

Arising amitraz and pyrethroids resistance mutations in the ectoparasitic *Varroa destructor* mite in
Canada

Rassol Bahreini^{1*}, Joel González-Cabrera^{2,3}, Carmen Sara Hernández-Rodríguez^{2,4}, Sara Moreno-Martí^{2,3},
Samantha Muirhead⁵, Renata B Labuschagne⁶, Olav Rueppell¹

¹Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

²Institute BIOTECMED, Universitat de València, Valencia, Spain.

³Department of Genetics, Universitat de València, Valencia, Spain.

⁴Department of Microbiology and Ecology, Universitat de València, Valencia, Spain.

⁵Plant and Bee Health Surveillance Section, Alberta Agriculture and Irrigation, Edmonton, Alberta,
Canada.

⁶Technology Transfer Program, Alberta Beekeepers Commission, Edmonton, Alberta, Canada.

*Corresponding author: rassol.bahreini@gmail.com

21 **Supplementary Table 1:** Frequency (%) of genotypes and associated phenotypes related to amitraz and
22 pyrethroids resistance in selected operations from Alberta. “*n*” indicates the number of mites were
23 analyzed in each location.

Apiary code	Region	County	Amitraz						Pyrethroids								
			Genotype (%)			n	Phenotype (%)		Genotype (%)						n	Phenotype (%)	
			Y215	Y215H	H215		Susceptible	Resistant	L925	L925I	L925M	I925	M925	I925M		Susceptible	Resistant
AB-17	North West	Smoky River	94.5	0	5.6	36	94.5	5.6	77.1	2.9	2.9	17.1	0	0	35	82.9	17.1
AB-18	North West	Fairview	2.5	2.5	95	40	5	95	5	2.5	0	80	0	12.5	40	7.5	92.5
AB-20	North West	Smoky River	17.9	2.6	79.5	39	20.5	79.5	20.5	7.7	0	56.4	2.6	12.8	39	28.2	71.8
AB-31	South	Rocky View	100	0	0	40	100	0	47.5	2.5	17.5	0	32.5	0	40	67.5	32.5
AB-32	Central	Tofield	57.5	7.5	35	40	65	35	27.8	2.8	0	52.8	0	16.7	36	30.5	69.5
AB-39	North West	Bezanson	82.5	5	12.5	40	87.5	12.5	60	5	2.5	30	0	2.5	40	67.5	32.5
AB-43	Central	Strathcona	100	0	0	7	100	0	42.9	0	5	57	0	0	7	42.9	57.1
AB-52	Central	Lacombe	47.5	5	47.5	40	52.5	47.5	28.2	10.3	0	59	0	2.6	39	38.5	61.5
AB-53	South	Calgary	55	7.5	37.5	40	62.5	37.5	16.2	5.4	0	70.3	2.7	5.4	37	21.6	78.4
AB-55	South	Lethbridge	35	5	60	40	40	60	22.5	7.5	0	57.5	10	2.5	40	30	70
AB-69	South	Brooks	3.8	0	96.2	80	3.8	96.2	6.5	5.2	1.3	74	10.4	2.6	77	13	87
AB-73	South	High River	5	0	95	80	5	95	16.5	10.1	0	63.3	1.3	8.9	79	26.6	73.4
AB-77	South	Oyen	0	4.2	96.8	72	4.2	96.8	0	2.6	0	89.6	0	7.8	77	2.6	97.4
AB-87	North West	Wanham	65	5	30	20	70	30	30	10	0	55	0	5	20	40	60
AB-88	North West	Wanham	40	15	45	20	55	45	15.8	15.8	0	47.4	0	21.1	19	31.6	68.4
AB-89	North West	Wanham	45	10	45	20	55	45	36.8	10.5	0	52.6	0	0	19	47.4	52.6
AB-90	North West	Wanham	75	15	10	20	90	10	20	30	0	20	0	30	20	50	50
AB-92	Central	Clyde	45	2.5	52.5	40	47.5	52.5	36.8	2.6	0	50	0	10.5	38	39.5	60.5
AB-93	Central	Clyde	25	15	60	40	40	60	5.1	5.1	2.6	46.2	0	41	39	12.8	87.2

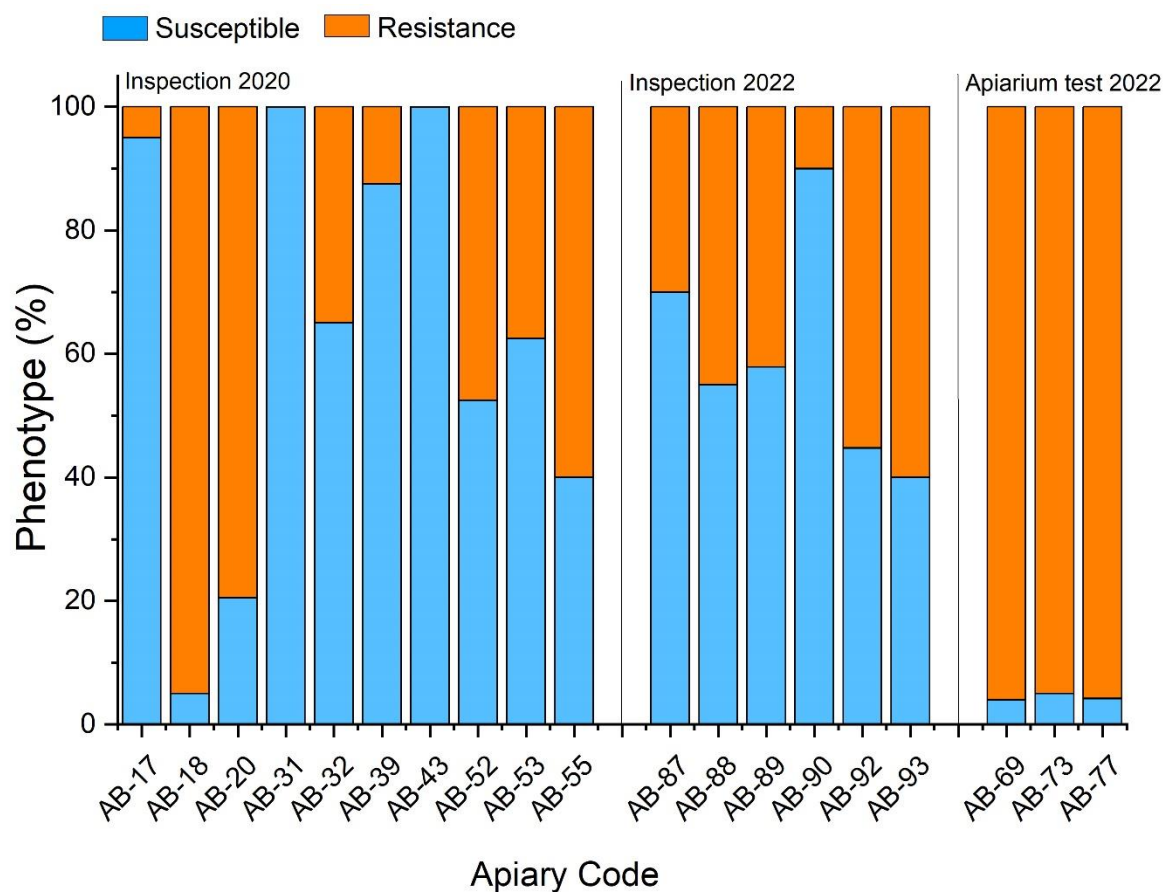
Supplementary Table 2: History of acaricide treatments in sampled operations from Alberta.

Apiary code	Region	County	Year of sampling	Acaricide treatment	
				Spring	Fall
AB-17	North west	Smoky River	2020	N/A	N/A
AB-18	North west	Fairview	2020	AP	OA
AB-20	North west	Smokey River	2020	AP	AP
AB-31	South	Rocky view	2020	OA	OA+FA
AB-32	Central	Tofield	2020	OA	OA
AB-39	North west	Bezanson	2020	OA	OA
AB-43	Central	Strathcona	2020	OA	N/A
AB-52	Central	Lacombe	2020	N/A	N/A
AB-53	South	Calgary	2020	N/A	N/A
AB-55	South	Lethbridge	2020	N/A	N/A
AB-66	Central	Edmonton	2022	AP	AP+OA+FA
AB-69	South	Brooks	2022	AP	AP
AB-73	South	High River	2022	AP	OA
AB-77	South	Oyen	2022	AP	AP+OA
AB-83	North west	Grim Shaw	2022	AP	N/A
AB-87	North west	Wanham	2022	OA	OA
AB-88	North west	Wanham	2022	OA	OA
AB-89	North west	Wanham	2022	OA	OA
AB-90	North west	Wanham	2022	OA	OA
AB-92	Central	Clyde	2022	OA	OA
AB-93	Central	Clyde	2022	OA	OA

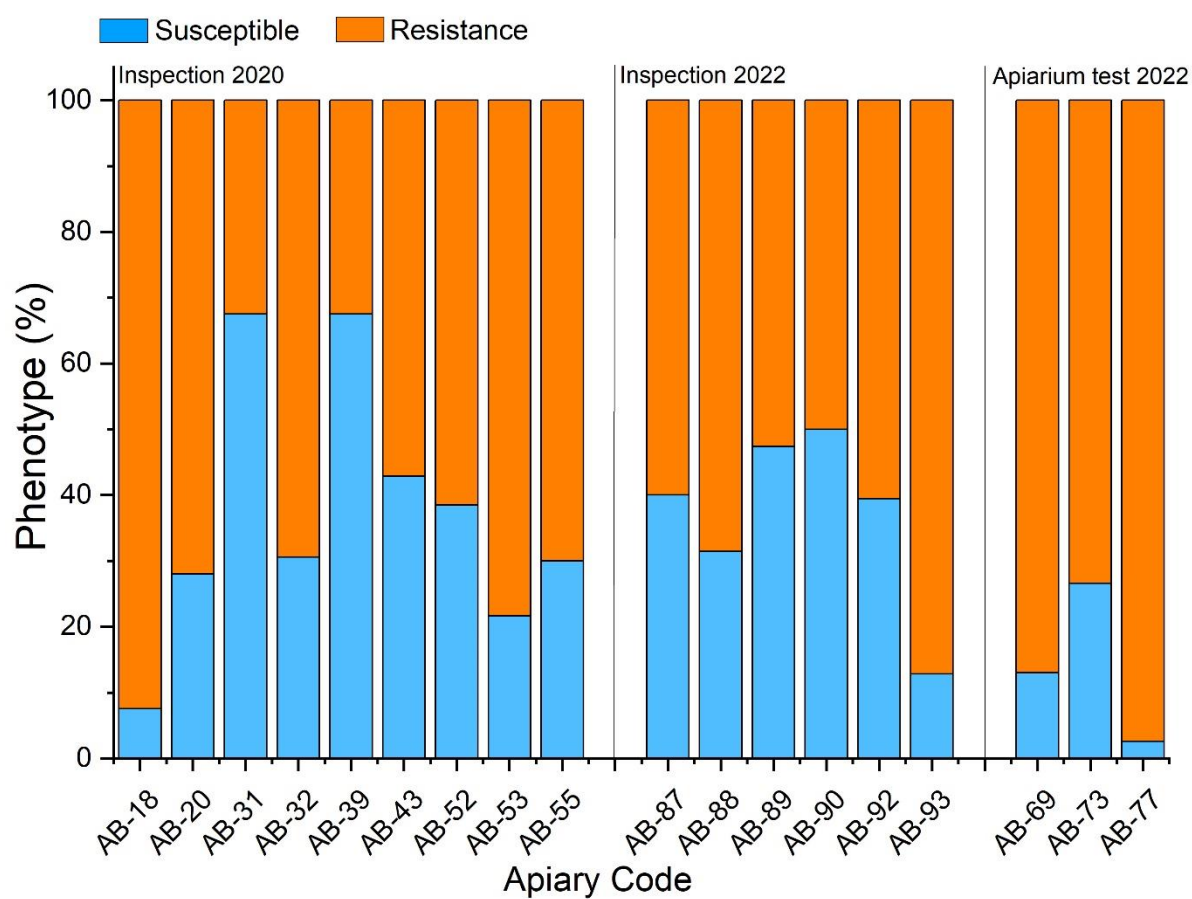
AP: Apivar, OA: oxalic acid, FA: formic acid, N/A: not available information.

Supplementary Table 3: Primers and probes used for TaqMan assays

Primer/probe	Sequence 5'-3'	Reference
Vd_L925V_F	CCAAGTCATGGCCAACGTT	González-Cabrera et al., PloS One (2016) 11: e0155332
Vd_L925V_R	AAGATGATAATTCCTAACACAAAGG	
Vd_L925_V	TTACCCAGAGCTCC	
Vd_L925I_M	AGGTTACCTAtAGCTCC	
Vd_L925M_N	TTACCCAtAGCTCCTATC	
OctR_Vd_215_F	GGATACCGTGCTCAGTAATGCT	Hernández-Rodríguez et al., J Pest Sci. (2022) 95:1179–1195
OctR_Vd_215_R	CTGTCGGGTCGCTTCTAGATAG	
Vd_Y215H_V	ATGCGCCAATAAGTGAAT	
Vd_Y215H_M	CGCCAATGAGTGAAT	



Supplementary Fig. 1: Frequency (%) of predicted amitraz-susceptible (dark blue) and amitraz-resistant (dark orange) mites detected in selected operations from Alberta.



Supplementary Fig. 2: Frequency (%) of predicted pyrethroids-susceptible (dark blue) and pyrethroids-resistant (dark orange) mites detected in selected operations from Alberta.