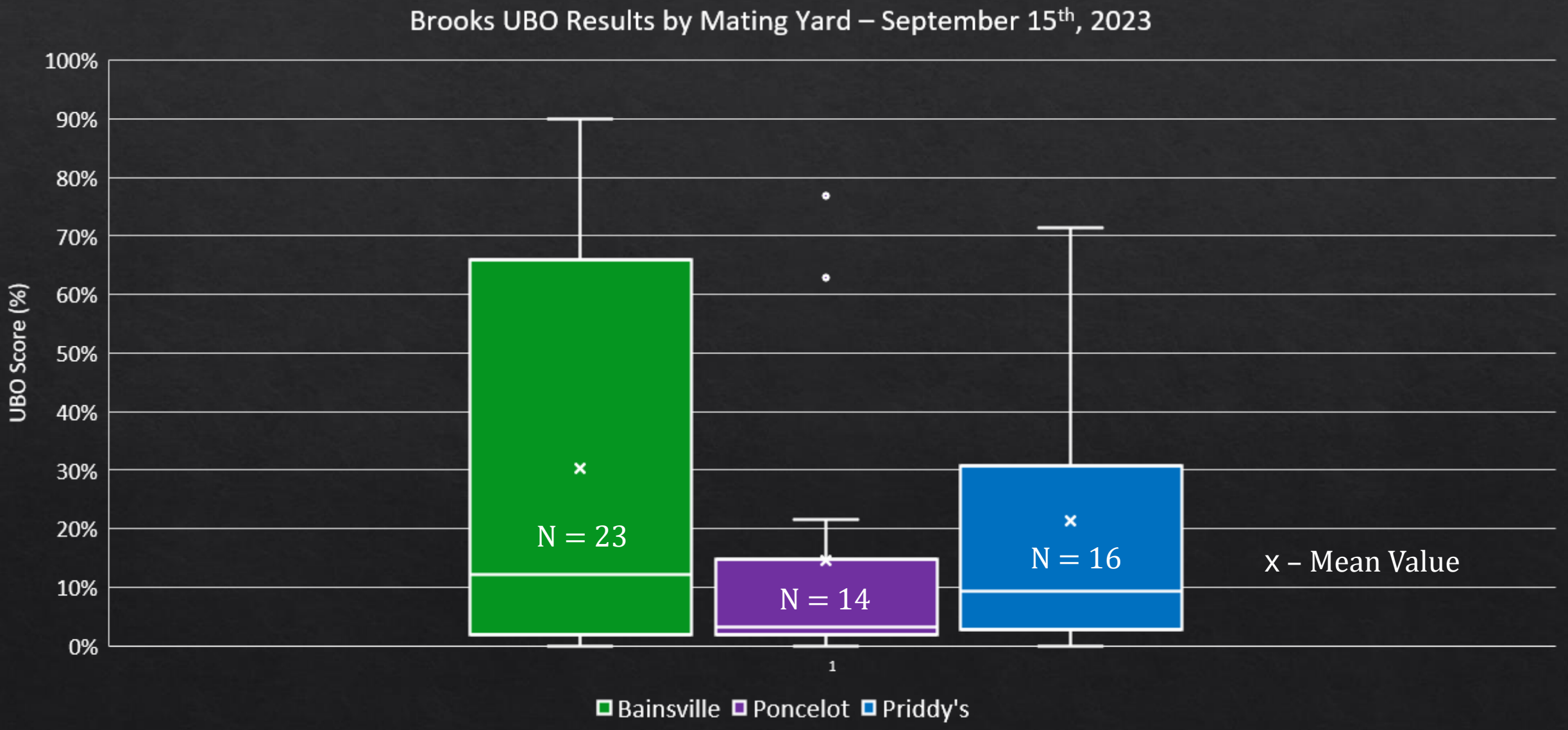
UBeeO Scores



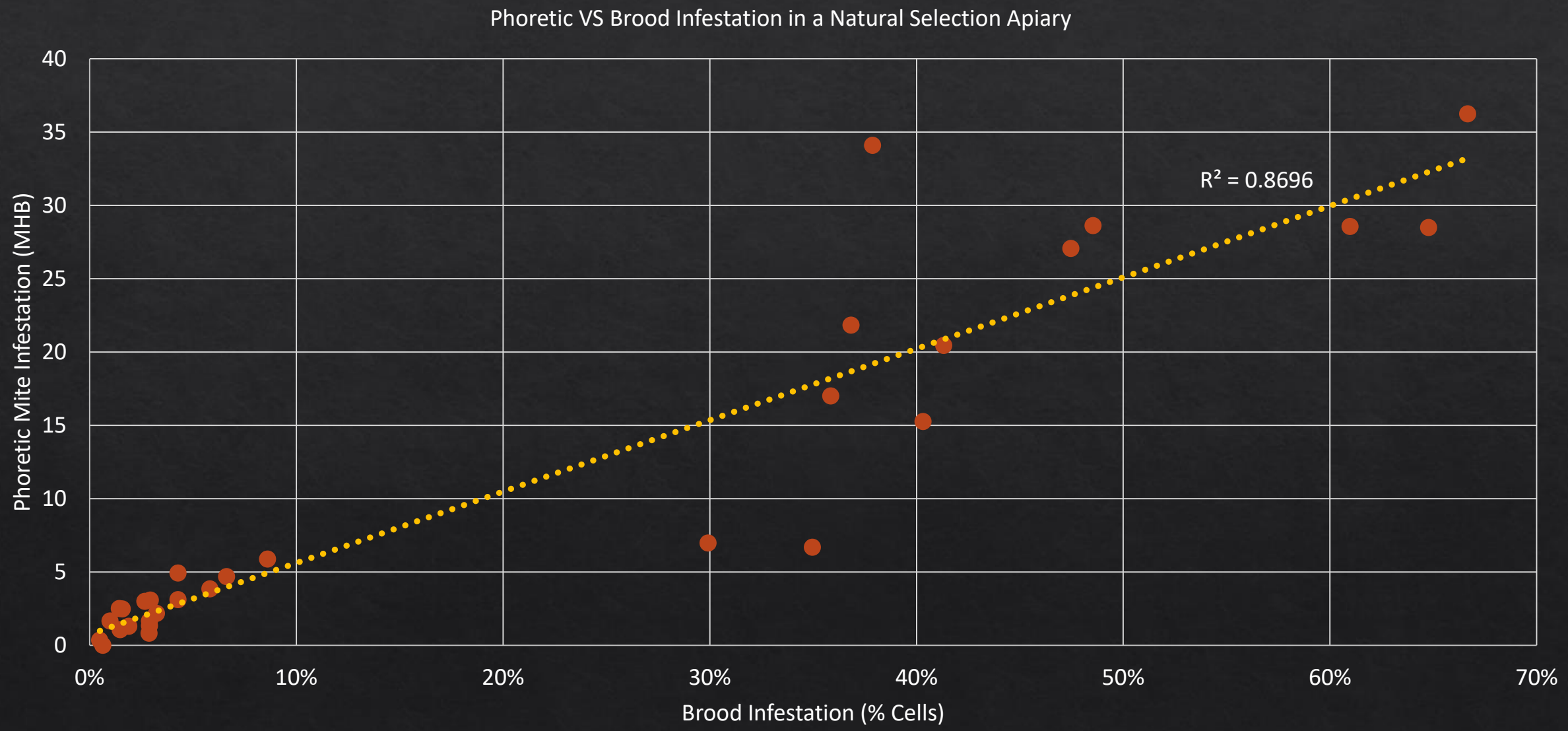
UBeeO – Natural Selection Apiary



UBO Progeny Analysis – SY26-123 Daughters



Correlation – Phoretic vs Brood Infestation



UbeeO Summary and 2024 Testing

The UBeeO Assay has been shown to be useful in predicting VSH phenotypes, but in our experience has considerable variability in response over the season. Some colonies that do not respond (S96-36 and SY26-116) show low Varroa population growth in Natural selection apiaries – possible different mechanisms?

- Select highest scoring colonies to establish a close population mating apiary.
- Select the best UBeeO scoring and Natural Selection maternal lines (S96 and SY26 116) and make crosses.
- Conduct more UBeeO assays on the progeny to assess heritability of the UBeeO response and effects of maternal and paternal lines.

This work is supported in part by:

Project *Apis m.*

Varroa Resistance Colonies Identified by Natural Selection – Survivor Colonies

- S88 for 58 Months and SY26 for 70 Months

- Varroa Sensitive Hygiene (VSH)
- Grooming Behaviour (Mite Biters)
- Supercedure, Brood Breaks, and Requeening Success
- Stress Resistance Biomarkers – Express higher levels of detoxification factors for pesticide, miticides and environmental stressors - Apolipoprotein D, Esterase E4, Cytochrome P450
- Kinome Phosphorylation Biomarkers – Virus Immunity (Innate Immunity)
- Vitellogenin Maintenance, Wintering Ability, and Longevity
- Better Foraging Activity = Better Nutrition

Summary

Goal – To combine and stabilize the traits associated with Varroa resistance by recurrent selection and close population mating.

Strategy: Screen economically selected Saskatraz colonies for Varroa resistance/tolerance using Natural Selection, UBeeO assay, and Harbo assays. Improve heritability by focusing on both the patriline and matriline.

Deploy marker assisted selection (kinome, transcripts, etc.) to further understand mechanisms of Varroa resistance and identify more informative markers.

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 and Varroa resistant traits in commercial populations.

Saskatraz stock distribution

- North America
- Iran
- Middle East (UAE, Saudi Arabia, etc.)
- Afghanistan
- Ukraine
- Turkey
- South Korea
- Virgin Islands, USA
- Lebanon
- 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.

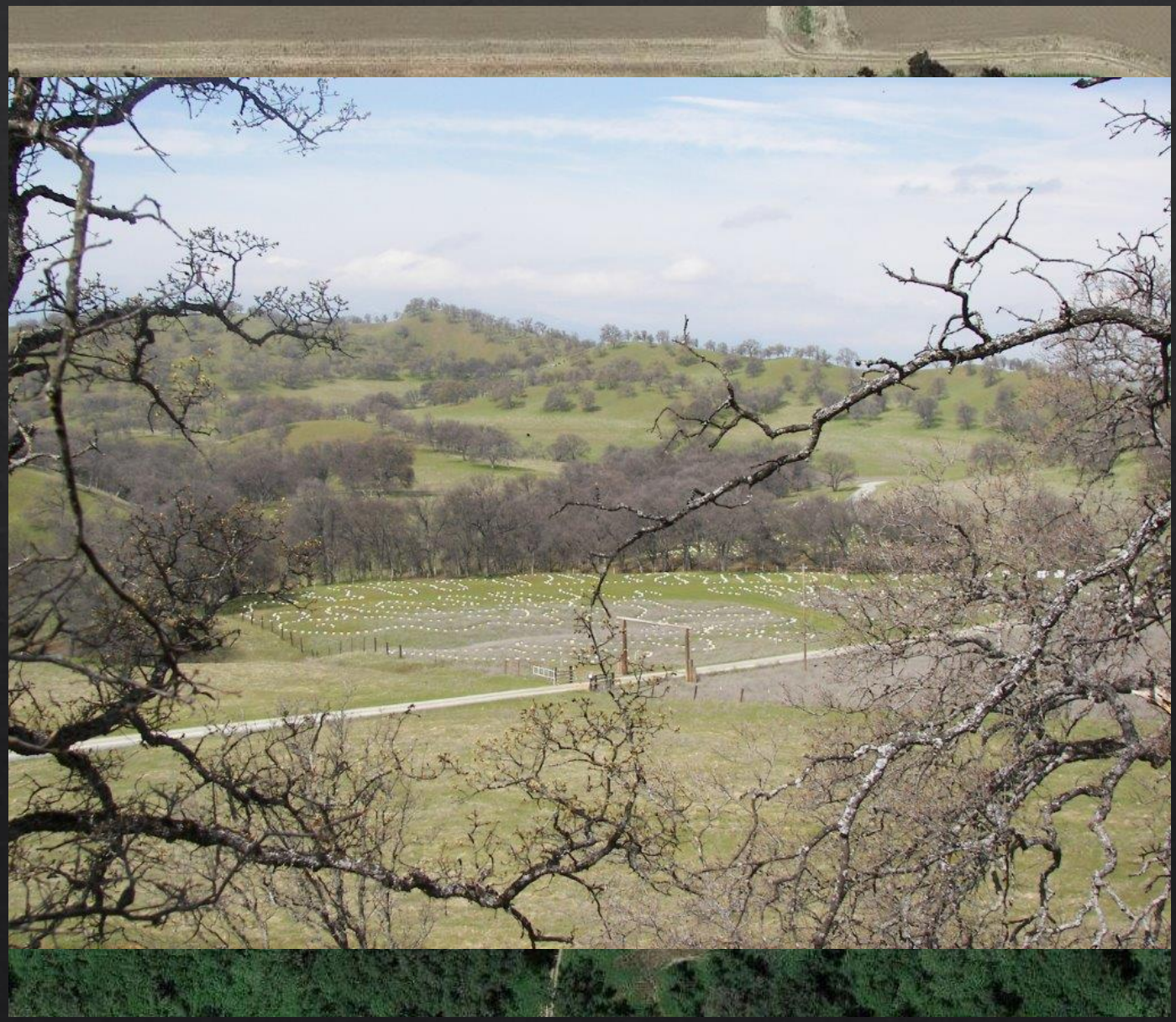
- Short queen production season
- Good location for selection criteria
- Can produce around 2500 queens/season
- Send about 150-200 breeders to be re-selected in California

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 Hybrid Project

Behaviour Assays – Reselect pre-selected breeders:

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. Drone brood Varroa assay and VSH assays

MHBA Meeting – March 12th, 2024

Varroa Assay


S96 RCD~15 x BV ~18
The Saskatraz Project

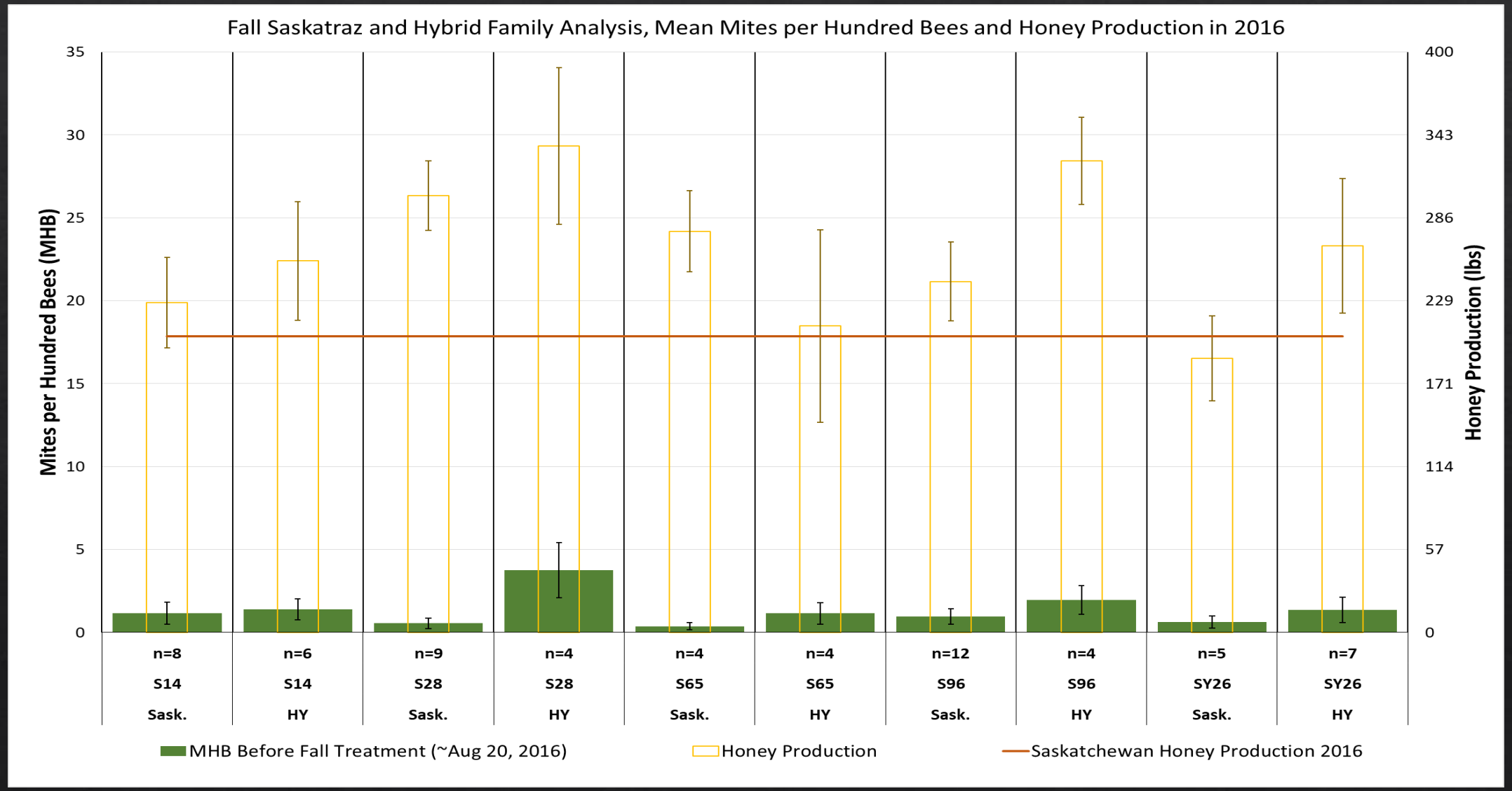
Saskatraz – Orland, USA

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)

Saskatraz Hybrid Performance



Questions?

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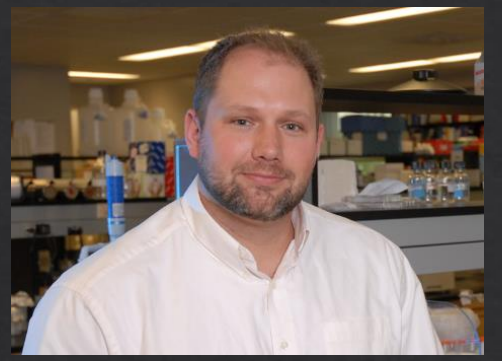

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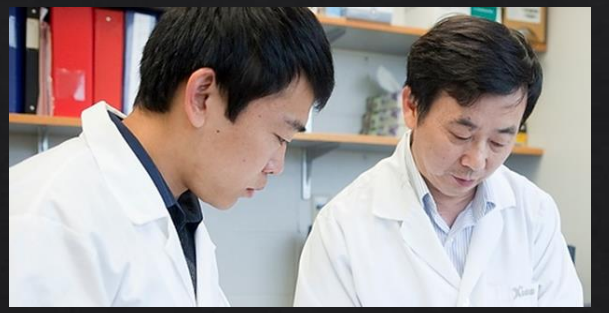
Dr. Philip Griebel Wayne Connor



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