The Saskatraz™ Project

Queens Produce Superior Workers

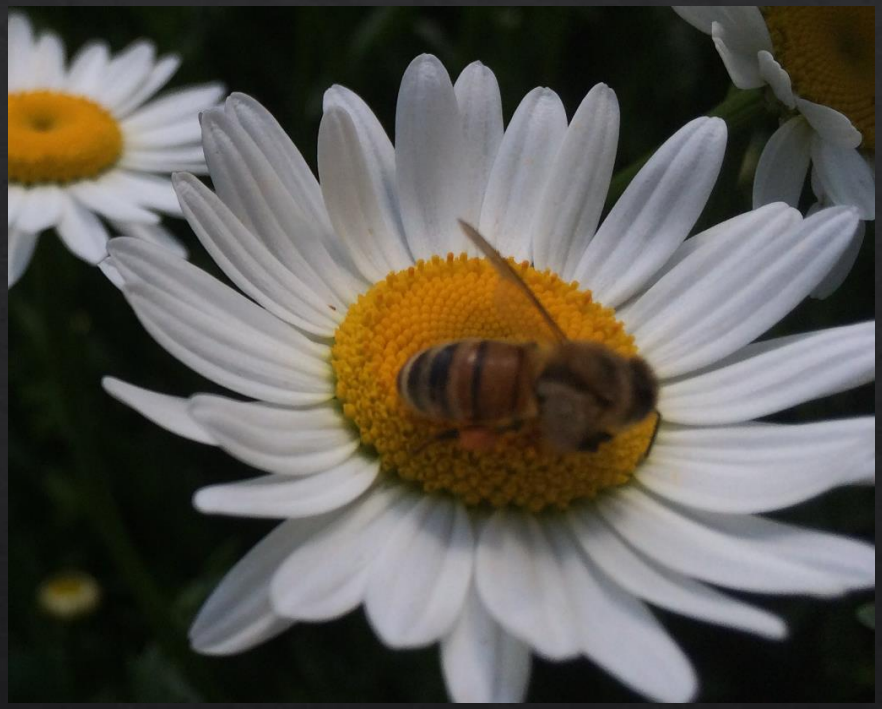
Objective: To develop productive, gentle honeybees with tolerance to mites and brood diseases

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

Saskatraz Research Team

Current Honeybee Health Issues

- Varroa
- Viruses
- Miticides
- Pesticides
- Nutrition



Outline

- Review of Saskatraz Breeding
- Saskatraz Hybrid Project
- Selection and Molecular Analysis of Extreme and Intermediate Phenotypes for Varroa Resistance and Susceptibility - Biomarker Development (Micro and Kinome Arrays) and Virus Screening
- Proteomic and PCR analyses for Pathogen Identification
- Management of Varroa Populations by Selective Treatment Strategies Using Varroa Tolerant and Susceptible Phenotypes
- Screening of Hive Products and Honeybees for Miticides (Toxicology Collaboration, U of S)
- Efficacy of Combined Treatments of Synthetic and Organic Miticides
- Please find Published Papers and More Information on the Saskatraz Project at www.saskatraz.com

Current Objectives

1. To propagate, maintain and improve (stabilize) productive and varroa tolerant phenotypes (17 Saskatraz families established), using re-current natural selection and newly discovered biomarker tools.

2. To continue identification and characterization of key genes (genomic analyses, microsatellites, micro arrays), and proteins (SDS-PAGE-LC-ESI-MS) and enzymes (kinome arrays) involved in signal transduction mechanisms.

3. Investigation of pesticide residues (OP-NI) in hive products and bees.

4. Evaluation of spring and fall miticide treatments both alone and in different combinations to improve efficacy. Determine rates of varroa population regrowth after miticide treatments in different Saskatraz breeding lines, including hybrids.

Saskatraz Breeding Program

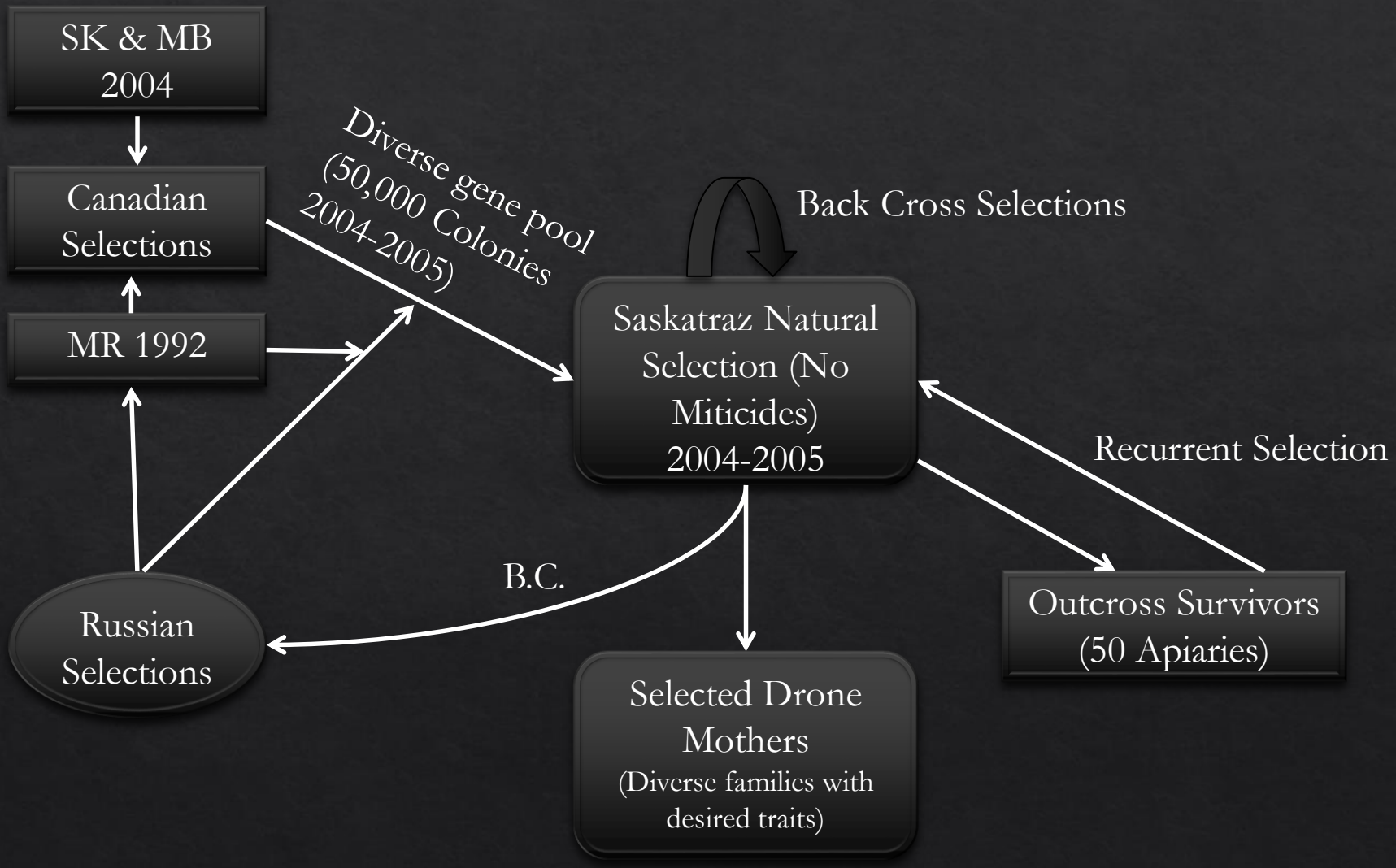
Primary Selection Criteria:

1. Honey Production
2. Wintering Ability
3. Mite Resistance and Suppression
4. Resistance to Brood Diseases (chalk brood etc.)
5. 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).


North Pole
Norwegian Sea
Norway
Polar Ice
Greenland Sea
UK
Russia
Arctic Ocean
Ellesmere Island
Alert
Iceland
Kalaallit Nunaat (Grønland) (Denmark)
North Magnetic Pole
Bering Strait
Arctic Circle
Beaufort Sea
Banks I.
Resolute
Baffin Bay
Alaska (USA)
Inuvik
Victoria Island
Davis Strait
Dawson
Great Bear Lake
Gjoa Haven
Baffin Island
Yukon Territory
Whitehorse
Mackenzie River
Iqaluit
Northwest Territories
Nunavut
Labrador Sea
Yellowknife
Baker Lake
Fort Nelson
Great Slave Lake
Ungava Bay
Nain
Newfoundland
Atlantic Ocean
CANADA
British Columbia
Kuujjuaq
Fort McMurray
Churchill
Hudson Bay
Churchill Falls
St John's
Prince George
Alberta
Saskatchewan
Manitoba
Pacific Ocean
Fraser River
Edmonton
L. Winnipeg
Sept-Îles
La Grande Rivière
Saint-Pierre et Miquelon (France)
Vancouver
Victoria
Kamloops
Calgary
Saskatoon
Sandy Lake
James Bay
Quebec
Prince Edward Island
Charlottetown
Winnipeg
Thunder Bay
Ontario
Québec
New Brunswick
Nova Scotia
Halifax
Fredericton
Montréal
United States of America
Ottawa
Lake Superior
Toronto
Lake Michigan
Lake Huron
Lake Ontario
Lake Erie
U of S Saskatoon
LEGEND
National capital
Provincial or Territorial capital
Other populated places
Scale
300 0 300 600 900
km km

Saskatraz Breeding Program Logistics

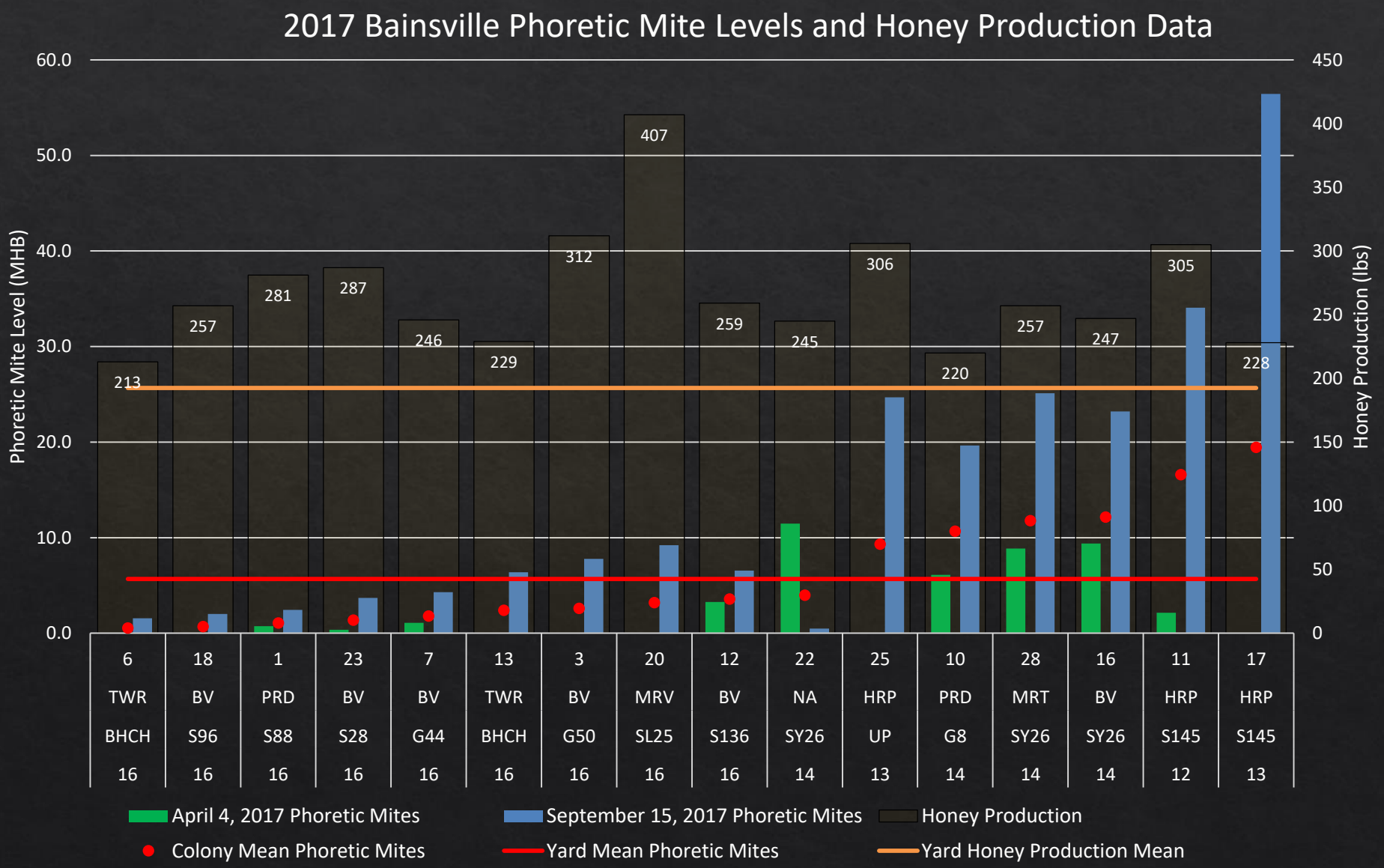


See www.saskatraz.com for review

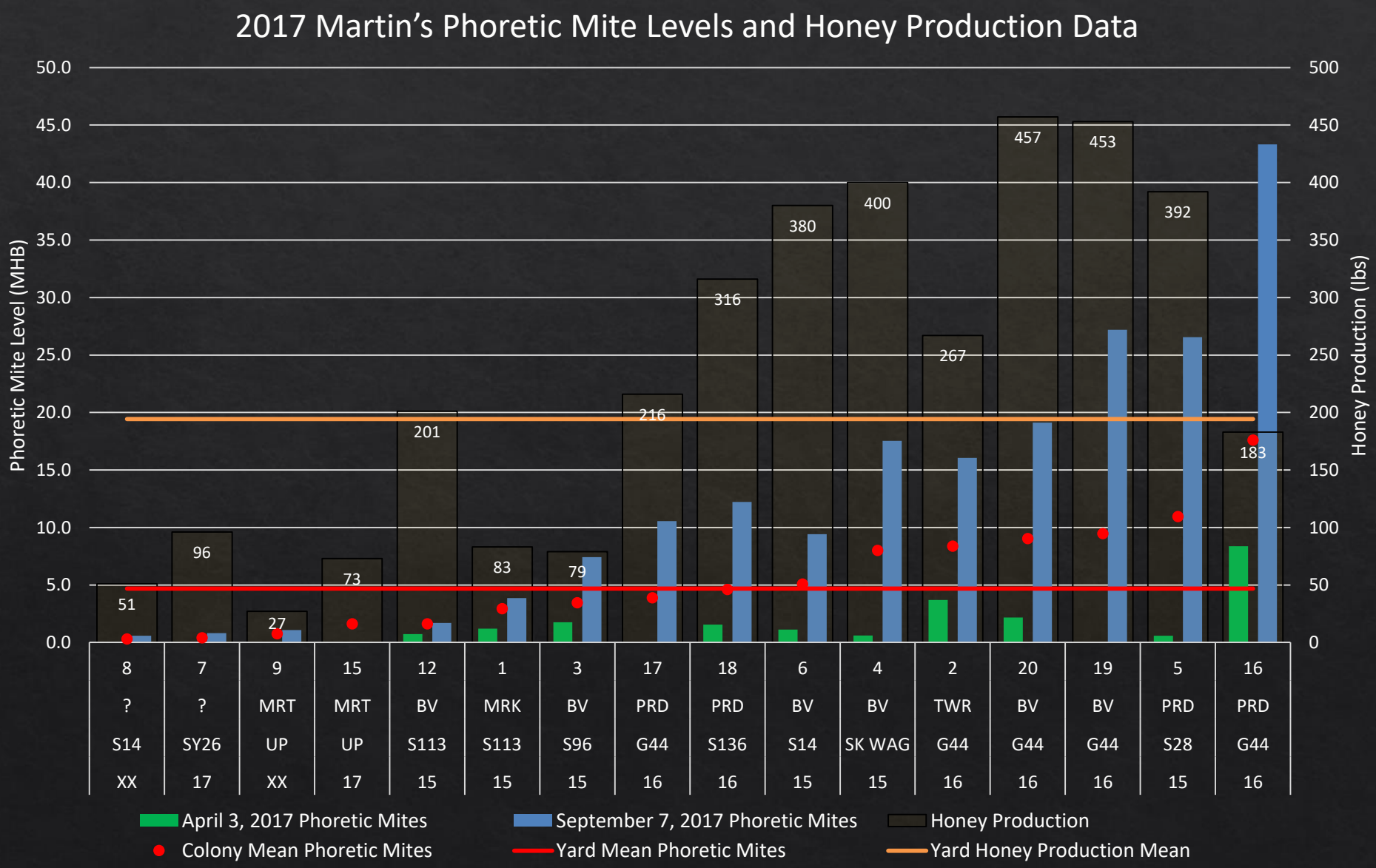
The Original Saskatraz Apiary

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

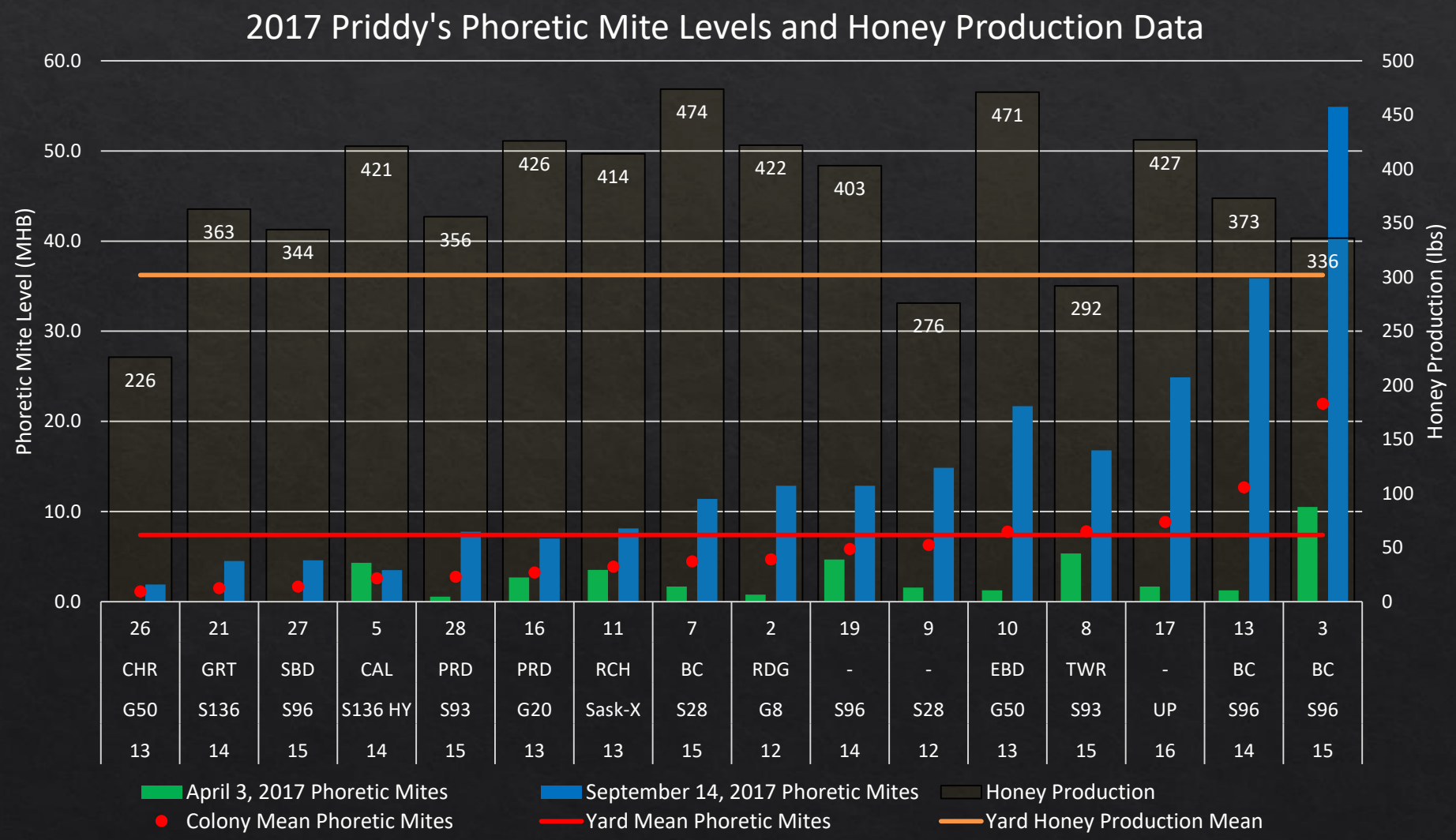
Natural Selection for Varroa Tolerance



Natural Selection for Varroa Tolerance

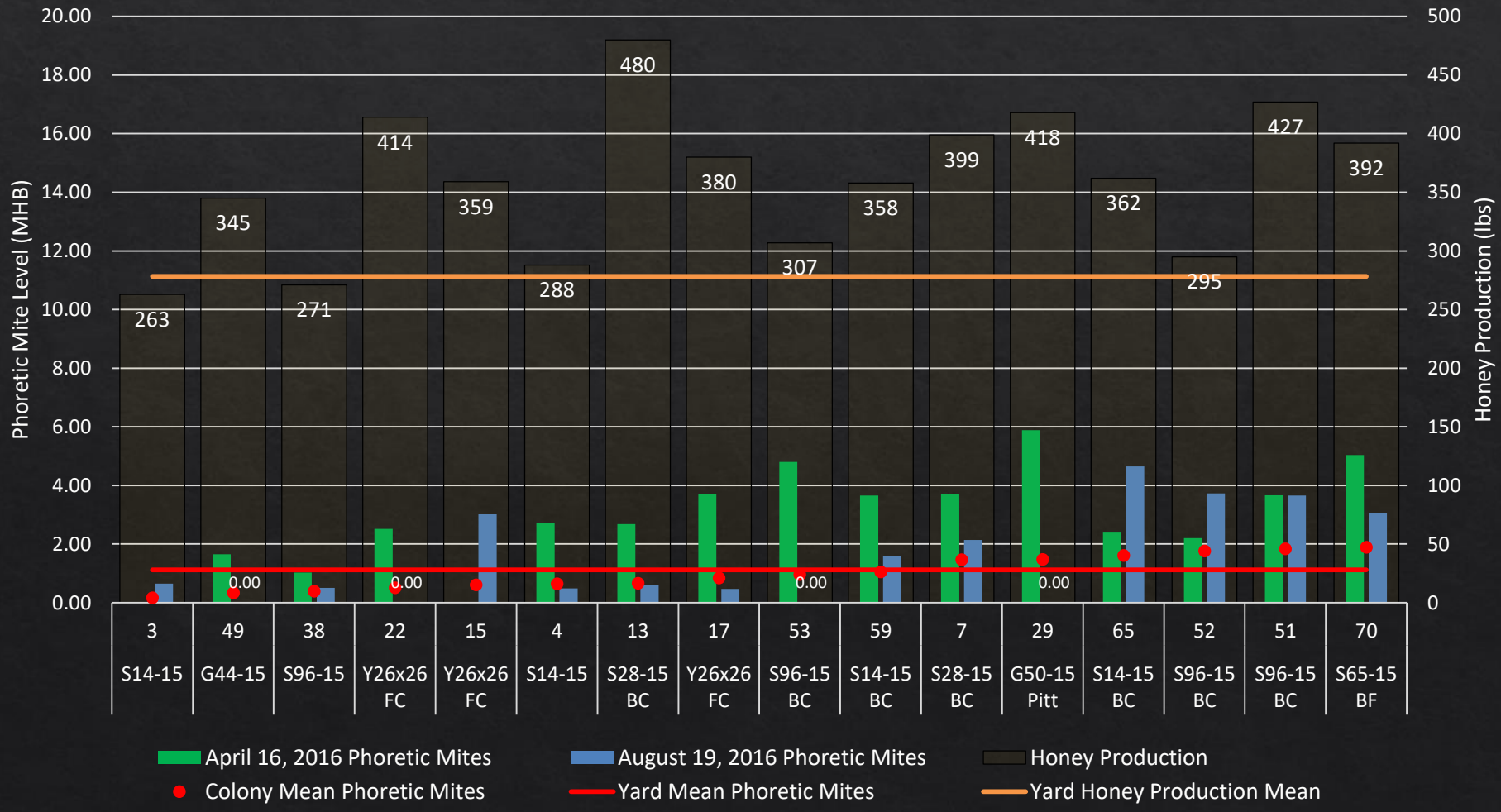


Colony Selection for Honey Production and Wintering – Closed Pop. Mating



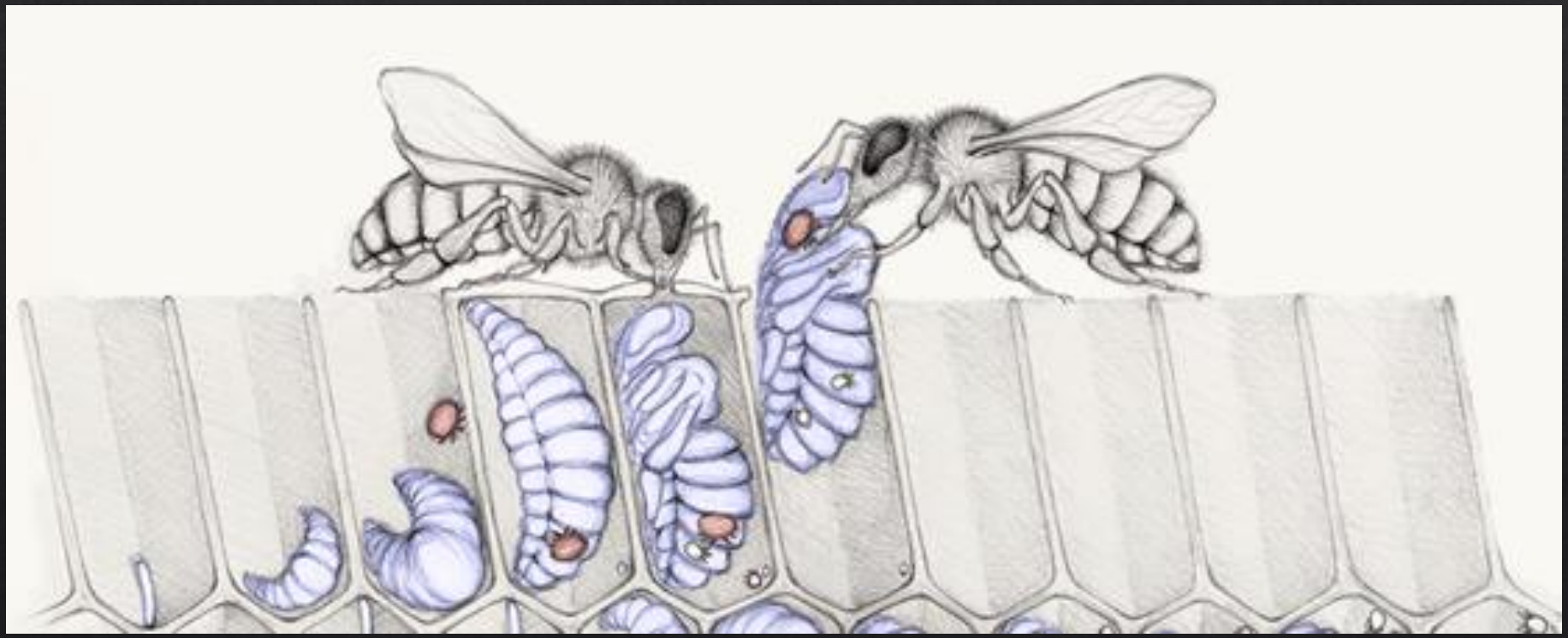
Saskatraz Hybrid Yard for Closed Population Mating – Drone Mothers

2016 Tower Phoretic Mite Levels and Honey Production Data



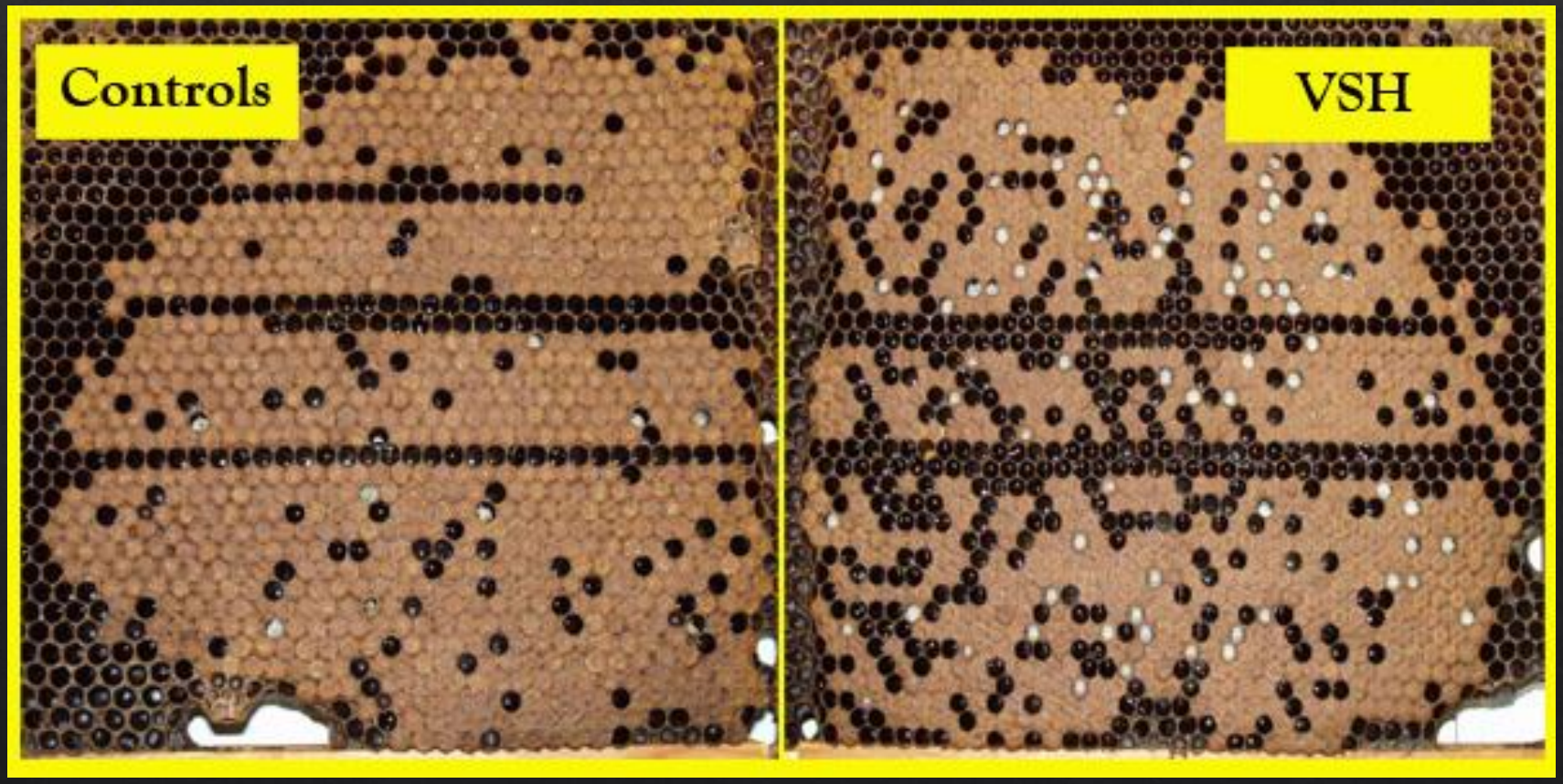
Example of VSH Activity

High levels of Varroa-Sensitive Hygienic (VSH) behavior



Slide from USDA – Baton Rouge.

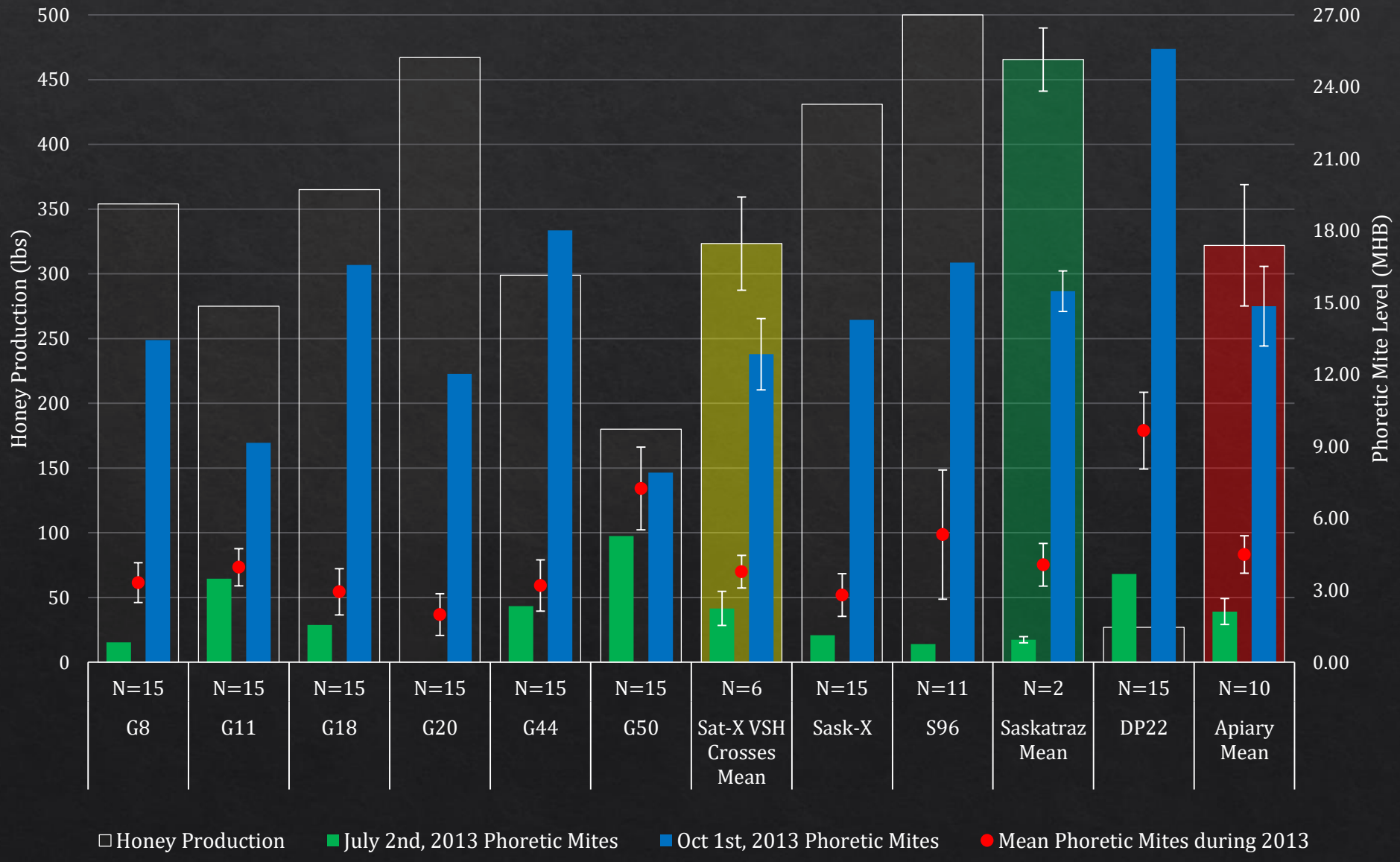
Example of High VSH Activity



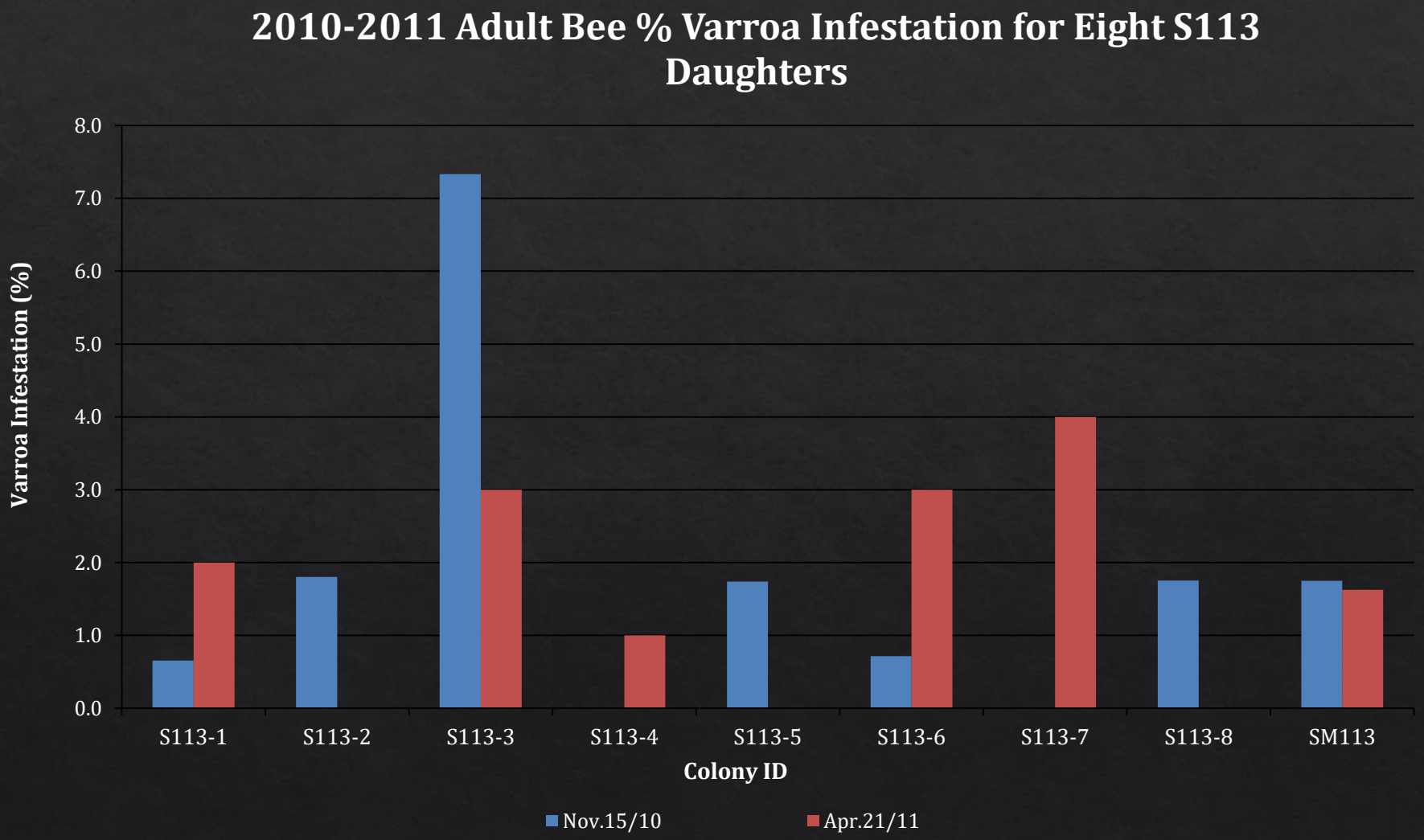
Slide from USDA – Baton Rouge.

Global Forum for Innovations in Agriculture, ApiArab Expo - February 5th 2018

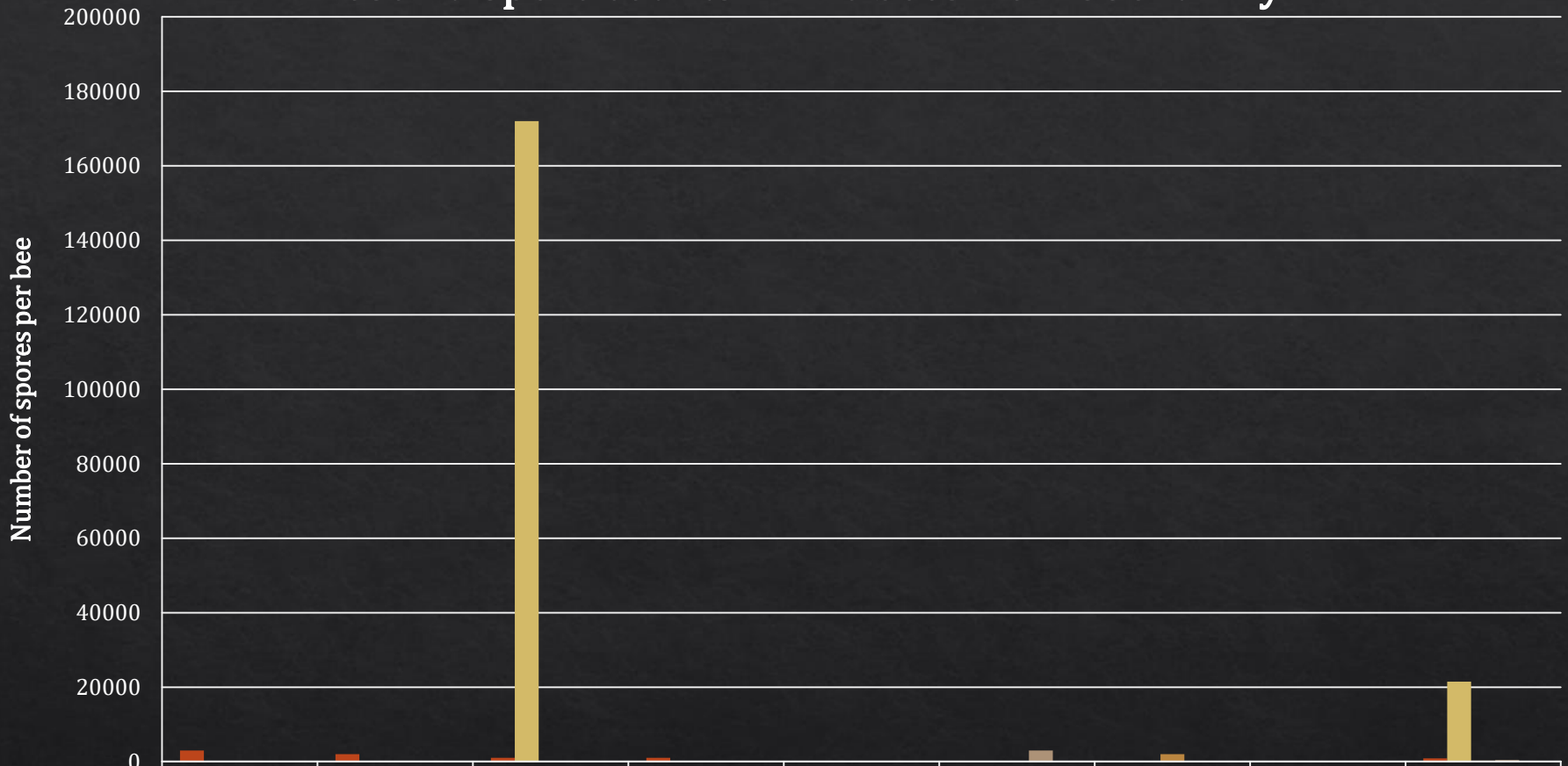
Effects of Adding VSH Traits to Naturally Selected Lines



Progeny Analyses – S113

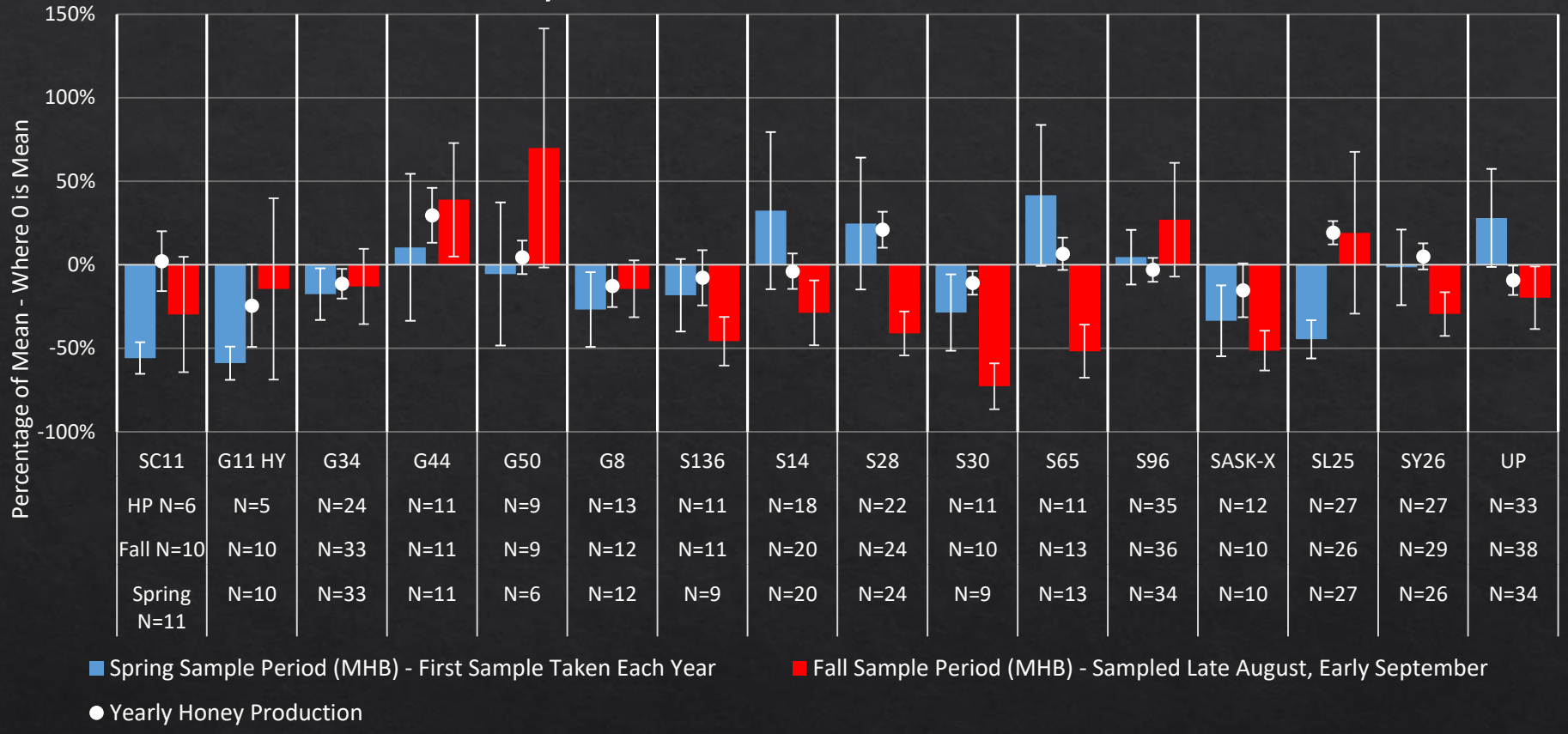


Nosema Spore counts in live bees from S88 family



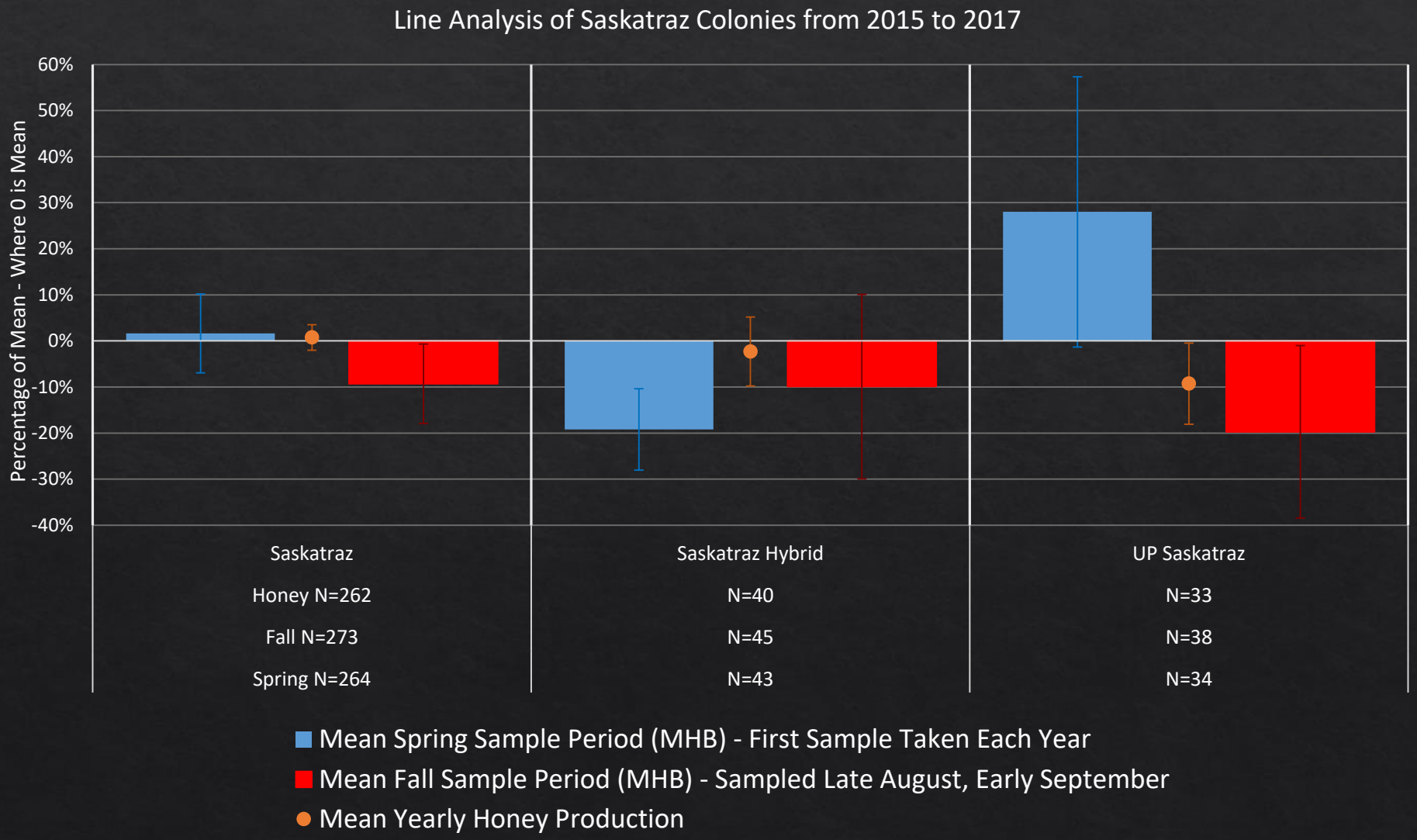
	S88-1	S88-2	S88-3	S88-4	S88-5	S88-6	S88-7	S88-8	Mean
Dec28-10	3000	2000	1000	1000	0	0	0	0	875
Jan-13-11	0	0	172000	0	0	0	0	0	21500
Jan-28-11	0	0	0	0	0	0	2000	0	250
Feb-13-11	0	0	0	0	0	3000	0	0	375
Feb-28-11	0	0	0	0	0	0	0	0	0

Analysis of Individual Saskatraz Families for Phoretic Mite Levels and Honey Production from 2015 to 2017



Saskatraz breeding lines were scored for spring and fall phoretic mites and honey production at 17 different apiaries between 2015 and 2017. The mean honey production and phoretic mites were calculated for each apiary. Individual families were then scored according to percent of the yard mean for each parameter.

Saskatraz – SKHY – UP Analysis



Saskatraz Hybrid Project

Introduction

- To commercialize and distribute the breeding stock to the end users.
- Every year colonies are selected for honey production, overwintering ability, temperament, mite resistance and brood diseases.
- In 2017 we sent 130 queens to be reselected in March 2018.
- The California Tech Transfer Team, Bee informed Partnership has independently evaluated our Saskatraz breeding stock in late February early March.

Colony Number	Colony ID	Brood Pattern	Chalk-brood Presence (+/-)	Tempera-ment	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	Y26 x 26 Martin 14	Good	-	1	Average	+	-	0	0	-	99%O / 99%R	No drone brood; no visible mark on queen
25	Y26 x 26 Martin 14	Excellent	-	1	Average	+	+	0	0	0	100%O / 100%R	-
37	G44 JHN 12-9 B.V. 14	Excellent	-	1	Average	+	+	0	0	-	93%O / 75%R	No Drone

California Tech Transfer Team

Evaluation included hygienic testing (uncapped, removed), colony strength (frames of brood), brood pattern (1-poor to 5-best), queen status, temperament (1- best to 5-poor), color, varroa infestation (Mites per Hundred Bees) and nosema spore count.

Colony ID	Hive Body	Frames of Bees	Brood Pattern	Queen Status	Temperament	Color	Mites Per Hundred Bees	Millions of Spores/Bees	% Uncapped	%Removed
1469	1D	6.5	4.5	QR	1	3.75	0	0.25	71%	53%
1470	1D	7.5	4.5	QS	1	3.5	0	0.3	88%	82%
1471	1D	7	4.25	QR	1	3.25	0	0.3	52%	42%
1472	1D	7	5	QR	1	3.75	0	0.15	76%	63%
1473	1D	5	4.5	QS	1	3.25	0	2.2	63%	51%
1474	1D	3.5	4.5	QR	1	3.5	0	0.35	98%	67%
1475	1D	5.5	5	QR	1	3.5	0	2	64%	56%
1477	1D	7.5	5	QR	1	3.5	0	0.9	89%	70%
1478	1D	7	4.5	QR	1	3.25	0	0.65	88%	85%
1479	1D	6.5	5	QR	1	3	0	0.6	87%	82%
1480	1D	8	5	QR	1	3.75	0	0.05	93%	80%
1481	1D	6.5	4.75	QR	1	3.75	0	0.4	66%	54%
1482	1D	7	4.75	QS	1	3.75	0	2.25	79%	76%
1483	1D	7	5	QR	1	4	0	0.35	100%	99%
1484	1D	7	4.75	QR	1	3.5	0	2.75	58%	50%
1485	1D	3	5	QS	1	3.75	0	0.95	89%	74%

Global Forum for Innovations in Agriculture, ApiArab Expo - February 5th 2018

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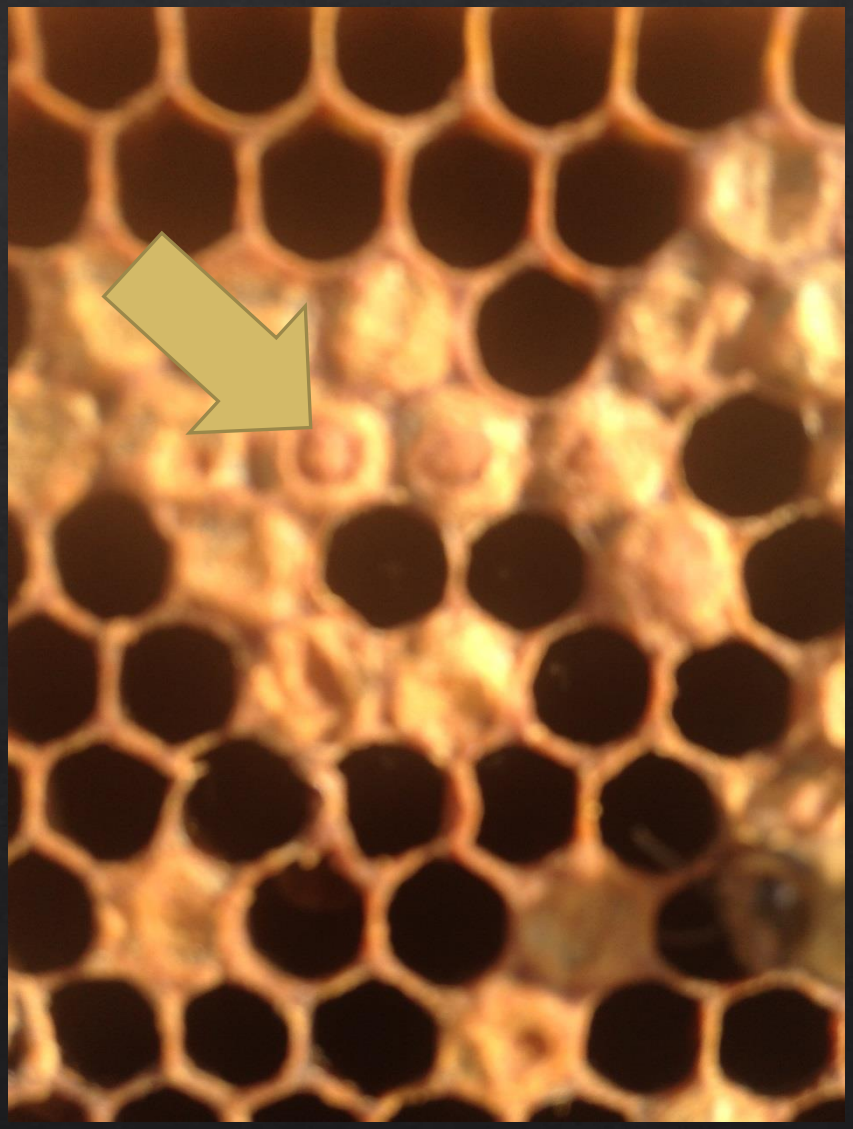
S96 RCD-14 (Hygienic Behavior; 89%U+70%R)

Y26x26 Martins (Hygienic Behavior; 100%U+100%R)

S65 SW 09 (A), S65 ROB 14 (B), S28 PRD 14 (C), S28 MRT 14 (D), S28 MRT 14 (E), S146 GP (F)

Bee Hygienic Behaviour – Recapping Cells



Queen

Retinue

#47-S96 CHR 14

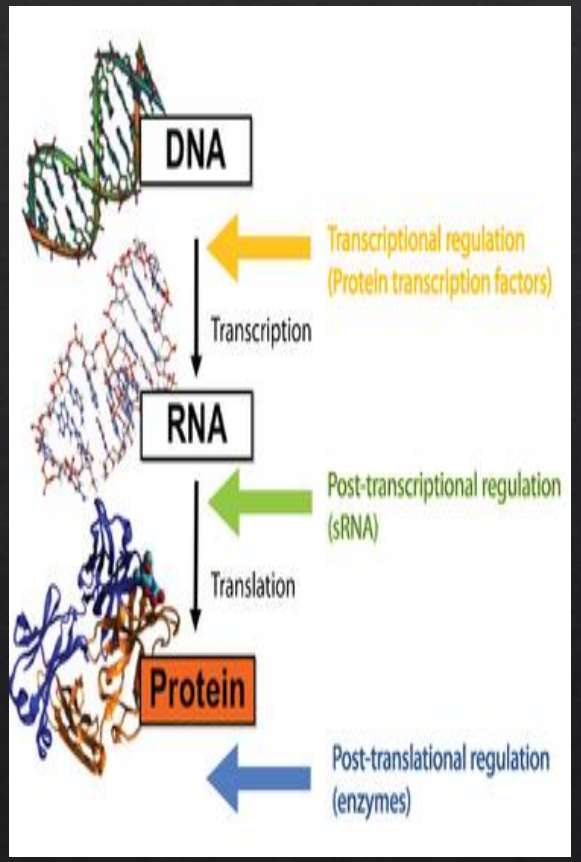
Benefits of the Saskatraz Hybrid Project

Saskatraz hybrids are a valuable source of drones. Useful for distributing alleles for selected traits (honey production, over-wintering, varroa resistance, brood disease) amongst breeding population.

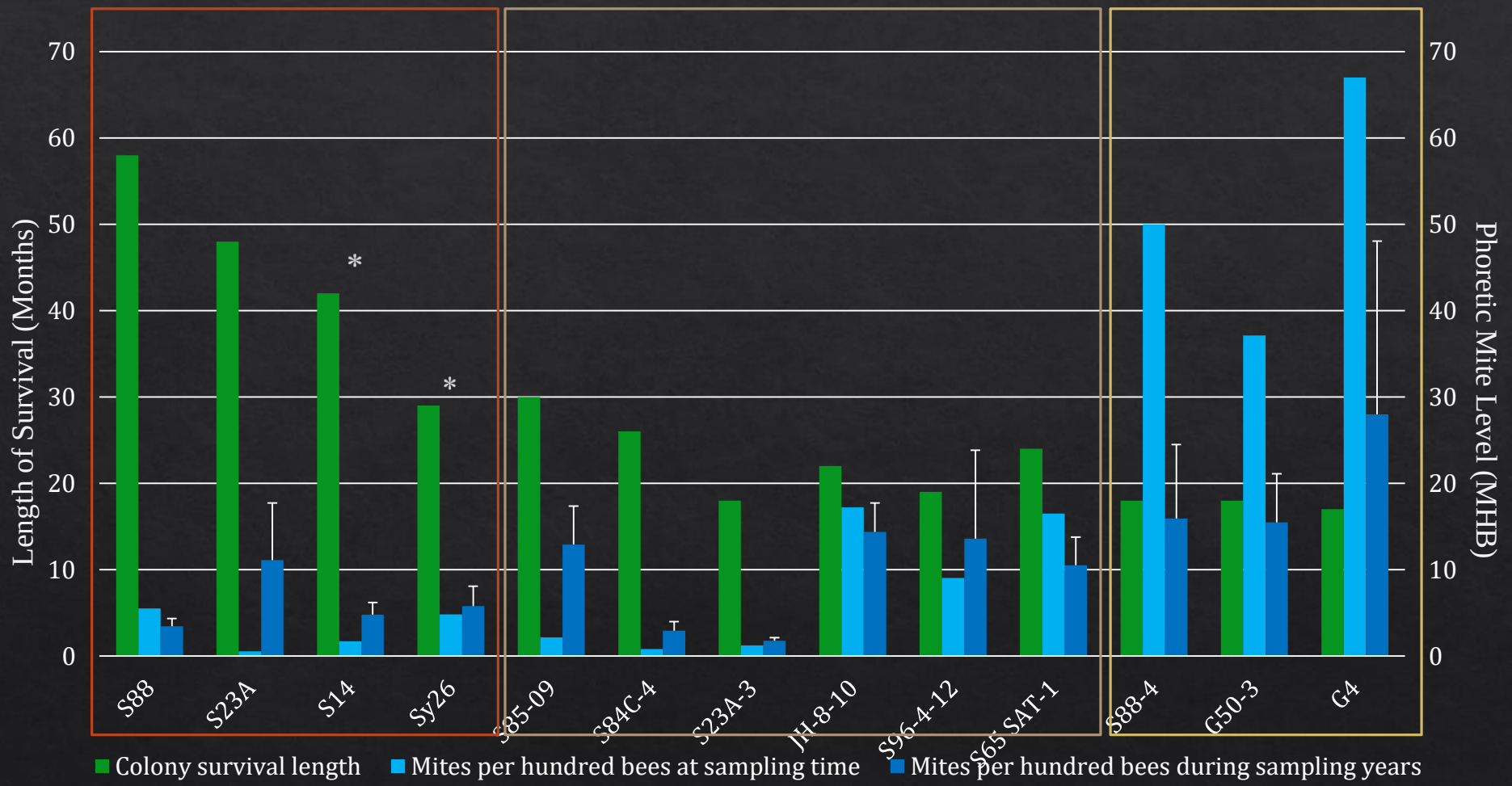
Biomarker Development

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

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



Colony Phenotyping



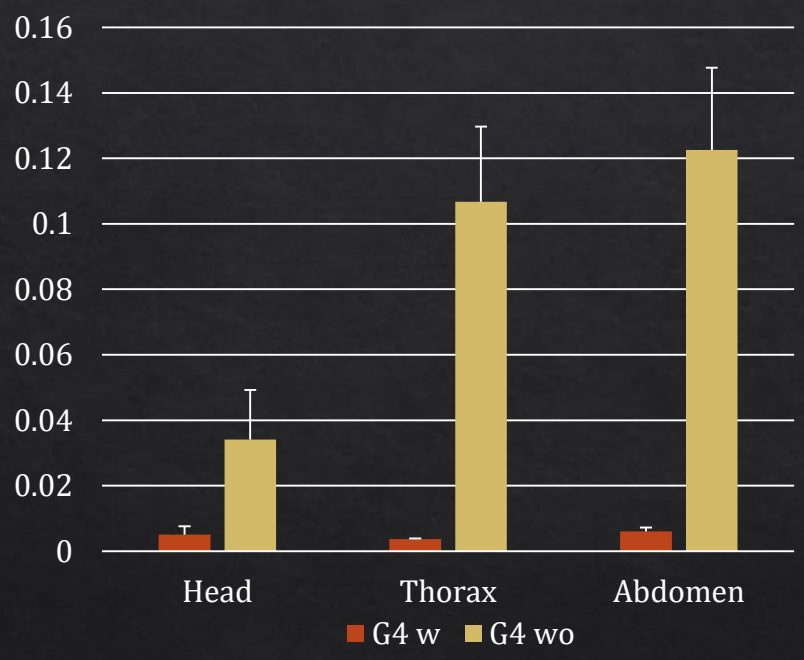
The colony survival time and varroa mite infestation of selected honey bee colonies.

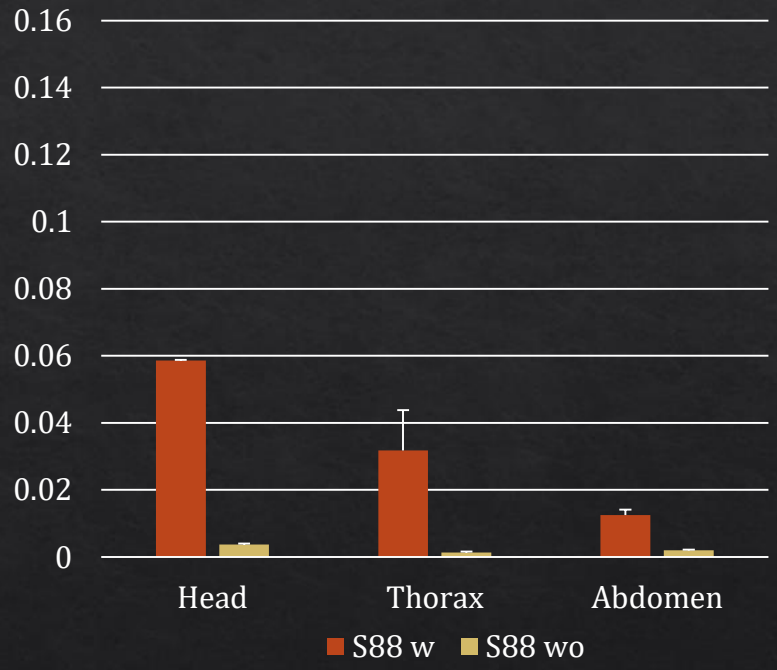
The survival time (green bar) is presented in months, and the Varroa mite infestation (blue bars) is presented in Mites per Hundred Bees (MHB). The light blue bar shows the varroa infestation rate at the sampling time. The dark blue bar shows the varroa infestation rate in the sampling years (mean ± SEM). A colony with a single star is still alive.

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

Relative gene expression of Esterase *E4 transcripts* in different honeybee tissues

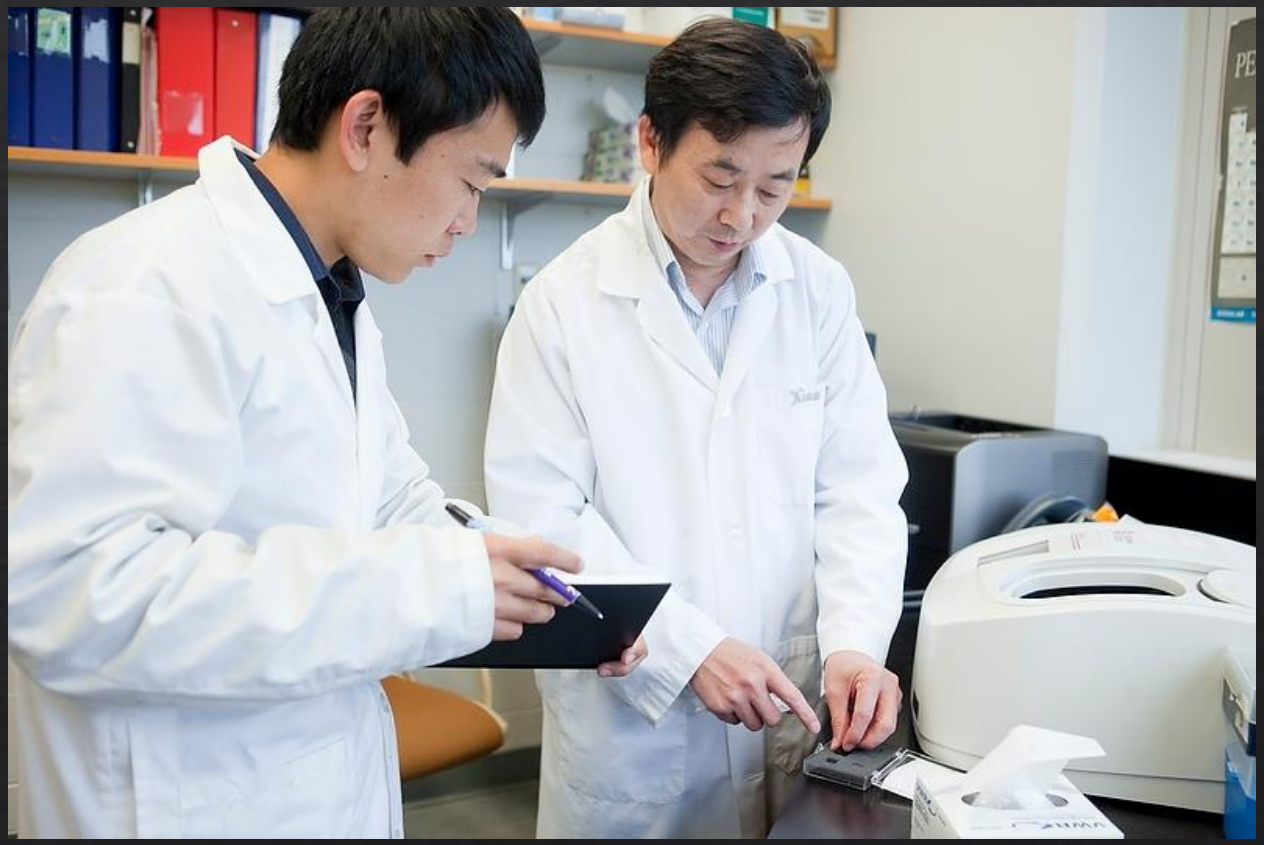




Susceptible phenotype G4

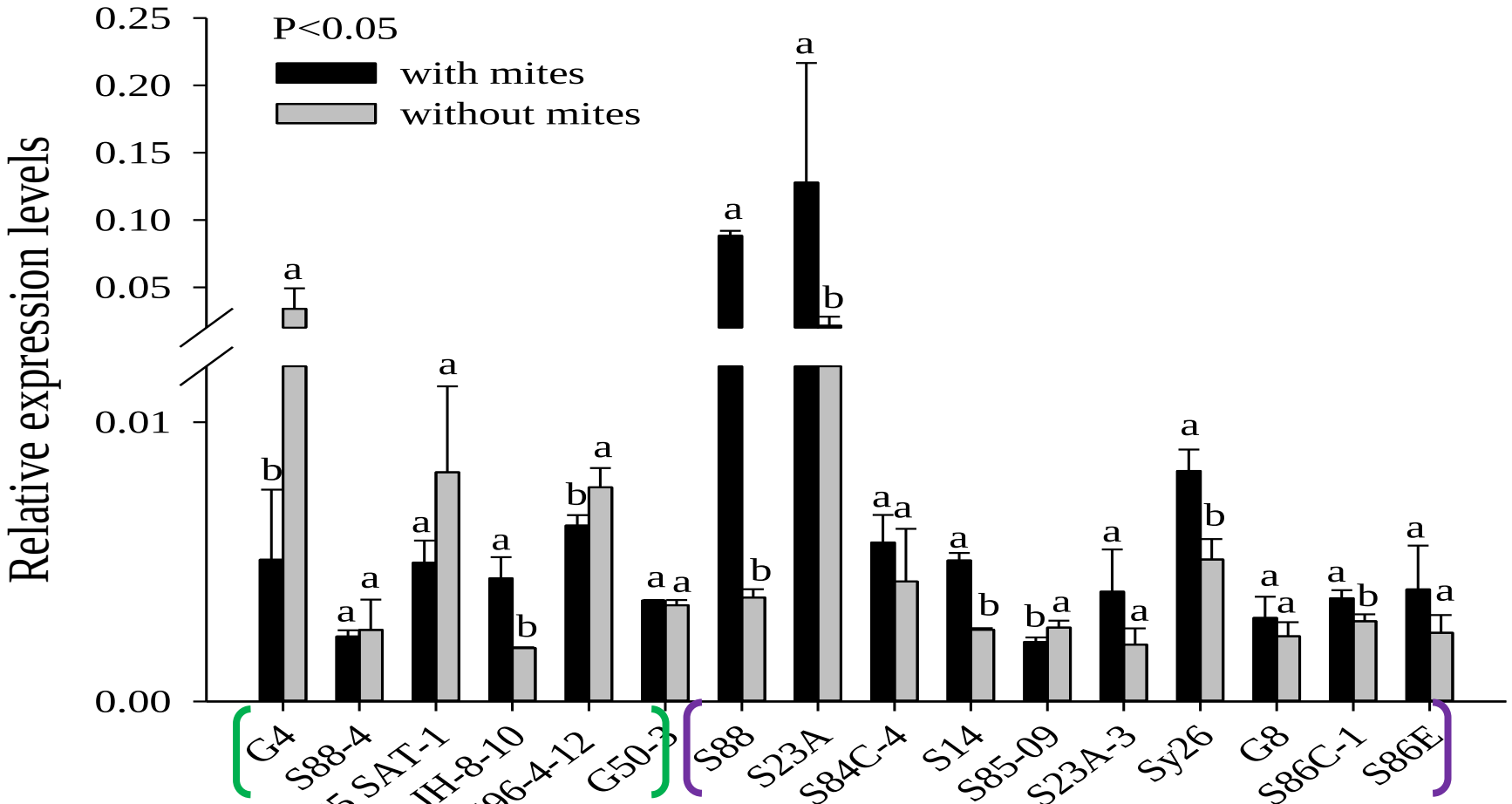
Tolerant phenotype S88

Biomarkers



Dr Xiao Qiu and Sanjie Jiang Food and Bio products , University of Saskatchewan

Differential expression analyses of **Esterase E4** in a wide range of colony phenotypes

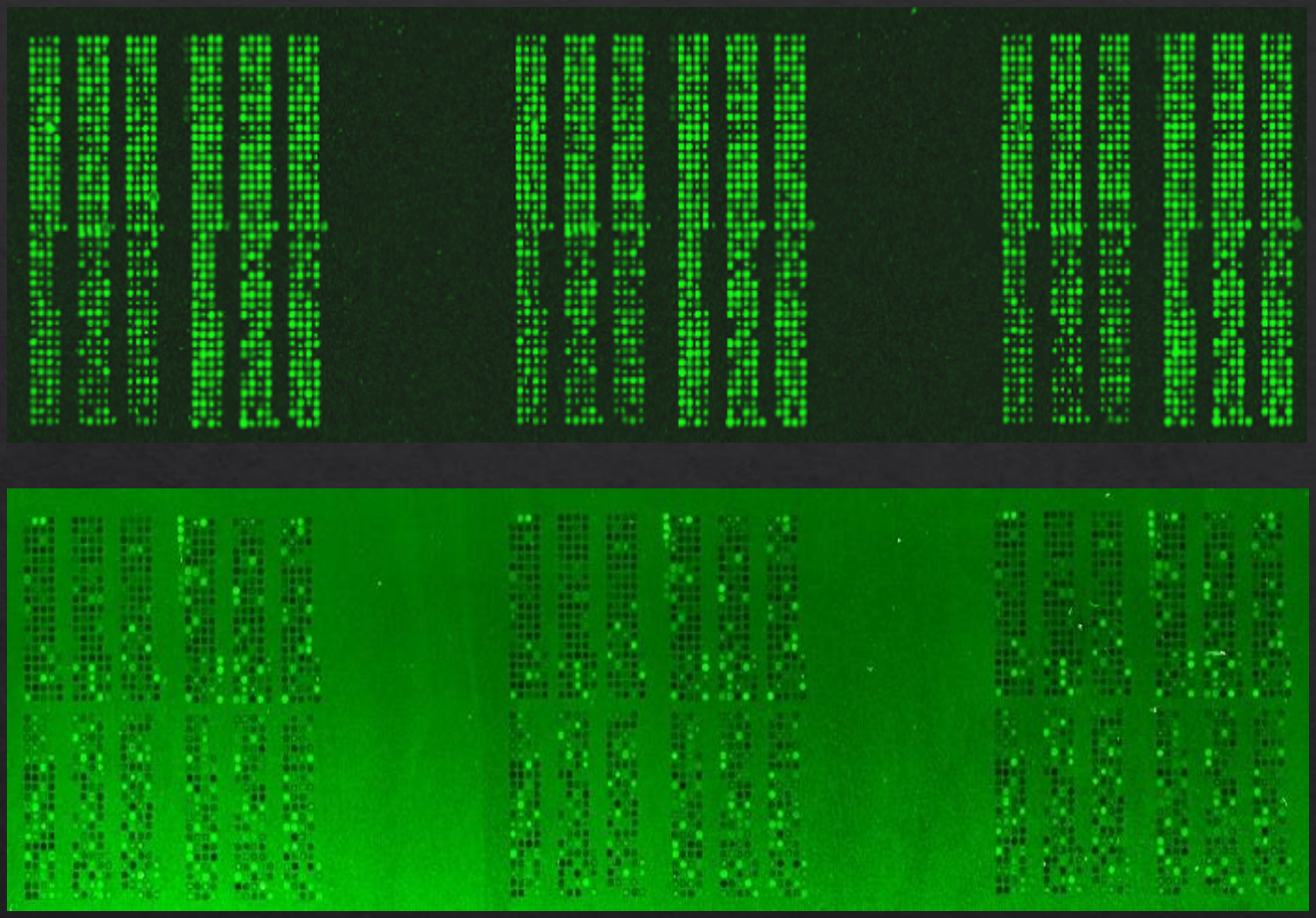


Kinome Analysis of Colony Phenotypes and Honey Bee Immune Responses

- There is a growing appreciation that cellular kinase activity (the kinome) is one of the most informative levels to characterize host.

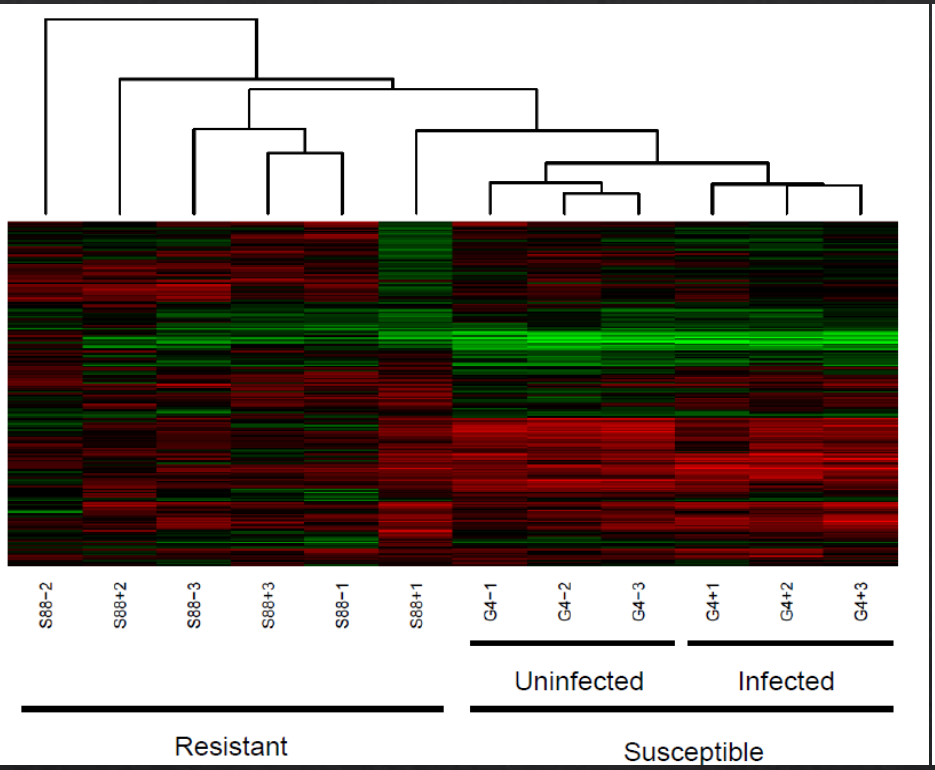
- Provides information on signal transduction.

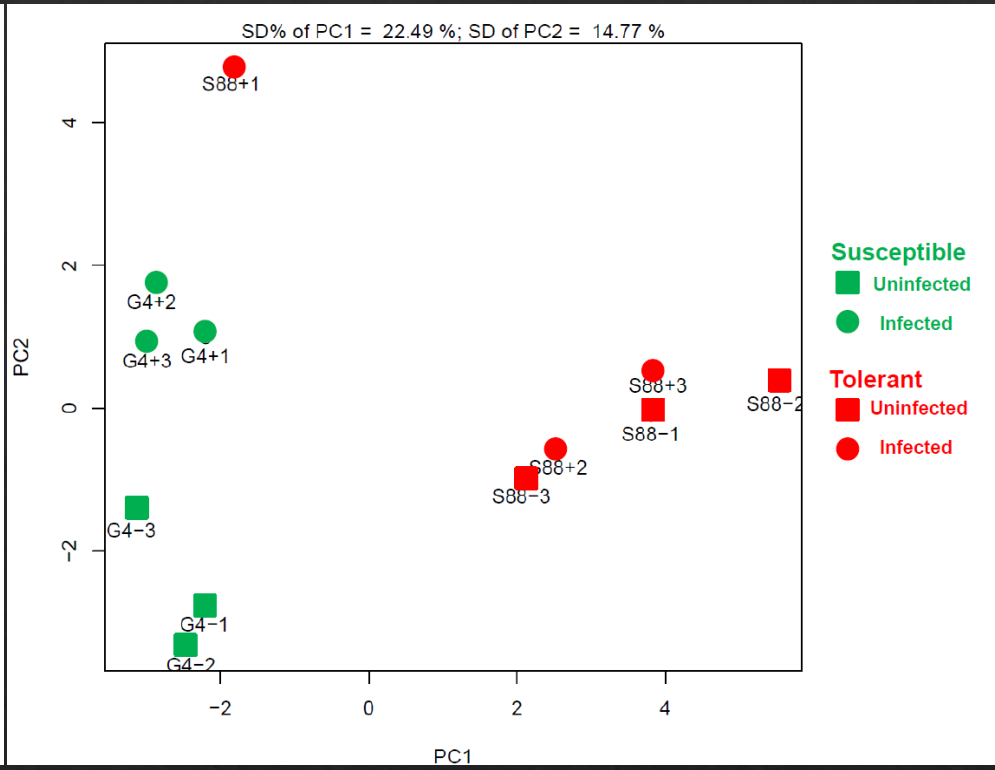
- Information on how different honey bee phenotypes respond to varroa, viruses and other pathogens.



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.



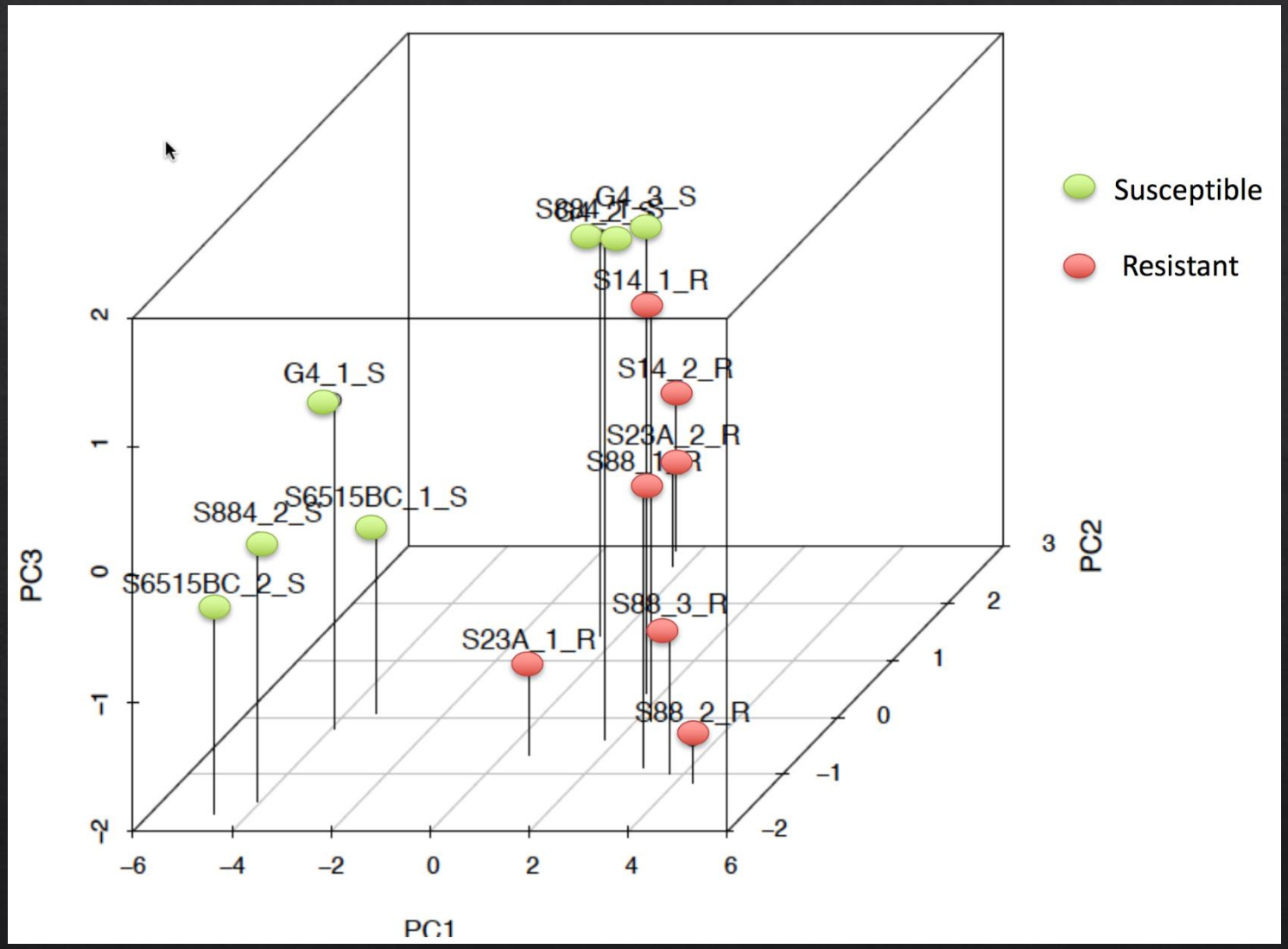


Clustering of Kinome Data. Kinome datasets were subjected to hierarchical clustering and PCA analysis. Pupae from two colonies (G4 and S88) were selected for either the presence (+) or absence (-) of Varroa mites. A) Heat Map Clustering "Average Linkage + (1 - Pearson Correlation)" was used for clustering both the bee-treatments (columns) and the peptides (rows). Each column represents the kinome activity of individual pupae (n=3/treatment). Cluster analysis segregated kinome profiles first by colony phenotype (S88: Tolerant; G4: susceptible) and then segregated G4 larvae by response to Varroa infection. B) 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.

Table 1: Peptides with Differential Phosphorylation between Varroa Mite Susceptible and Resistant Honeybees.

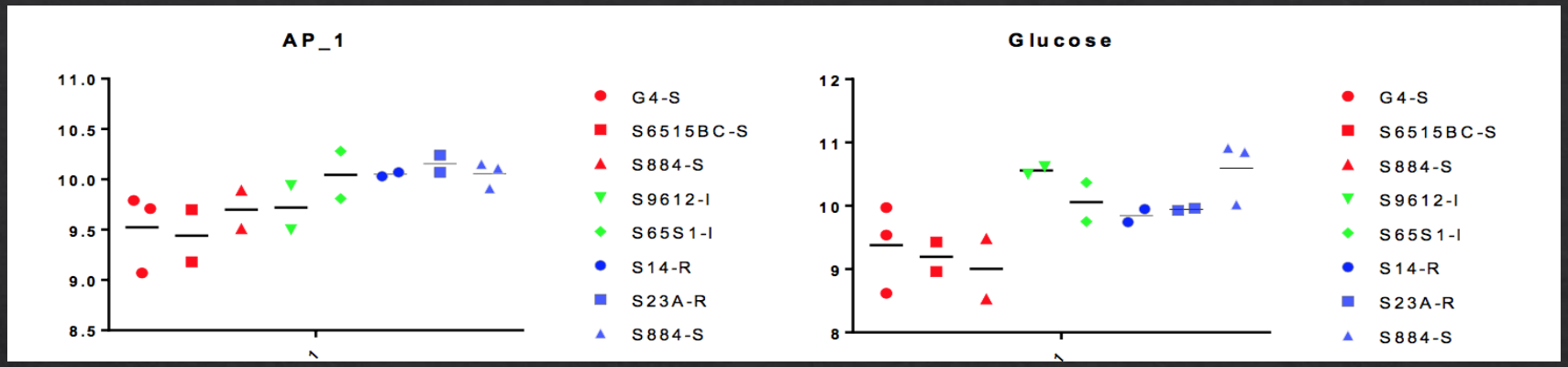
Accession #	P	Accession #	p	Accession #	p	Accession #	p
P15172	0.0003	P23572	0.01	Q99459	0.02	P11413	0.04
Q16584	0.0004	Q06609	0.01	P53349	0.02	P09467	0.04
P06493	0.001	Q9Y2H1	0.01	P28482	0.02	P04626	0.04
P13639	0.002	Q05397	0.01	Q05397	0.03	P67775	0.04
O54950	0.001	Q13153	0.01	P37173	0.03	O00311	0.04
Q06187	0.002	P10398	0.01	Q9UPZ9	0.03	P84243	0.04
O35643	0.003	P07949	0.01	Q8WUM4	0.02	Q13557	0.04
P06744	0.003	Q13188	0.01	P49327	0.03	Q13526	0.04
Q9P0L2	0.004	P11021	0.01	Q920L2	0.03	O17732	0.05
P54764	0.006	P05023	0.02	Q00610	0.03	P31645	0.05
P15056	0.007	P49137	0.02	Q12972	0.04	P24941	0.05
Q6P9R2	0.007	O43837	0.02	P10809	0.04	P00558	0.05
P10860	0.008	P53350	0.01	Q9NQU5	0.04		
P29320	0.009	P06576	0.02	Q06187	0.04		

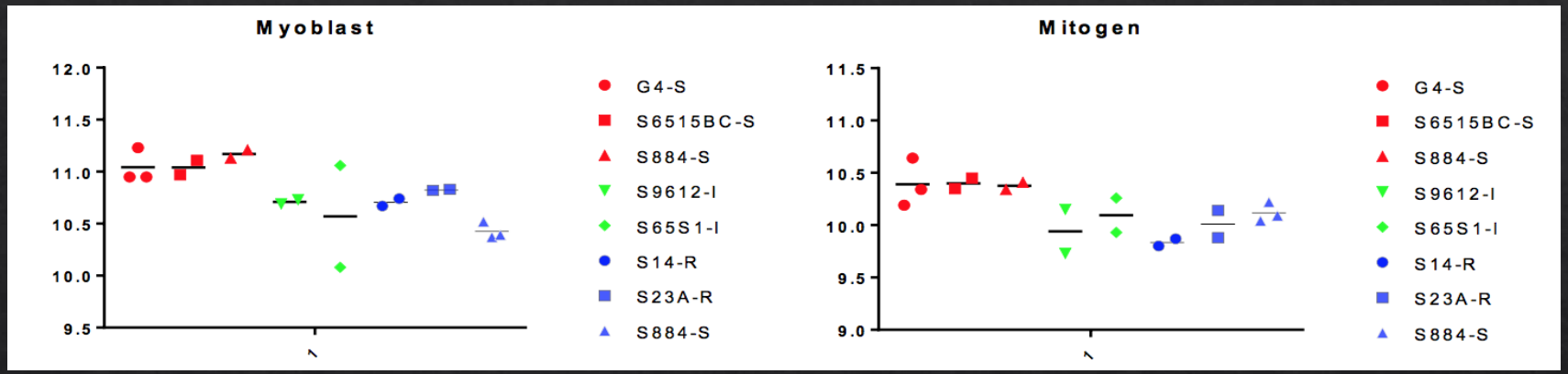
Using an expanded honeybee specific peptide array developed for this project kinome analysis was performed on whole organism samples representing bees (n=3) representing a range of susceptibilities to varroa mite infestation. From most susceptible to most resistant these phenotypes were G4, S88-1, S651B6 (susceptible), S9612 and S65S1 (Intermediate susceptibility), and GS14, S23A, S88 (resistant). T-tests were performed to identify peptides with differential patterns of phosphorylation between the phenotypes. A total of **54 peptides** were identified which demonstrated consistent differential ($p<0.05$) phosphorylation between the bees of the three susceptible phenotypes vs bees of the three resistant phenotypes [**Table 1**].



Clustering of the kinome profiles of bees of different phenotypes at different developmental stages.
Principal component analysis.
The first three principal components are shown. The points are as follows: resistant phenotypes (red) and susceptible phenotypes (green).

VIDO


AP_1
11.0
10.5
10.0
9.5
9.0
8.5
G4-S
S6515BC-S
S884-S
S9612-I
S65S1-I
S14-R
S23A-R
S884-S
Glucose
12
11
10
9
8
G4-S
S6515BC-S
S884-S
S9612-I
S65S1-I
S14-R
S23A-R
S884-S


Myoblast
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11.5
11.0
10.5
10.0
9.5
G4-S
S6515BC-S
S884-S
S9612-I
S65S1-I
S14-R
S23A-R
S884-S
Mitogen
11.5
11.0
10.5
10.0
9.5
9.0
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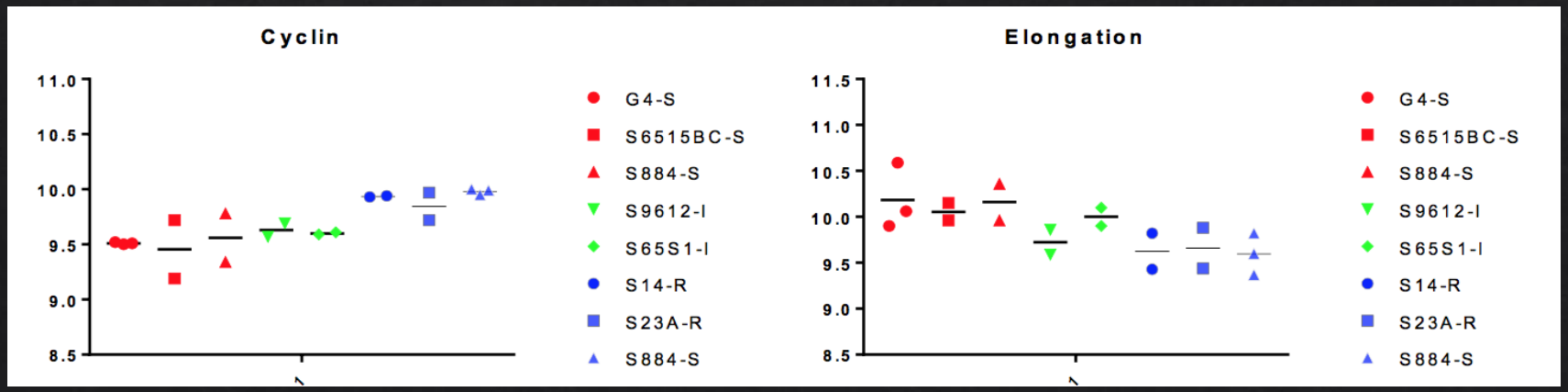
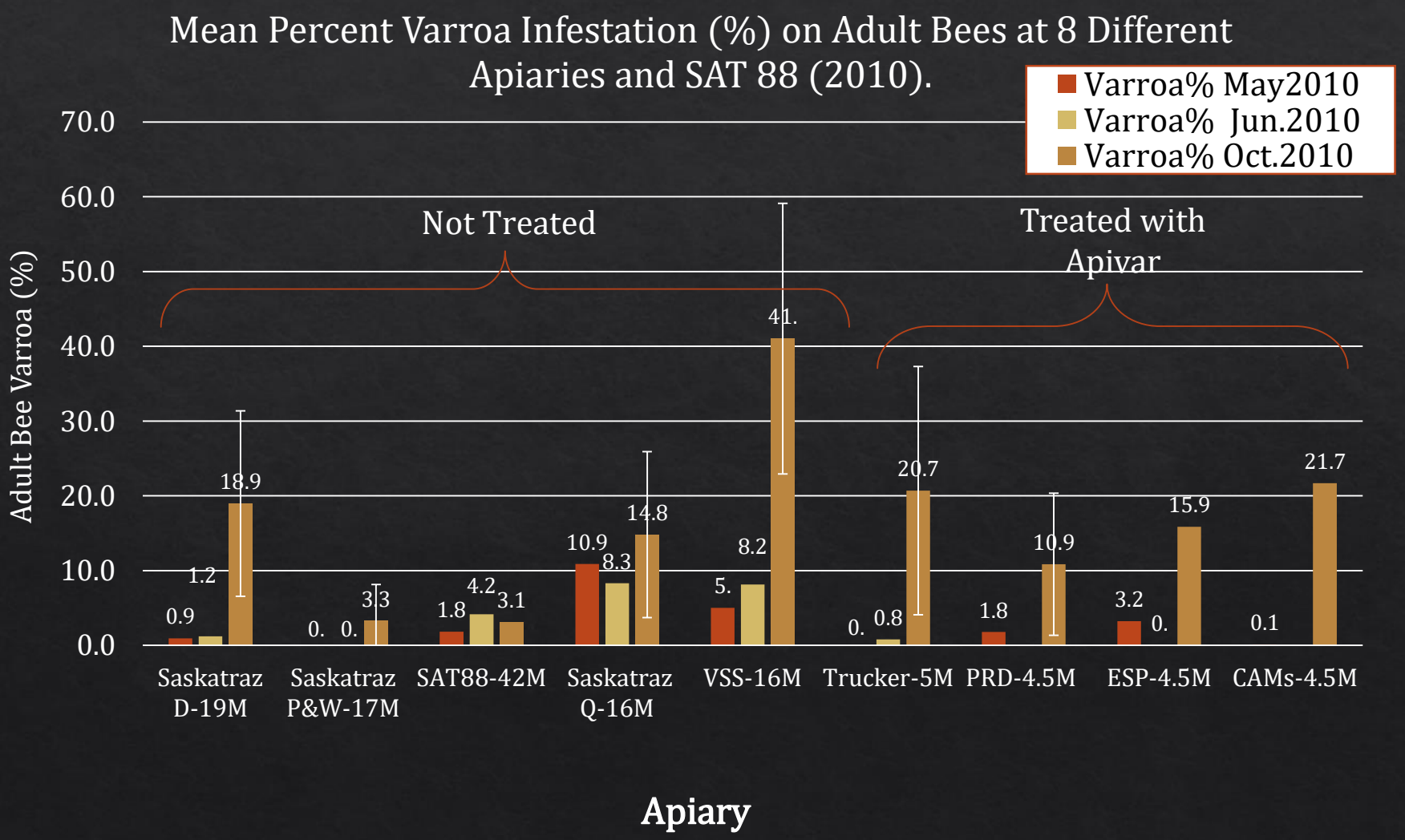
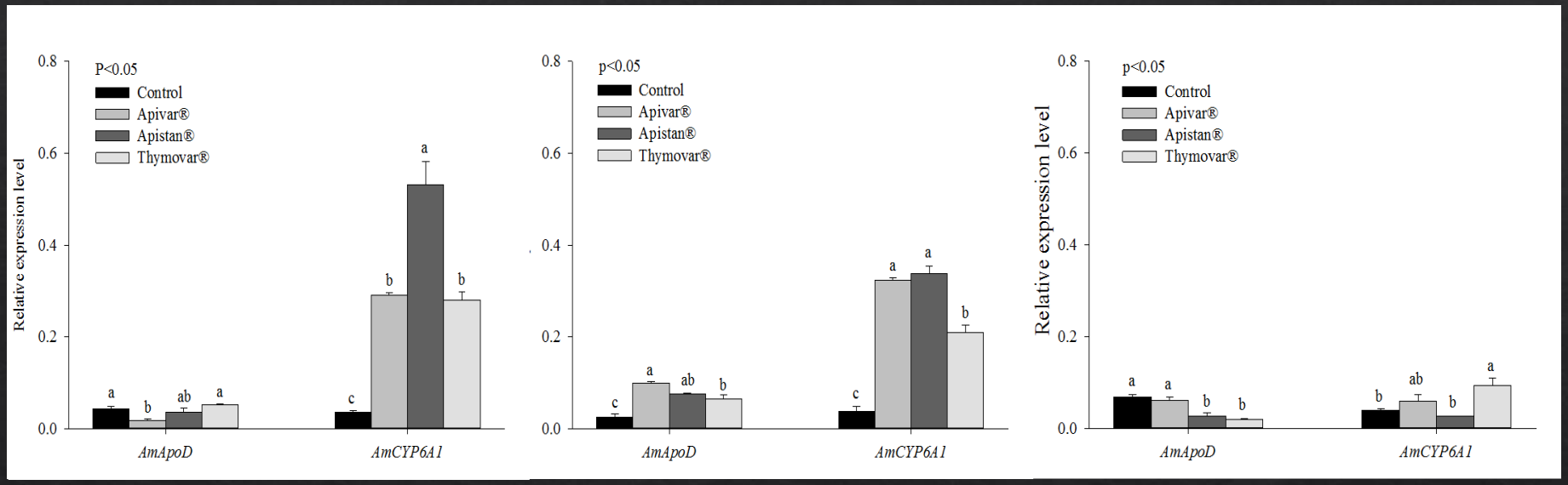
S23A-R
S884-S


Cyclin
11.0
10.5
10.0
9.5
9.0
8.5
G4-S
S6515BC-S
S884-S
S9612-I
S65S1-I
S14-R
S23A-R
S884-S
Elongation
11.5
11.0
10.5
10.0
9.5
9.0
8.5
G4-S
S6515BC-S
S884-S
S9612-I
S65S1-I
S14-R
S23A-R
S884-S

Effects of Miticides on Varroa Population Growth

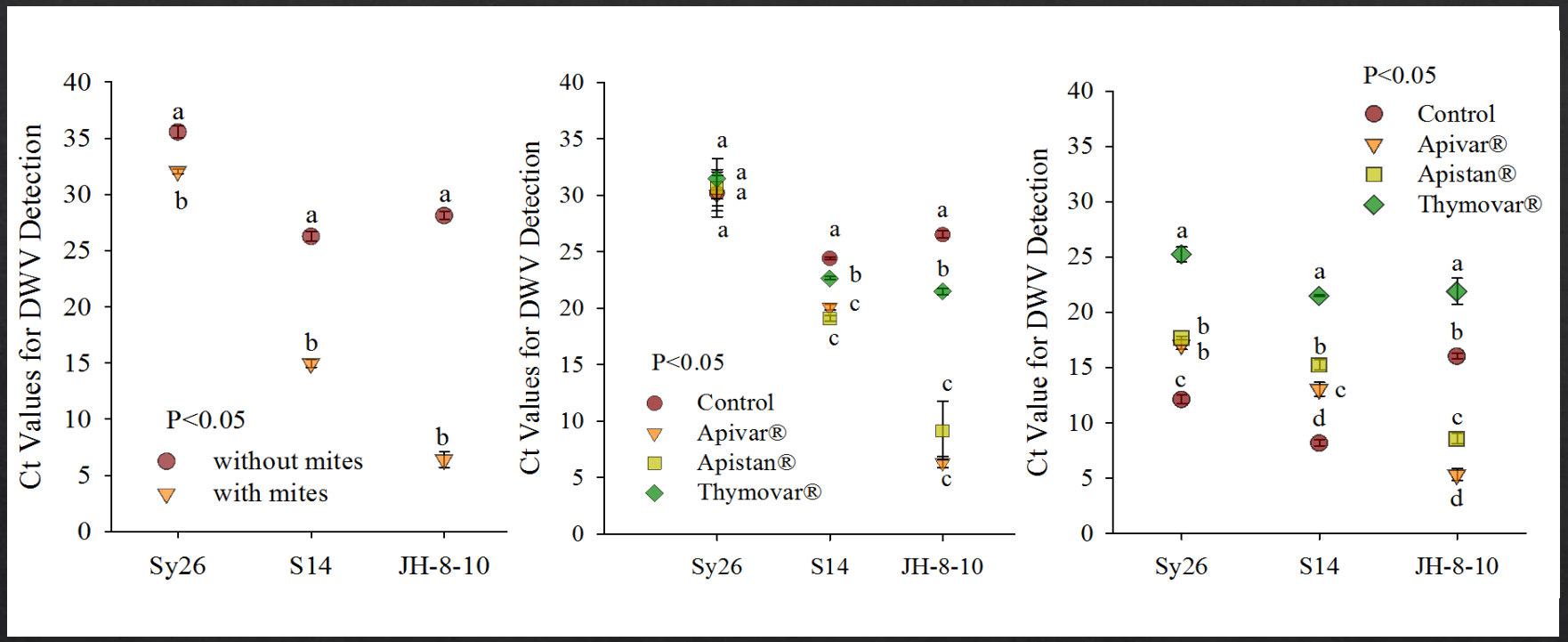


Relative Expression of Transcripts in Response to Miticide Treatments



SY26 S14 JH-8-10

Relative expression level of transcripts from two selected biomarker genes, *AmApoD* and *AmCYP6A1* in pupae abdomens from two varroa tolerant (SY26 and S14) and one susceptible (JH-8-10) honey bee colony in response to Apistan®, Apivar®, and Thymovar® miticide treatments. Relative gene expression (mean±SEM, N=3) was normalized by the expression of internal reference genes (*actin* and *RpS5*), and error bars indicate the expression variability of each gene. A. SY26; B. S14; C. JH-8-10. Significantly different ($p<0.05$) values are depicted by different letters above columns. The treatment comparisons used the LSD (least significant difference) method for difference analysis of each gene.



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

DWV in Head Tissue in Miticide Experiment

DWV in Abdomen 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; C. DWV in the abdomen with and without miticide treatments. Notes: Data points followed by different letters are significantly different ($p<0.05$). The multi-treatment comparisons of Ct values used the LSD (least significant difference) method for difference analysis.

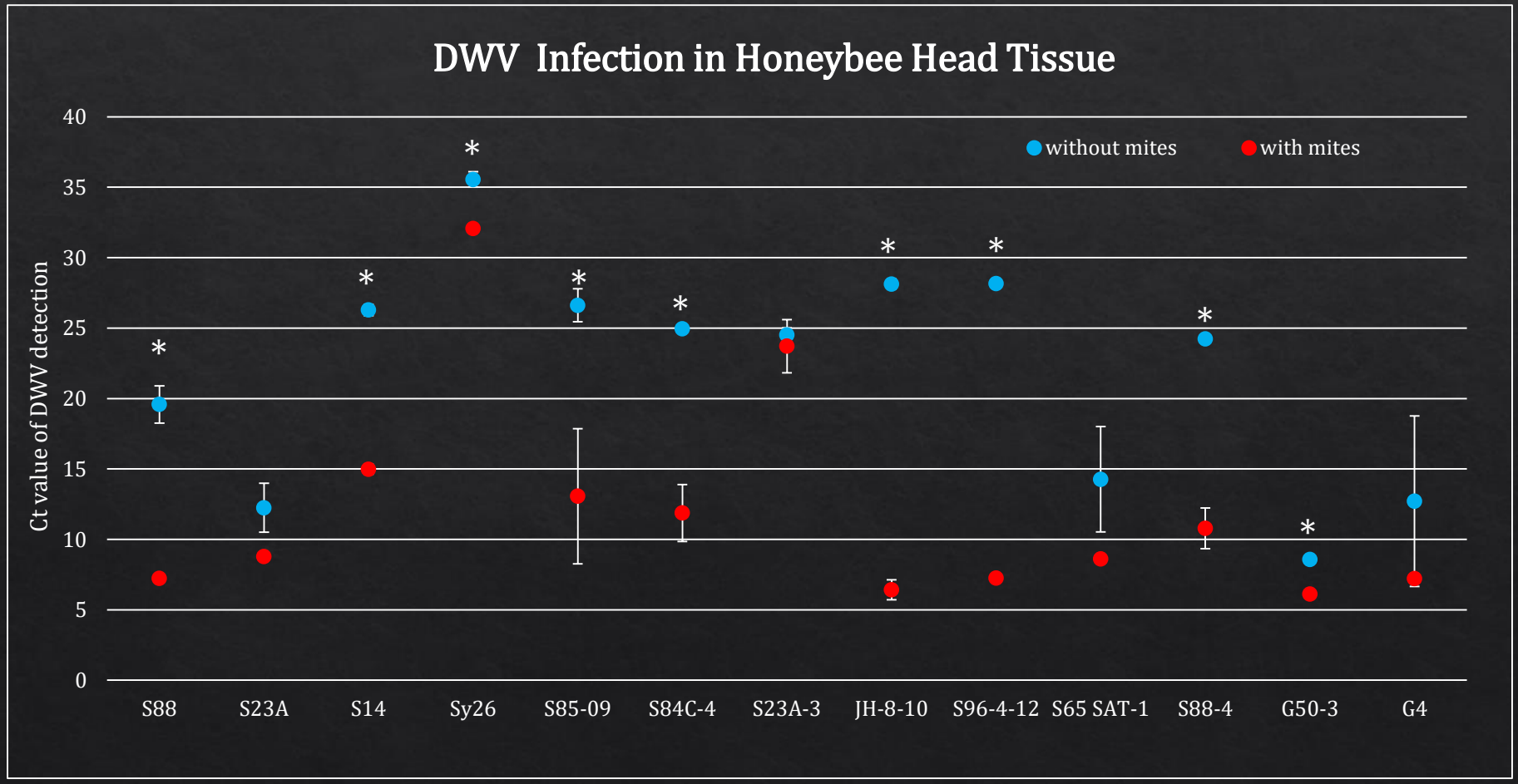
Quantitative measurement of DWV infection in head tissues in three different colonies in response to the varroa mite infestation

Honeybee Colony ID	Ct Value of DWV in non-infested bees	Ct Value of DWV in mite-infested bees	Fold change of DWV (infested/non-infested)
Y26×26	35.55±0.54[a]	32.06±0.22[b]	11.24
S14	26.28±0.39[a]	14.97±0.35[b]	2,544.79
JH-8-10	28.13±0.36[a]	6.42±0.70[b]	3,430,529.88

Quantitative measurements of DWV infection in head tissues in three different colonies treated with Apivar, Apistan and thymol miticides

Honeybee Colony ID	Non-treated bees	Apivar-treated bees	Apistan-treated bees	Thymol-treated bees	Fold change (Apivar-treated/non-treated)	Fold change (Apistan-treated/non-treated)	Fold change (Thymol-treated/non-treated)
Y26×26	30.19±1.57[a]	30.07±1.99[a]	30.66±1.60[a]	31.46±1.78[a]	1.09	0.72	0.41
S14	24.43±0.12[a]	20.10±0.22[b]	19.11±0.25[c]	22.66±0.14[d]	20.07	39.95	3.40
JH-8-10	26.53±0.33[a]	6.36±0.49[b]	9.19±2.60[c]	21.50±0.29[d]	1,176,986.76	165,905.20	32.60

DWV Analysis of Saskatraz Phenotypes with and without Mites



Proteomic and PCR analyses for Pathogen Identification

Virus and Nosema species identified by Proteomic and PCR Analyses of Honey Bees from Western Australia and Saskatchewan Colonies with and without Varroa.

Varroa destructor, an ecto parasitic of the domestic honey bee, has invaded most countries around the world with the exception of Australia, although some isolated areas in some other countries still do not have the parasite. Recent studies have concluded that the Varroa mite spreads viruses throughout infested colonies and increases the virulence of deformed wing virus[3], causing honey bee health problems and increased overwintering losses. In our kinome array studies the data implies that Varroa mites may cause immune suppression in susceptible phenotypes, making these colonies more susceptible to pathogens[2].

In this study our objective was to investigate what honey bee viruses were detectible in honey bees sampled from package bees imported from Western Australia in 2014, colonies from Saskatchewan with no detectable Varroa infestation, and colonies with high and low Varroa mite infestations. Our approach was to use RT-PCR to detect pathogenic RNA viruses with known sequences and to further investigate the presence of possible unknown (virus flora) or recombinant forms of previously characterized honey bee viruses by proteomic methods. We also tested Varroa collected from the infested colonies to determine what viruses were carried by Varroa in highly infested colonies and those with low infestations. In addition ,the presence or absence of nosema species was determined in honey bee samples as well as Varroa.

Phoretic Varroa Level on Colonies used for Proteomic Study

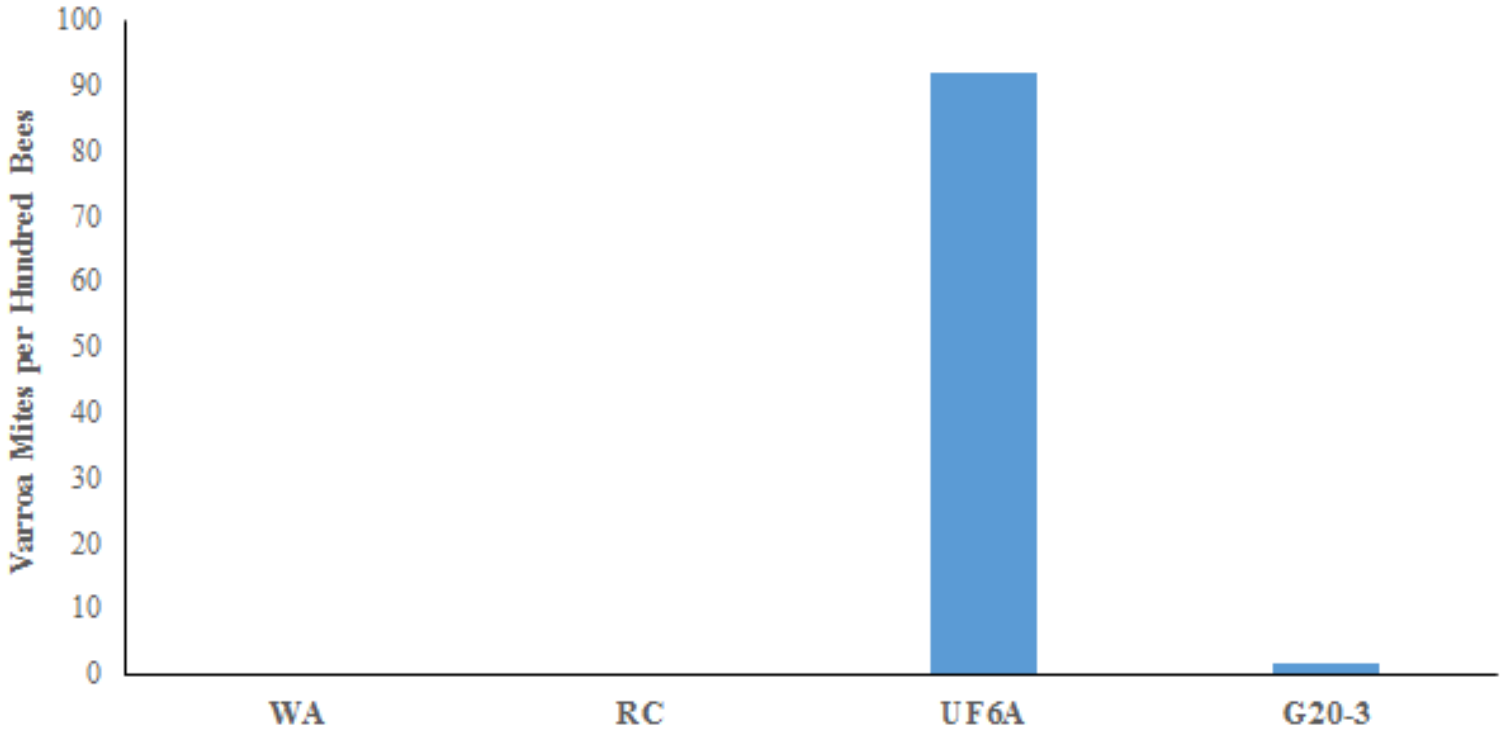


Figure 1. Blue bars stand for Varroa mites per hundred bees from Western Australian package (WA) bees, Saskatchewan Varroa free (RC) bees, Saskatchewan bees with high (UF6A) and low (G20-3) Varroa infestations when their worker bees were sampled. No Varroa mites were in WA and RC bees, while UF6A bees had 92 Varroa

Virus and Nosema Analyses

Sample Type	Colony Name	Pathogenic Virus					Pathogenic Fungi	
		Deformed Wing Virus	Israeli Acute Paralysis Virus	Black Queen Cell Virus	Acute Bee Paralysis Virus	Varroa destructor Macula-like virus	Nosema Apis	Nosema Ceranae
Honeybee	WA						√	√
	RC							
	UF6A	√	√	√			√	√
	G20-3	√	√				√	√
Varroa	UF6A	√	√		√	√	√	√
	G20-3					√	√	√

Table 1. Pathogenic virus and fungi detected in honeybees from Western Australian package bees in 2014 (WA), Saskatchewan bees free of Varroa (RC), and with high (UF6A) and low (G20-3) Varroa infestations and in Varroa collected from infected colonies. Viruses were detected by proteomic analyses and RT-PCR analyses.

Varroa Proteins Found in Highly Infected UF6A Honeybees

accession #	protein description	protein mass	peptide mass	peptide score	expect value	# of peptides
gi|114842185	cytoplasmic actin, partial	13088	2214.0627	149.94	1.00E-15	8
gi|396582590	vitellogenin 1	214402	2321.0115	1.52	1.2	23
gi|545698343	hemelipoglycoprotein precursor, partial ; large lipid transfer protein	182577	853.4059	3.21	0.48	12
gi|317160955	cytochrome oxidase subunit I, partial (mitochondrion)	8337	1647.8265	1.47	0.71	5
gi|735659418	acetylcholinesterase, partial	61430	1330.6026	13.18	0.048	2

Honeybee Proteins Found in Varroa from UF6A

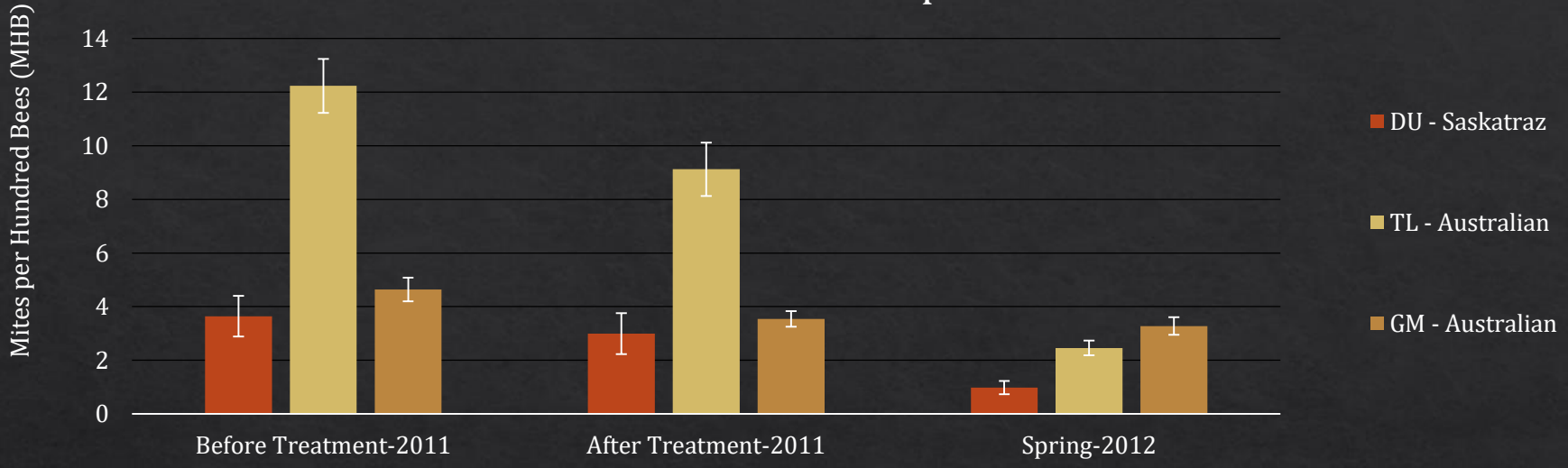
#	Accession #	Protein Description	Species	Protein Score	Protein Mass	Protein Matches	Protein Sequences
1	58585098	major royal jelly protein 1 precursor	Apis mellifera	1222	49311	32	15
2	156637469	hexamerin 110	Apis mellifera	1173	111976	44	16
3	58585104	vitellogenin precursor	Apis mellifera	499	113067	22	9
4	571543905	PREDICTED: LOW QUALITY PROTEIN: apolipophorins	Apis mellifera	592	375552	26	21
5	58585148	hexamerin 70b precursor	Apis mellifera	394	79535	9	7
6	58585086	transferrin 1 precursor	Apis mellifera	371	80033	13	9
7	56422035	major royal jelly protein 3	Apis mellifera	339	66055	12	8
8	571546900	PREDICTED: myosin heavy chain, muscle isoform (X1 to X13)	Apis mellifera	163	225783	5	4
9	94158822	odorant binding protein 14 precursor	Apis mellifera	304	15590	8	5
10	58585108	major royal jelly protein 2 precursor	Apis mellifera	302	51441	15	9
11	209969888	silk fibroin 3 precursor	Apis mellifera	275	33567	4	4
12	572298373	PREDICTED: apolipophorins-like	Apis dorsata	272	375916	13	10
13	58585138	major royal jelly protein 5 precursor	Apis mellifera	253	70531	7	6
14	820842768	PREDICTED: LOW QUALITY PROTEIN: transferrin	Apis florea	244	80066	7	5
15	110756609	PREDICTED: protein NPC2 homolog	Apis mellifera	236	16454	6	4
16	48094573	PREDICTED: uncharacterized protein LOC408608	Apis mellifera	233	19479	4	1
17	572260854	PREDICTED: uncharacterized protein LOC102681889	Apis dorsata	214	162484	7	7
18	58585172	phospholipase A2 precursor	Apis mellifera	193	19615	13	5
19	62198227	major royal jelly protein 7 precursor	Apis mellifera	195	50851	7	5
20	110749126	PREDICTED: glucose dehydrogenase [FAD, quinone] isoform 3	Apis mellifera	194	70764	5	4
21	571574384	PREDICTED: chymotrypsin inhibitor	Apis mellifera	89	8695	1	1
22	149939405	hexamerin	Apis mellifera	129	81490	6	6
23	571549928	PREDICTED: putative ATP-dependent RNA helicase DHX30-like	Apis mellifera	0	84787	2	1
24	58585184	short-chain dehydrogenase/reductase	Apis mellifera	155	27631	6	5
25	229892203	heat shock protein cognate 5	Apis mellifera	0	75642	1	1
26	110748949	PREDICTED: fructose-bisphosphate aldolase-like isoform X2	Apis mellifera	153	39975	4	4
27	571578633	PREDICTED: chitinase-like protein Idgf4-like, partial	Apis mellifera	201	45169	5	4
28	571506700	PREDICTED: nucleoplasmin-like protein-like isoform X1	Apis mellifera	130	21562	2	2
29	94158810	odorant binding protein 13 precursor	Apis mellifera	122	15494	2	1
30	58585170	major royal jelly protein 4 precursor	Apis mellifera	119	53225	7	6

Management of varroa population growth by genetics and selective treatment strategies

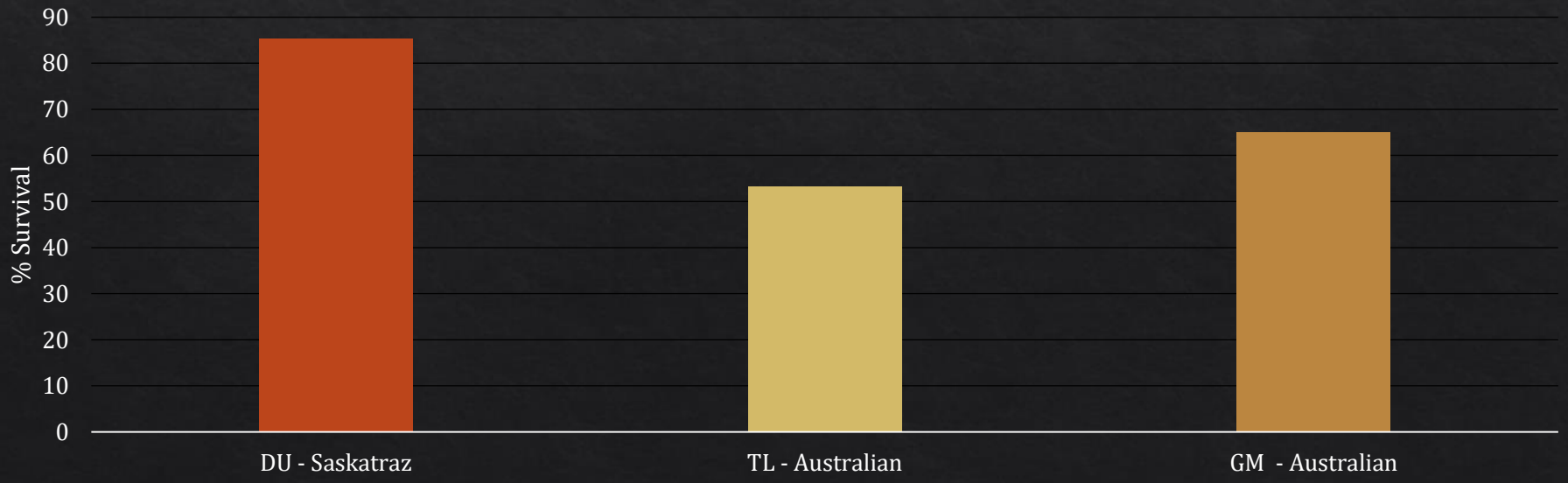
Objective:

- To control varroa with resistant stock and naturally occurring miticides (oxalic and thymol)

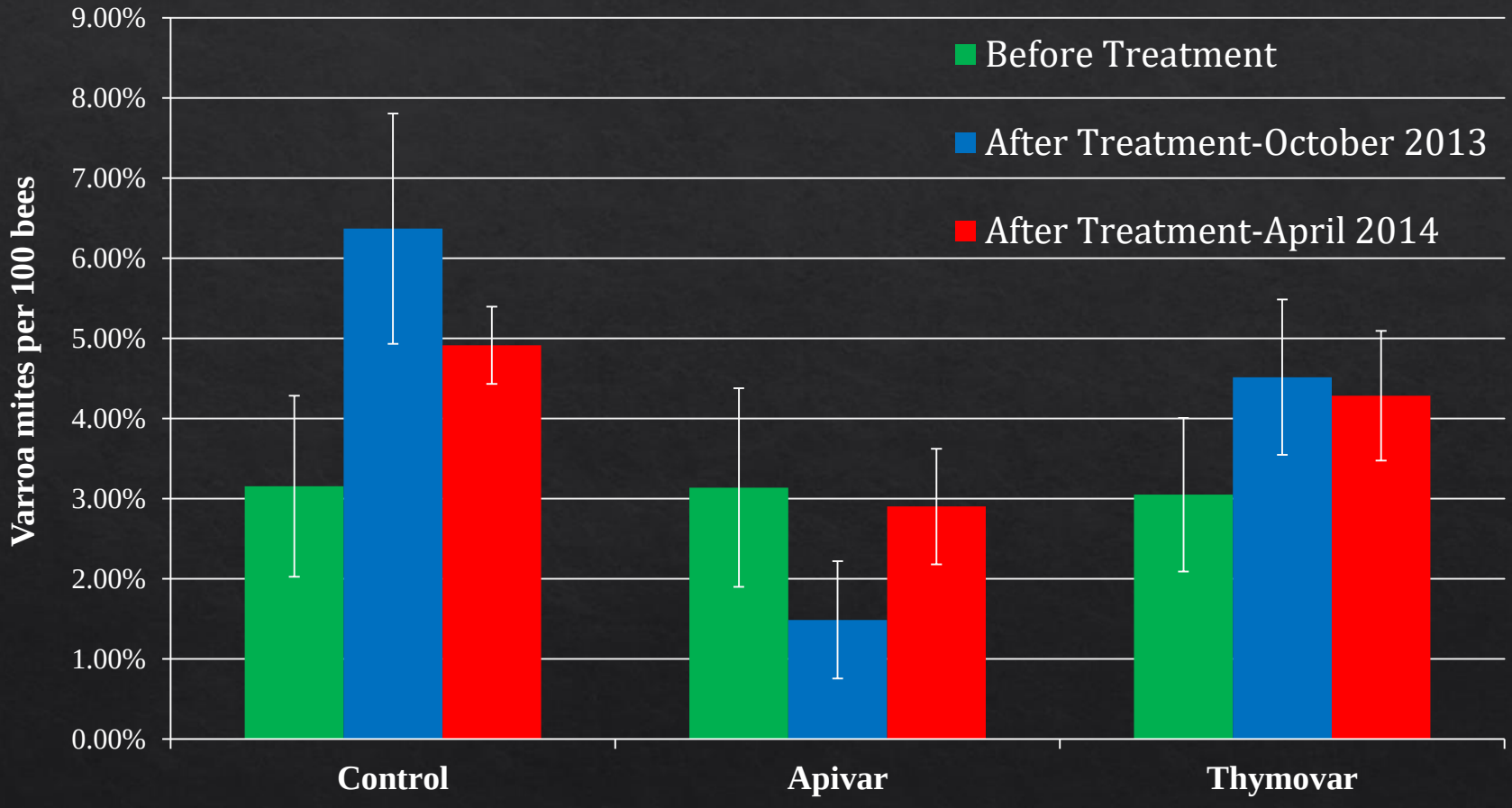
Miticide Treatment Effects in Varroa Susceptible and Tolerant Colonies



Surviving Colonies Spring 2012



Effects of Apivar® and Thymovar® vs Control



2013 Phoretic Varroa Infestation of Adult Bees in Merv's Apiary

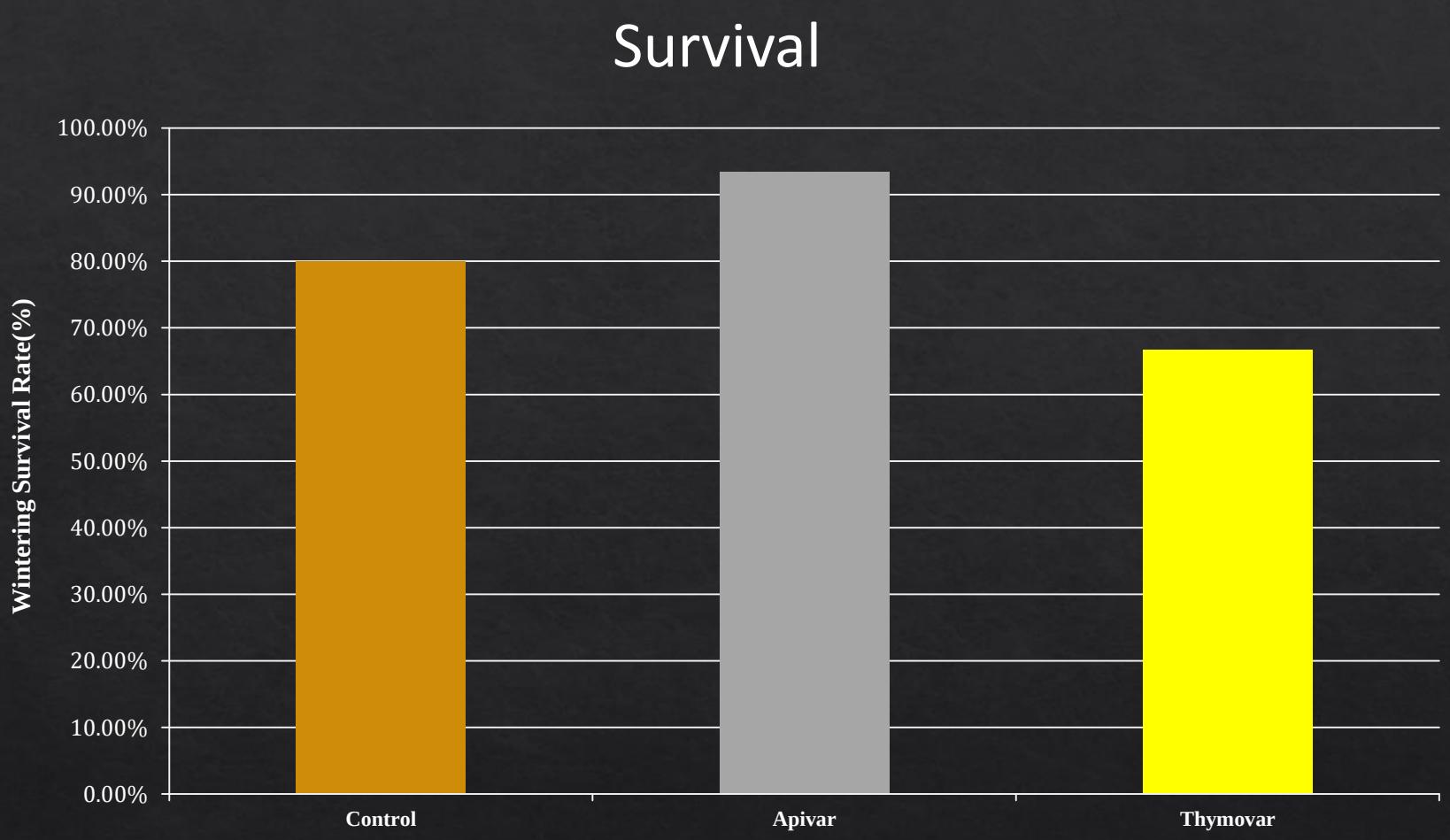
Error bars are means ± standard error, n=15.

Miticide Residues

Compound	Concentration (ng/g, wm)				LOD (ng/g)
	Before treatment	After treatment			
		Control	Apivar®	Thymovar®	
Amitraz	ND	ND	15.4	ND	4
2,4 DMA	ND	ND	ND	ND	50
2,4 -DMPF	ND	ND	ND	ND	4
Thymol	408	245	1660	64800	50

Table 1. Concentrations of miticides (ng/g, wm) in bees before and after treatment with Apivar® and Thymovar® October 2013.

LOD = Limit of detection



Wintering Survival Rate of Fall Miticides Treatment(2013) - No Treatment(Control), Apivar and Thymovar.
The Control(no treatment) survival rate was 80%, Apivar treatment was 93%, and Thymovar treatment was 67%.

Efficacy of Combined Fall Miticide Treatments and Effects on Colony Winter Survival

Treatments Tested in 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

This initial experiment was tested on a total of 50 colonies, 5 per treatment to gain an idea of the most effective combinations.

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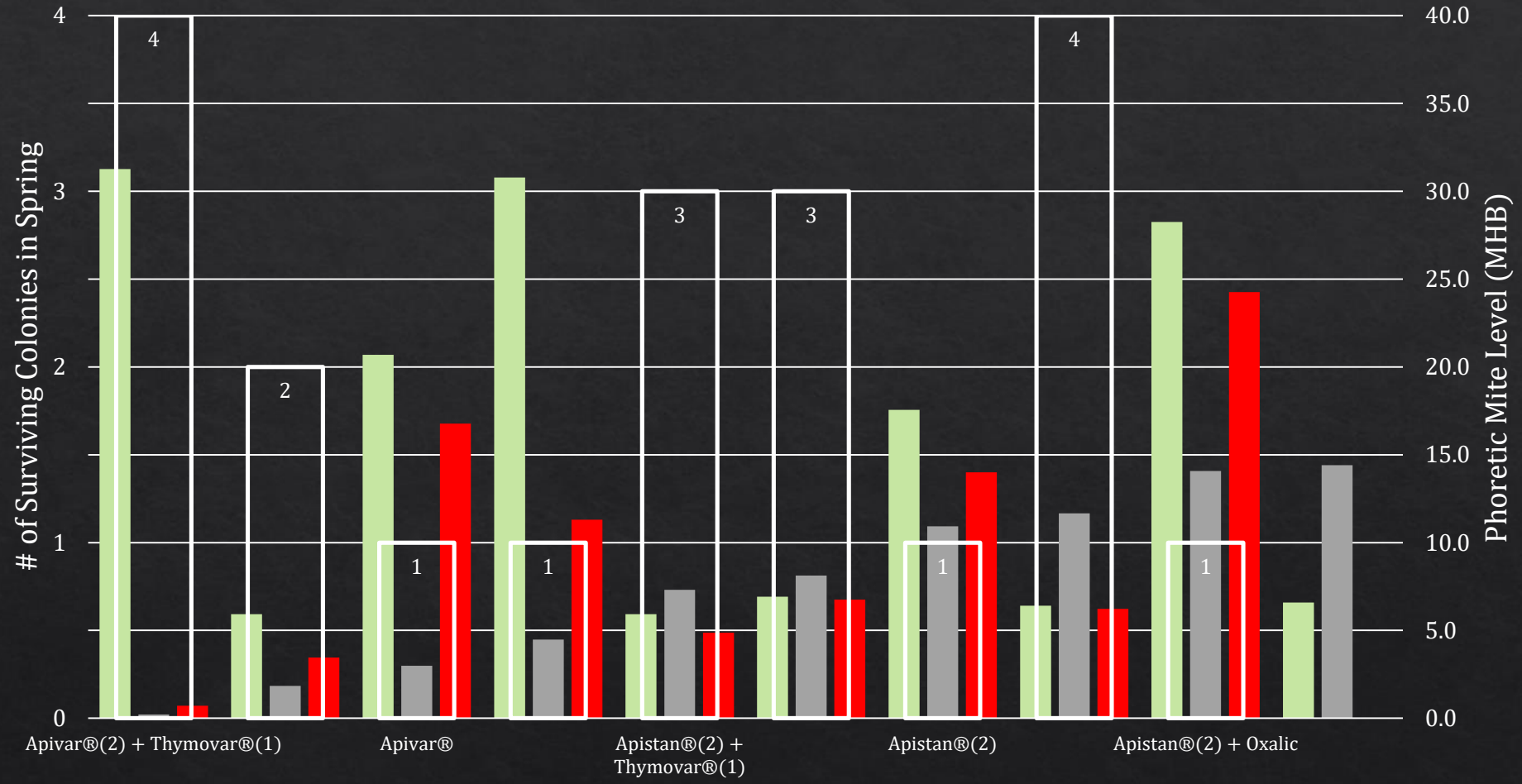
2015 Combined Treatment Experiment Winter Survival and Mean Mites per Hundred Bees For Combined Treatments Before, After, and in Spring 2016



Efficacy of Combined Spring Miticide Treatments and Effects on Colony Winter Survival

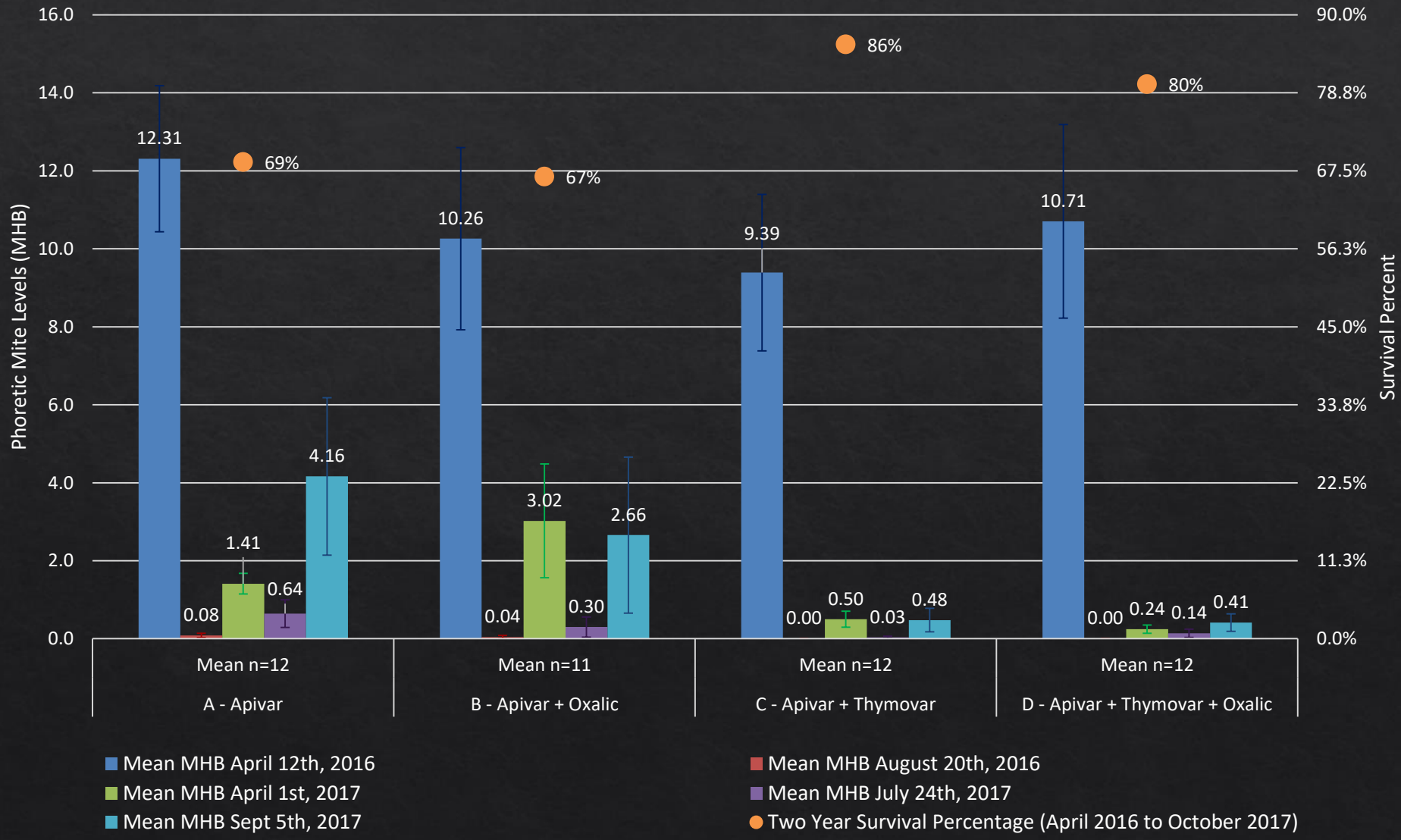
Treatments Tested in 2016

- 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

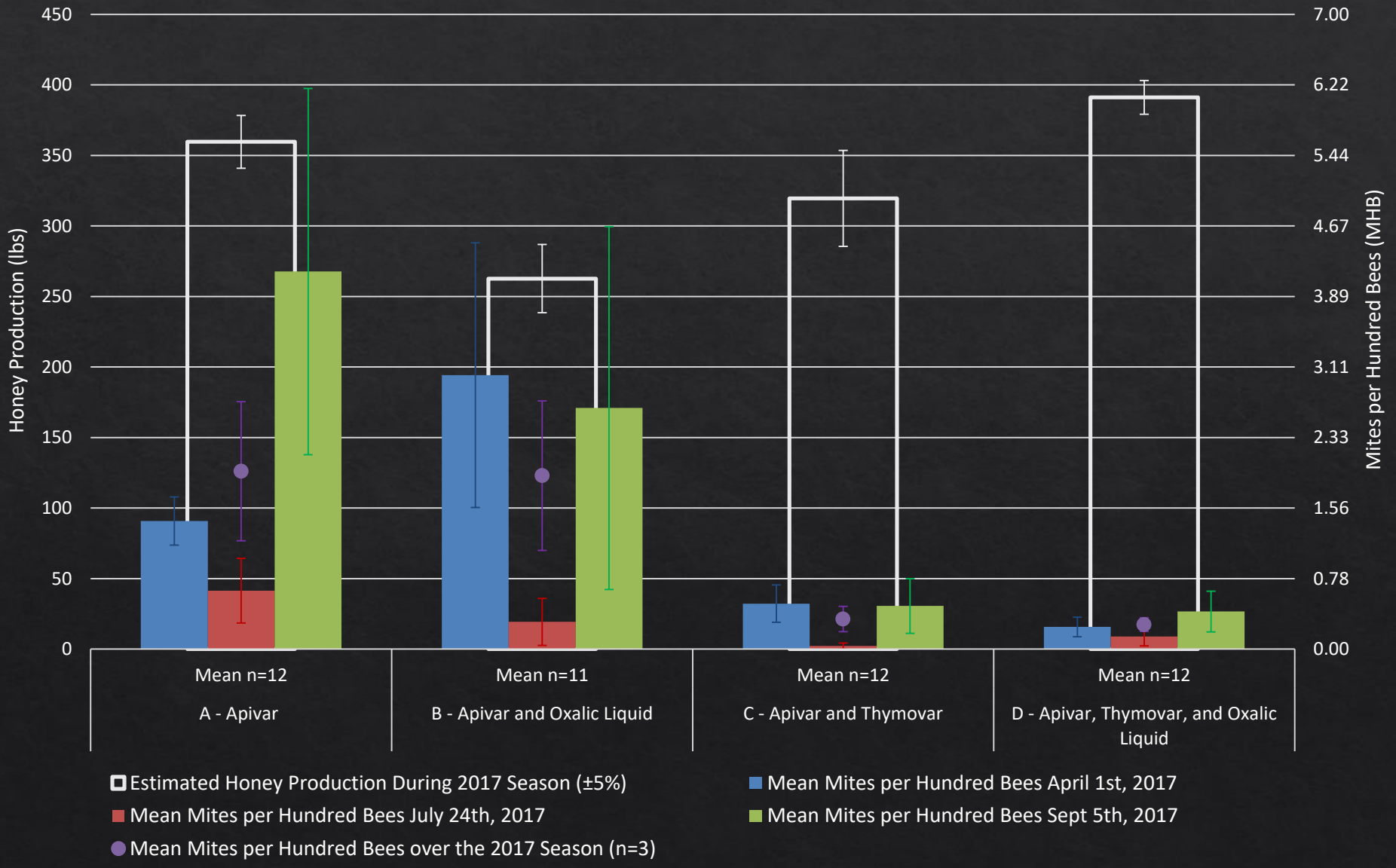
Three different combinations were determined to be effective enough to trial further. We then selected a total of 60 colonies, 15 per treatment plus an additional 15 for a control group that received just Apivar.

This experiment has continued for two years with an identical treatment applied in the spring of the second year.

Summary of Phoretic Varroa Levels (MHB) and Colony Survival After Four Different Miticide Treatments from April 12th, 2016 to September 5th, 2017



Summary of Phoretic Mite Levels (MHB) and Honey Production After Four Different Miticide Treatments in 2017



Conclusions:

• Possible to identify productive and mite tolerant colony phenotypes, and improve by established breeding procedures (back crossing, out crossing, re-current selection and progeny analyses).

• Colony phenotypes are difficult to stabilize because of considerable variability in progeny, from selected breeders. This may be due, in part, to multiple mating (sub-families), queen events (supersedure), high recombination rates, epistatis, and genotype – environment interactions (epigenetic effects). Evolutionary characteristic?

• New selection tools (biomarkers) for identifying phenotypes expressing genes involved in varroa tolerance, pathogen resistance immunity, and productivity should help to stabilize phenotypes and assist with breeding procedures.

• Improvement in techniques for the diagnosis of honey bee pathogens will help to improve the quality of breeding stock, and bee health in general.

• Use of non synthetic miticides (organic acids, thymol, etc.) in a timely fashion, with tolerant stock will improve honey bee health and colony longevity.

• Combined miticide trials are showing better efficacy, good colony survival, and no observable effects on bee mortality. This should delay development of resistance by varroa.

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