Confirmation of the Y215H mutation in the β_2-octopamine receptor in *Varroa destructor* is associated with contemporary cases of amitraz resistance in the US

Amitraz Resistance Genotyping in *Varroa*

Frank D. Rinkevich[1*] *Sara Moreno-Martí*[2], *Carmen Sara Hernández-Rodríguez*[2], *and Joel González-Cabrera*[2]

[1]USDA-ARS Honey Bee Breeding, Genetics, and Physiology Laboratory, Baton Rouge, LA USA

[2]Institute BIOTECMED, Universitat de València, Burjassot 46100, Spain

*Corresponding author frank.rinkevich@usda.gov ORCID 0000-0001-8591-9910

Author Contribution Statement

FDR performed amitraz resistance tests and collected Varroa samples; JGC designed the genotype analysis; SMM and CSHR performed the genotyping experiments; FDR, JGC, SMM, and CSHR analyzed the data and wrote the manuscript.

ABSTRACT

BACKGROUND: The parasitic mite, *Varroa destructor* (Anderson and Trueman), is a leading cause of honey bee colony losses around the world. Application of miticides such as amitraz are often the primary method of Varroa control in commercial beekeeping operations in the US. It is likely that excessive and exclusive amitraz application has led to the development of amitraz resistance in Varroa. A mutation of tyrosine at amino acid position 215 to histidine (Y215H) in the β_2-octopamine receptor was identified in putatively amitraz resistant Varroa in the US. This research investigated the presence of the Y215H mutation in quantitatively confirmed amitraz resistant Varroa from the US.

RESULTS: There was a strong association of susceptible and resistant phenotypes with the corresponding susceptible and resistant genotypes, respectively, and vice versa. The resistance bioassay may understate resistance levels due to the influence of environmental conditions on the outcome of the test whereby Varroa with an amitraz resistant genotype may appear with a susceptible phenotype.

CONCLUSION: Confirmation of the Y215H mutation in the β_2-octopamine receptor of amitraz resistant Varroa encourages the development and validation of low-cost, high throughput genotyping protocols to assess amitraz resistance. Resistance monitoring via genotyping will allow for large-scale passive monitoring to accurately determine the prevalence of amitraz resistance rather than directed sampling of apiaries with known resistance issues. Genotyping of Varroa for amitraz resistance early in the beekeeping season may predict late season resistance at the colony level and provide beekeepers with enough time to develop an effective Varroa management strategy.

Keywords: Honey bee, *Varroa destructor*, amitraz, resistance, β-octopamine receptor

1 INTRODUCTION

The honey bee, *Apis mellifera* (L.), is unquestionably one of, if not the most, agriculturally beneficial insects. Commercially managed honey bees contribute more than $17B to agricultural productivity in the US alone where they are critically important pollinators of more than 130 different crops, especially almonds, apples, cherries, blueberries, and cranberries [1, 2]. In 2021, more than 57 million kg of honey was produced in the US for a total value of more than $321 million [3].

Honey bee colony losses are a frustrating reality for beekeepers. While on the surface, the overall number of honey bee colonies in the US has stabilized over the past 15 years at around 2.7 million colonies per year [4], annual colony losses have also averaged approximately 40% over this same period [5, 6]. This apparent state of equilibrium between colony numbers and annual losses belies the increasing financial costs of replacing colonies and lost revenue from pollination and honey production. Even with stable honey bee populations in the US, crop production is limited by an insufficient supply of pollinators [7]. This is especially emphasized in almonds where the demand for pollination (i.e., acres of almonds) is growing faster than the price of pollination contracts despite a shortage of colonies shipped for pollination [8, 9]. This inefficiency in pollination contracts, coupled with sustained low honey prices and declining honey production [10] forces beekeepers to run their operations as efficiently as possible.

Commercial beekeepers understand that the parasitic mite, *Varroa destructor* (Anderson and Trueman), henceforth referred to as Varroa, is the most likely culprit in colony losses [11]. Beekeepers who utilize synthetic miticides (mostly amitraz and to a much lower degree tau-fluvalinate and coumaphos) experience significantly reduced colony losses [12]. The efficacy and ease of miticide applications may lead to it being the sole measure used to control Varroa. Theoretically, this pattern of overuse and overreliance may lead to resistance. Demonstrably, widespread resistance to tau-fluvalinate and

coumaphos has developed within a few years of the registration of each product [13-16]. The first report of resistance to amitraz in Varroa was more than 20 years ago [17], but amitraz has remained mostly efficacious over the years to the point where it is almost used exclusively [12]. However, an increasing number of anecdotal reports of Varroa control failure with amitraz have been mentioned by US beekeepers, thus providing the impetus to investigate amitraz resistance in Varroa. In the US, amitraz resistance was documented in 2019 where reduced amitraz efficacy was due to reduced sensitivity to technical grade amitraz [18]. Subsequent amitraz resistance monitoring using the amitraz efficacy test in 2020 and 2021 showed that about 25% of all apiaries sampled had levels of resistance that may result in amitraz control failure at the colony level (Rinkevich, manuscript in preparation).

Studies on identifying the molecular markers associated with amitraz resistance focused on mutations in octopamine receptors in Varroa as they are the target site of amitraz and other formamidine pesticides [19]. Mutations in the β_2-octopamine receptor of Varroa (*Vd_octβ_2r*) were associated with amitraz resistance but the exact mutation was dependent on geography. In France, a substitution of asparagine to serine at position 87 in the amino acid sequence of the β_2-octopamine receptor (N87S) was identified in amitraz resistant Varroa, but amitraz resistant Varroa from the US possessed a tyrosine to histidine mutation at position 215 (Y215H) [20]. The N87S mutation from France was strongly associated with amitraz resistance as treatment efficacy was quantitatively measured at the colony level. The association of the Y215H mutation in Varroa from the US was probable, but amitraz resistance was based on qualitative observations of treatment failure. While association of the Y215H mutation with amitraz resistance is not as definitive as the association with N87S, it does warrant confirmation with Varroa that were quantitatively phenotyped for amitraz resistance.

In this study, Varroa samples from the US that were phenotyped as amitraz resistant or susceptible based on the amitraz efficacy tests were genotyped for

the Y215H mutation. The ability to reliably associate the Y215H genotype with an amitraz resistance phenotype will confirm its role in amitraz resistance in the US and determine the utility of the molecular diagnostic to monitor amitraz resistance in the future.

2 MATERIALS AND METHODS

2.1 Sample Collection

Varroa were collected as part of amitraz resistance monitoring activities in the US from 2020 to 2021 (Rinkevich, manuscript in preparation). Amitraz resistance phenotype in Varroa was determined by the amitraz efficacy assay [18]. Briefly, a sample of approximately 300 bees from each colony was placed into a container with a 16 cm^2 piece of Apivar® (3.3% amitraz, Veto-Pharma, Palaiseau, France), sealed with a screened lid, and inverted over a tray with petroleum jelly for 3 h. The amitraz-susceptible Varroa on the tray were counted and collected into a 1.5 mL microcentrifuge tube with 70% (v:v) ethanol. Varroa remaining on the bees after the exposure period were washed off with soapy water. These amitraz-resistant Varroa from the wash were counted and collected into a separate 1.5 mL microcentrifuge tube with 70% ethanol. Amitraz efficacy was calculated as the number of Varroa on the tray divided by total Varroa in the sample (#Varroa on tray + #Varroa in wash). An amitraz efficacy of <70% is associated with control failure at the colony level and considered amitraz resistant ([18] and Rinkevich, manuscript in preparation). All Varroa samples were stored at -80°C at the USDA-ARS Honey Bee Breeding, Genetics, and Physiology Lab (HBBGPL) until genotyping. Information on collection date, location, amitraz resistance phenotype, and Varroa infestation levels can be found in Table S1.

2.2 Amitraz Resistance Genotyping

A total of 125 individual Varroa from 21 apiaries across 7 states were sub-sampled to provide a range of amitraz resistant phenotypes (Fig 1, Table S1). Varroa were collected into individual tubes with 70% ethanol. The samples were then randomized, assigned a numerical identifier, and sent blind to amitraz resistance phenotype to Universitat de València. Genomic DNA was extracted from in individual Varroa using the alkaline extraction method [21]. A TaqMan® assay was used to distinguish between susceptible (Y215) and resistant (H215)

mutations in the β_2-octopamine receptor (*Vd_octβ_2r,* GenBank LOC111251882, [20]). Genotypes were classified as homozygous susceptible (YY215, SS), heterozygous (YH215, SR), or homozygous resistant (HH215, RR). Genotype data corresponding to sample number was sent back to HBBGPL and assigned to the matching randomized amitraz resistance phenotype.

2.3 Statistical Analyses

All statistics were performed with JMP 14.2.0 (SAS Institute, Cary NC).

3 RESULTS

Individuals with a heterozygous genotype were assigned a susceptible phenotype since the proportion of samples with <70% amitraz efficacy is similar in samples with SS or SR genotypes compared to the RR genotype (X^2=14.671, df=2, p=0.0007; Fig 2). Therefore, SS and SR genotypes are designated amitraz susceptible and the RR genotype is designated as amitraz resistant.

There was a strong association of the amitraz resistant phenotype with the amitraz resistant genotype (i.e., RR), where 88.1% of the Varroa with the amitraz resistant phenotype possessed the amitraz resistant genotype (Tables 1 and 2). Phenotypically susceptible Varroa had a susceptible genotype in 60.3% of those samples (Table 2).

The resistant genotype was detected in 72.0% of Varroa with a resistant phenotype. Varroa that had a susceptible genotype were highly likely to have a susceptible phenotype with 81.4% susceptible genotypes displayed an amitraz susceptible phenotype (Tables 1 and 2). Overall, there was a highly significant relationship of the phenotype matching the corresponding genotype and vice-versa (Odds Ratio=11.22, 95%CI=4.53-27.79). The complete results of the genotyping assays along with amitraz resistance phenotype and other collection information are shown in Table S1.

Samples in which the amitraz resistance phenotype did not match the amitraz resistance genotype (i.e., susceptible phenotype with a resistant genotype and vice versa) was observed significantly more frequently in Varroa with a susceptible phenotype (Likelihood Ratio=13.119, p=0.0003, n=125, df=1; Fisher's Exact Test, left tail, p=0.0003, Table 1).

4 DISCUSSION

The significant association between resistant phenotype and resistant genotype confirms that the Y215H mutation in the β_2-octopamine receptor is a reliable indicator of amitraz resistance in the US. Furthermore, this congruency demonstrates that the amitraz efficacy test is very accurate at detecting amitraz resistant Varroa. This is an important feature of this test since these samples were collected by 8 different researchers who performed this test under a variety of conditions.

The genotype assay is able to reliably diagnose amitraz susceptible Varroa. The ability to accurately identify susceptible individuals with this molecular diagnostic test is extremely valuable. The value of the molecular diagnostic test is best conceptualized as a tool to identify amitraz susceptible individuals to predict amitraz treatment success. Therefore, the molecular test for the absence of amitraz resistance is an important Varroa management tool.

The largest disagreement between genotype and phenotype was seen in phenotypically susceptible Varroa that had 39.7% of individuals with a resistant genotype. This is due to a disproportionate amount of phenotypically susceptible individuals possessing a resistant genotype. It is possible that genotypically resistant Varroa appeared phenotypically susceptible in the amitraz efficacy test due to enhanced Varroa drop due to environmental conditions. Higher temperatures increase the rate at which Varroa fall off of adult honey bees [22]. Honey bees utilize a variety of grooming behaviors to dislodge and kill Varroa [23]. Higher temperatures increase the rate of Varroa removal from honey bees and this effect is amplified in stocks selected for high grooming behavior [24]. The background levels of Varroa dislodging under control conditions at room temperature (22-24^{o}C) in the amitraz efficacy test is very low, although variation is still possible outside of these conditions [18]. More detailed studies on the effects of temperature and other experimental conditions on the outcomes of the Amitraz efficacy test and amitraz sensitivity are currently underway.

A small proportion of phenotypically resistant Varroa possessed a susceptible genotype (6.4% of all samples, Table 1). A phenotypically resistant Varroa with a susceptible genotype may be due to Varroa escaping amitraz exposure by hiding between abdominal sclerites of the honey bee. In some instances, dislodged Varroa, which should be phenotypically susceptible, become trapped along the body of the honey bee, stuck to the inside of the plastic container, or tangled in the screen (Rinkevich, personal observation). This is a particular problem in low humidity environments where static electricity can build up. It is important to check the inner surface of the test container and the screen before washing the bees to ensure the dislodged Varroa have been counted and phenotyped accurately. While the amitraz efficacy test is a reliable indicator of *bona fide* amitraz resistance, it is important to better understand the influence of the environmental conditions in which the amitraz efficacy test is performed to improve the reliability and reproducibility of this enormously important tool for Varroa management.

The Varroa that are maintained at the HBBGPL have been used as the amitraz-susceptible reference population for comparison in amitraz resistance studies [18]. While this sample set did not lend itself to rigorous quantitative analyses, all of the Varroa that were genotyped from this population were homozygous susceptible. This suggests this particular Varroa population is a suitable amitraz-susceptible population due to only susceptible genotypes found in this population.

The heterozygous genotype was classified as having a susceptible phenotype because colonies that contained heterozygotes displayed levels of amitraz efficacy that were similar to colonies that contained individuals with the homozygous susceptible genotype (Fig 2). Target-site resistance mutations, such as the Y215H mutation in the β_2-octopamine receptor reported here, are typically inherited in a recessive manner so that heterozygotes are functionally susceptible [25, 26]. While the exact mutation was not identified, heterozygous individuals are

more similar to homozygous susceptible individuals in amitraz resistant cattle ticks, *Rhipicephalus* (*Boophilus*) *microplus* [27, 28]. Therefore, a recessive pattern of inheritance for target-site mutations as the basis for amitraz resistance in Varroa is plausible. It is important to understand the pattern of inheritance for amitraz resistance in Varroa so the results of the genotyping assays can be applied in a toxicologically relevant manner. For example, a population consisting of heterozygous individuals would have a resistance allele frequency of 0.5, but yet appear phenotypically as amitraz susceptible due to the recessive pattern of inheritance of amitraz resistance. Therefore, the development of a molecular diagnostic would need to report on the diploid genotype (i.e., SS, SR, RR) rather than simple allele frequency in order to yield results that can be utilized by the beekeeper to perform effective Varroa management.

5 CONCLUSIONS

This study confirms that amitraz resistance in Varroa is strongly associated with Y215H mutation in the β_2-octopamine receptor. The molecular diagnostic test is a reliable indicator of amitraz susceptible Varroa and should be used confidently to predict treatment success with amitraz at the colony level. Research is currently underway to adapt the current genotyping methodologies to be performed in a quantitative manner on pools of Varroa in order to reduce costs and speed up processing times so it may be used as a diagnostic tool by apiary inspectors and beekeepers. While the amitraz efficacy test is a valuable tool for monitoring for amitraz resistance, it is time consuming for those without expertise and the results are unreliable with low Varroa numbers in the sample. Resistance monitoring by genotyping will allow more beekeepers to participate in resistance monitoring since they will only need to supply a few Varroa from their apiaries that can be collected from regular Varroa infestation monitoring, therefore providing a more passive sampling regime to accurately determine the prevalence of amitraz resistance. It will be important to determine the reliability of detecting amitraz resistance though genotyping early in the season from a few Varroa to predict late season Varroa resistance at the colony level so beekeepers can procure the appropriate miticides and allocate resources for administering an effective Varroa management strategy.

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CONFLICT OF INTEREST DECLARATION

The authors have no relevant financial or non-financial interests to disclose.

DECLARATIONS

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Author Contributions

FDR performed amitraz resistance tests and collected Varroa samples; JGC designed the genotype analysis; SMM and CSHR performed the genotyping experiments; FDR, JGC, SMM, and CSHR analyzed the data and wrote the manuscript.

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Table 1. Association of amitraz resistant phenotype from the Amitraz efficacy test with genotypes for mutations in the β_2-octopamine receptor (*Vd_octβ_2r*) associated with amitraz resistance in Varroa. Numbers in the table represent the number of samples with the corresponding phenotype/genotype pairing.

		Phenotype		
		R	S	Row Total
Genotype	R	59	23	82
	S	8	35	43
	Column Total	67	58	125 Total Samples

Table 2. Reliability of amitraz resistance phenotype to predict resistance genotype and vice-versa. The predictor is the metric used to determine the outcome. Reliability is the proportion of samples where the predictor-outcome relationship agrees.

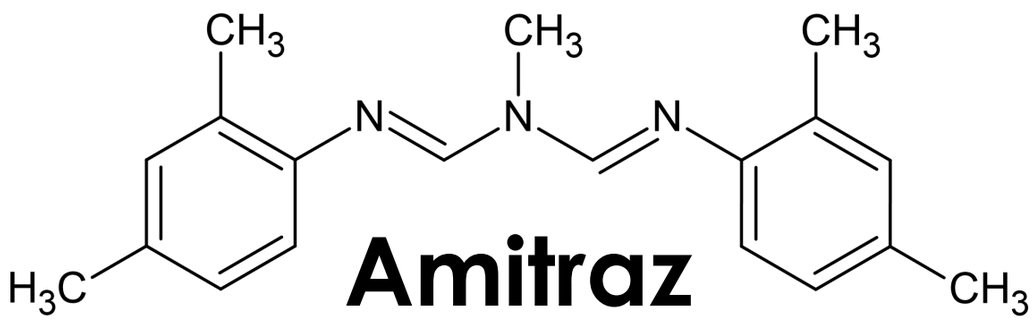
Predictor	Outcome	Reliability (%)
Resistant Phenotype	**Resistant Genotype**	**88.1**
Susceptible Phenotype	**Susceptible Genotype**	**60.3**
Resistant Genotype	**Resistant Phenotype**	**72.0**
Susceptible Genotype	**Susceptible Phenotype**	**81.4**

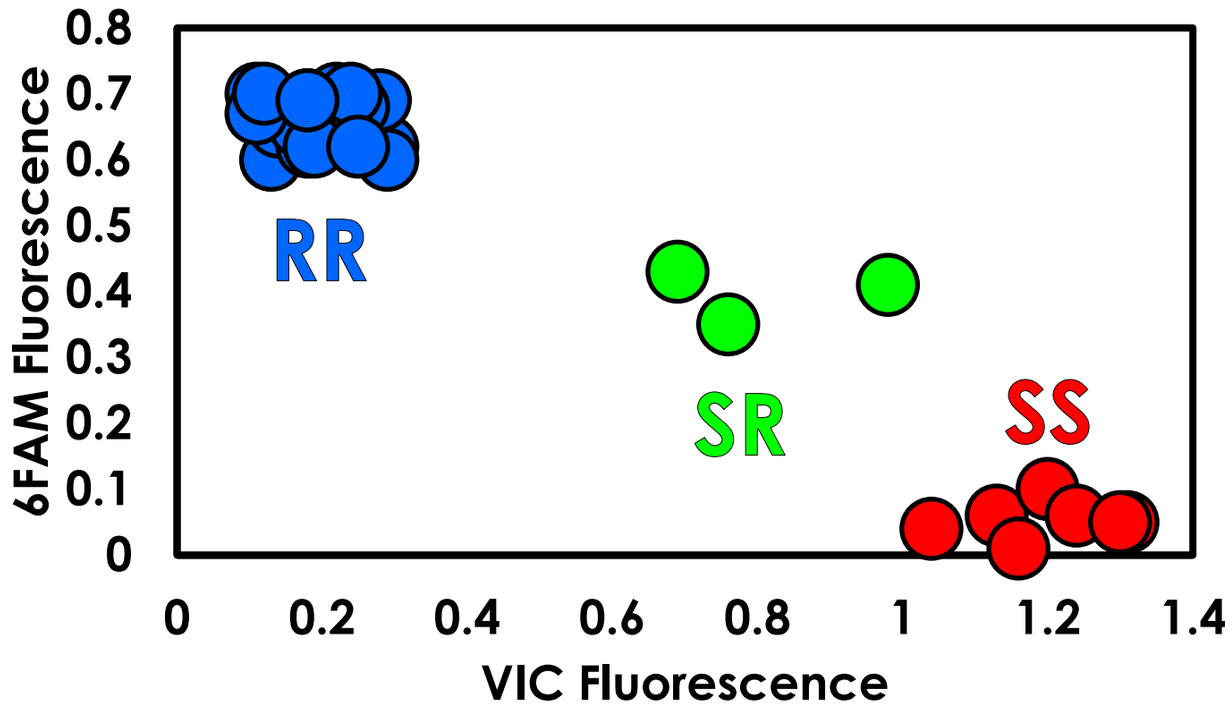
FIGURE LEGEND

Figure 1. Location and numerical distribution of Amitraz efficacy of Varroa genotyped for mutations in the β_2-octopamine receptor (*Vd_octβ_2r*) associated with amitraz resistance in Varroa. A) Geographic locations of apiaries that were sampled for amitraz resistance in Varroa. A total of 125 individual Varroa were genotyped from 21 apiaries across 7 states. The red dots show the approximate location of apiaries that were sampled. Some apiaries were too close to allow resolution between dots so only 19 apiaries are shown as dots. The map was used with permission from gisgeography.com. B) Numerical distribution of the Amitraz efficacy phenotype of Varroa that were genotyped. Varroa that were genotyped were distributed over a broad range of Amitraz efficacy phenotypes.

Figure 2. Association of proportion of samples with <70% Amitraz efficacy for each genotype. The <70% Amitraz efficacy threshold was chosen because that level of efficacy is associated with amitraz treatment failure at the colony level. Samples with SS and SR genotypes had significantly lower proportion of amitraz resistant samples compared to the RR genotype. Letters above bars indicate significant differences between genotypes. Numbers at the bottom of the bars indicate the number of samples that displayed the corresponding genotype.

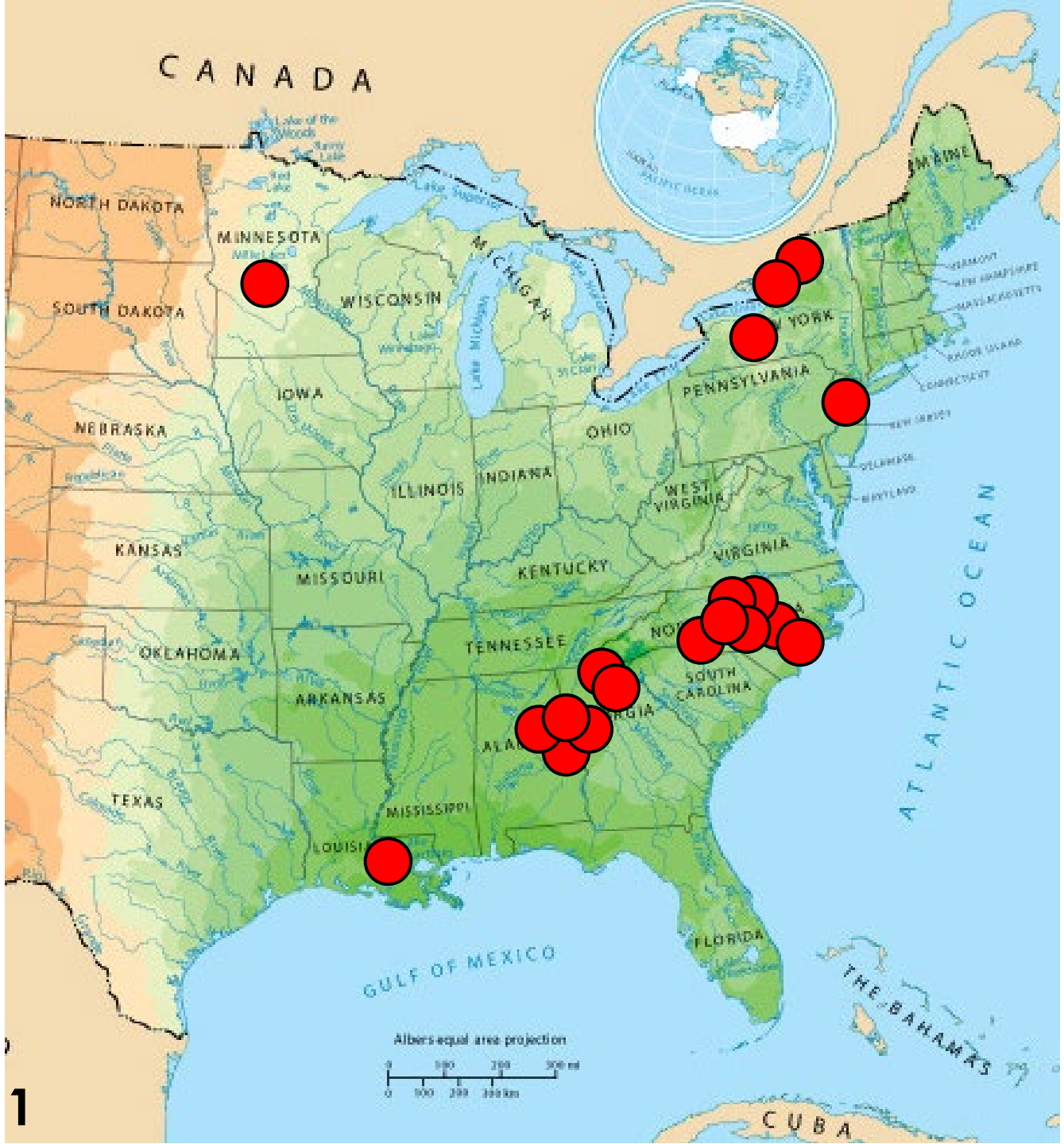
Varroa destructor





		Amitraz Resistance Phenotype	
		R	S
Vd_octβ$_2$r Genotype	R	59	23
	S	8	35

A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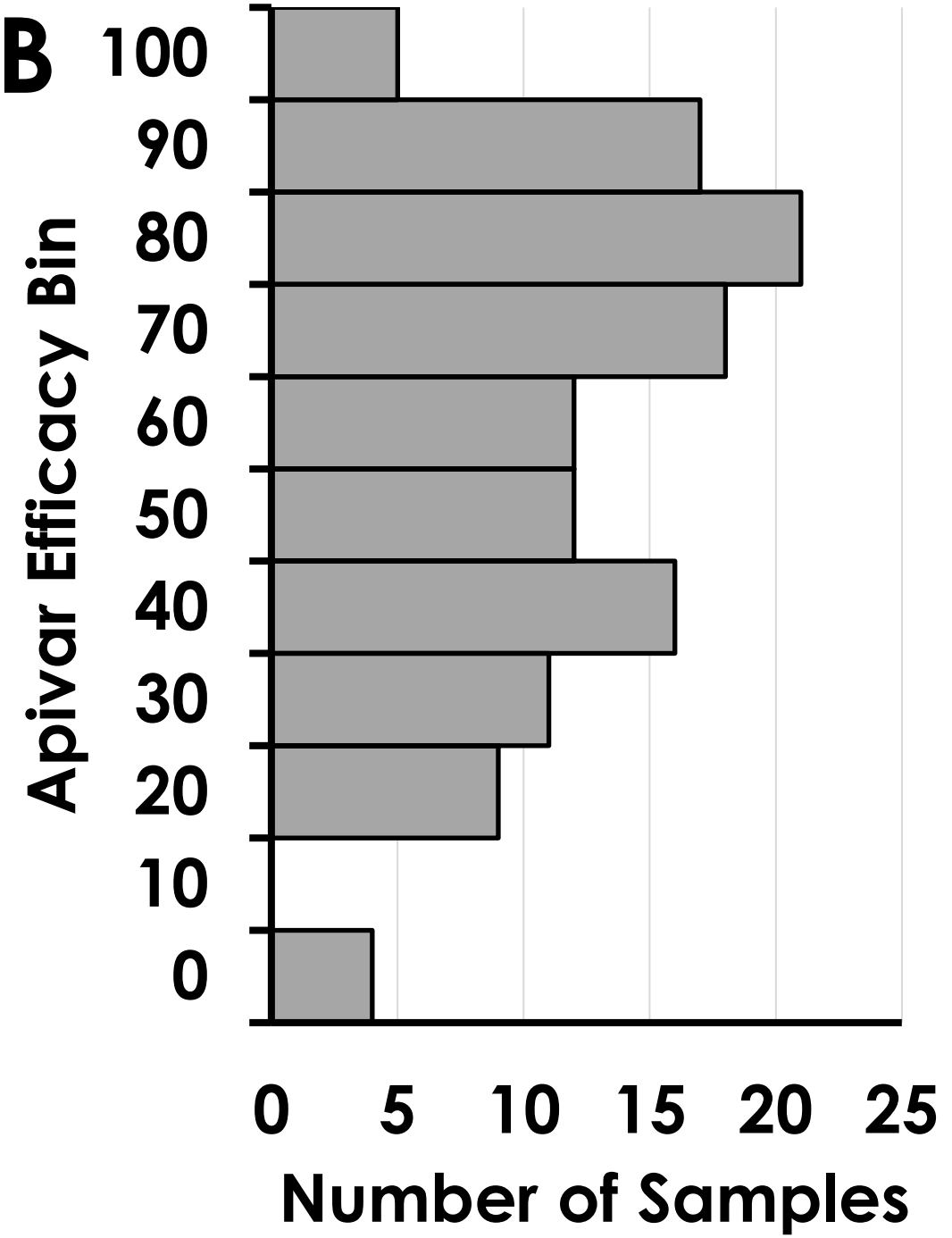
Apivar Efficacy Bin
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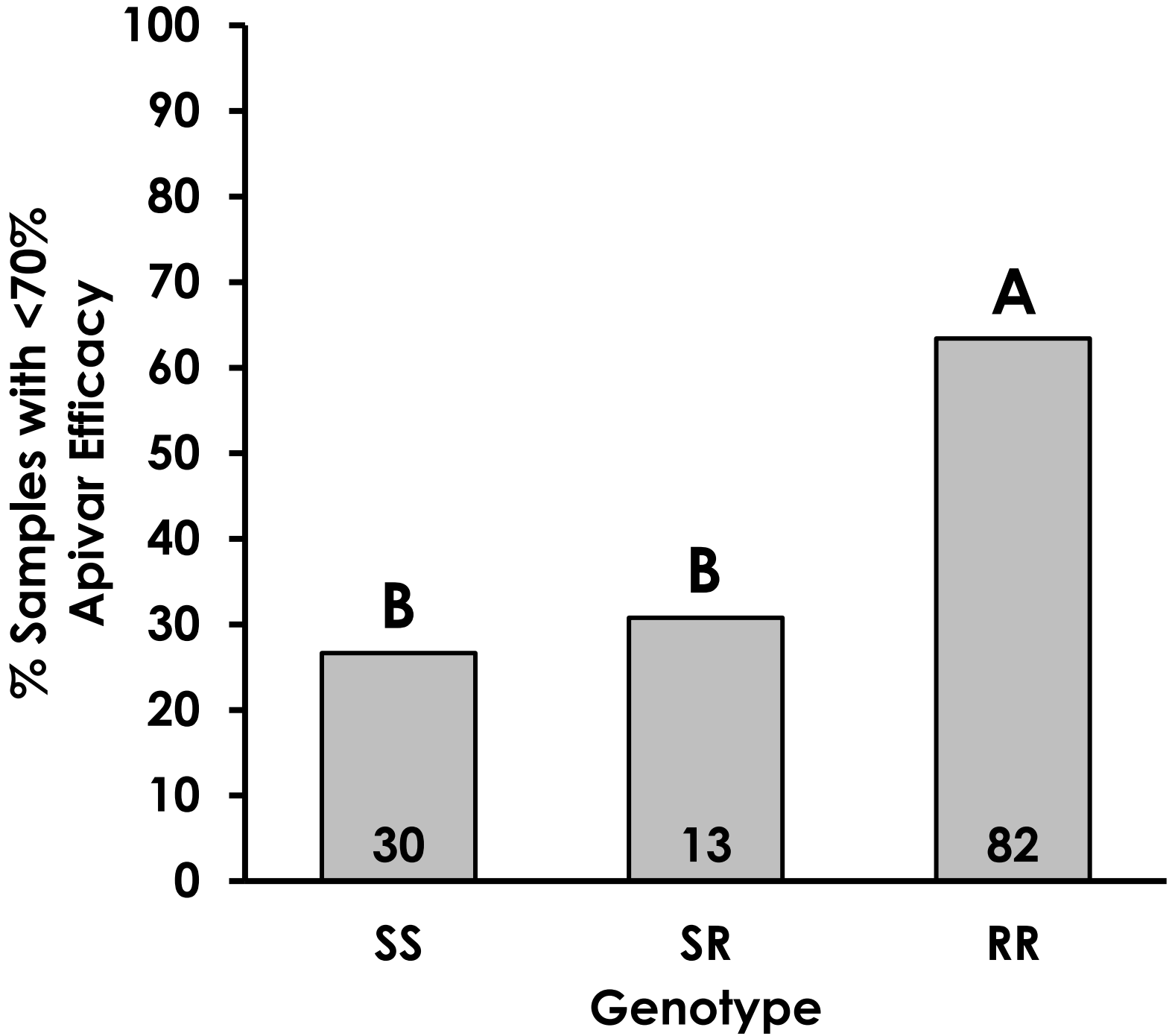
Fig 1


% Samples with <70%
Apivar Efficacy
100
90
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82
SS
SR
RR
Genotype

Fig 2

Sample #[1]	Phenotype[2]	Genotype[3]	Geno Tox[4]	Agree[5]
86	R	H	S	No
89	S	H	S	Yes
30	S	H	S	Yes
9	R	H	S	No
79	S	H	S	Yes
81	R	H	S	No
84	S	H	S	Yes
71	S	H	S	Yes
1	R	H	S	No
76	S	H	S	Yes
77	S	H	S	Yes
14	R	H	S	No
46	S	H	S	Yes
85	R	R	R	Yes
87	R	R	R	Yes
88	S	R	R	No
97	R	R	R	Yes
37	R	R	R	Yes
38	R	R	R	Yes
39	R	R	R	Yes
40	S	R	R	No
98	R	R	R	Yes
99	S	R	R	No
100	S	R	R	No
91	R	R	R	Yes
92	R	R	R	Yes
93	R	R	R	Yes
31	R	R	R	Yes
32	R	R	R	Yes
33	R	R	R	Yes
4	R	R	R	Yes
5	R	R	R	Yes
6	R	R	R	Yes
7	S	R	R	No
25	R	R	R	Yes
26	R	R	R	Yes
27	R	R	R	Yes
29	S	R	R	No
A11	R	R	R	Yes
A12	R	R	R	Yes
A24	R	R	R	Yes
A28	R	R	R	Yes
A31	R	R	R	Yes

A14	R	R	R	Yes
A21	R	R	R	Yes
A22	R	R	R	Yes
A23	R	R	R	Yes
8	R	R	R	Yes
10	R	R	R	Yes
55	R	R	R	Yes
56	R	R	R	Yes
58	S	R	R	No
59	S	R	R	No
61	R	R	R	Yes
62	R	R	R	Yes
63	R	R	R	Yes
64	S	R	R	No
65	S	R	R	No
66	S	R	R	No
82	S	R	R	No
83	S	R	R	No
67	R	R	R	Yes
68	R	R	R	Yes
69	R	R	R	Yes
70	S	R	R	No
72	S	R	R	No
2	R	R	R	Yes
3	R	R	R	Yes
19	R	R	R	Yes
20	R	R	R	Yes
21	R	R	R	Yes
22	S	R	R	No
23	S	R	R	No
24	S	R	R	No
73	R	R	R	Yes
74	R	R	R	Yes
75	R	R	R	Yes
A20	S	R	R	No
49	R	R	R	Yes
50	R	R	R	Yes
51	R	R	R	Yes
A8	R	R	R	Yes
A16	R	R	R	Yes
A18	R	R	R	Yes
A25	R	R	R	Yes
A33	R	R	R	Yes
13	R	R	R	Yes

15	R	R	R	Yes
17	S	R	R	No
18	S	R	R	No
43	R	R	R	Yes
44	R	R	R	Yes
45	R	R	R	Yes
47	S	R	R	No
48	S	R	R	No
90	S	S	S	Yes
41	S	S	S	Yes
42	S	S	S	Yes
94	S	S	S	Yes
95	S	S	S	Yes
96	S	S	S	Yes
34	S	S	S	Yes
35	S	S	S	Yes
36	S	S	S	Yes
28	S	S	S	Yes
A15	R	S	S	No
11	S	S	S	Yes
12	S	S	S	Yes
57	R	S	S	No
60	S	S	S	Yes
80	R	S	S	No
78	S	S	S	Yes
A1	S	S	S	Yes
A3	S	S	S	Yes
A5	S	S	S	Yes
A29	S	S	S	Yes
52	S	S	S	Yes
53	S	S	S	Yes
54	S	S	S	Yes
A2	S	S	S	Yes
A17	S	S	S	Yes
A19	S	S	S	Yes
A26	S	S	S	Yes
A35	S	S	S	Yes
16	S	S	S	Yes

1- Sample # is a randomized anonymous identifier assigned to a sample by th
2- Phenotype indicates if the Varroa tested was collected from the tray during
3- Genotype refers to S indicating homozygous susceptible, R indicating homo
4- Genotox refers to the susceptible genotypes S and H (S) or resistant genotyp
5- Agree denotes where phenotype and genotype match

6- Date is the date of sample collection
7- Year is the year the sample was collected
8- State is the location of the collection
9- Apiary code is an anonymous identifier to protect the identity of the coope
10- Tray indicates the number of Varroa on the tray at the end of the Apivar®
11- Wash indicates the number of Varroa that remained on the bees in the Ap
12- Bees is the total number of bees in the Apivar efficacy test
13- Total Varroa is the sum of Varroa from the Tray and Wash columns
14- Apivar efficacy is the number of Varroa in the Tray/number of Total Varroa
15- Varroa infestation is the total number of Varroa divided by the number of b

Date[6]	Year[7]	State[8]	Apiary Code[9]	Tray[10]	Wash[11]
2020Aug13	2020	NJ	JG20MAMA	52	13
2020Aug13	2020	NJ	JG20MAMA	52	13
2020Sep3	2020	NY	DW20MSMS	15	11
2021Aug10	2021	NC	LC21HEER	21	3
2021Aug2	2021	AL	GW21AUMOT	18	6
2021Aug2	2021	AL	GW21AUMOT	18	6
2021Aug2	2021	AL	GW21AUMOT	18	6
2021Aug24	2021	AU	AU-299	32	3
2021Aug25	2021	AU	AU-14	1	4
2021Aug5	2021	GA	LB21CLWA	7	3
2021Aug5	2021	GA	LB21CLWA	7	3
2021Oct1	2021	MN	SW21BUSW	3	9
2021Sept1	2021	NC	DH21NCBB	39	29
2020Aug13	2020	NJ	JG20MAMA	52	13
2020Aug13	2020	NJ	JG20MAMA	52	13
2020Aug13	2020	NJ	JG20MAMA	52	13
2020Aug19	2020	NY	JG20RWJO	121	19
2020Aug19	2020	NY	JG20RWSM	42	12
2020Aug19	2020	NY	JG20RWSM	42	12
2020Aug19	2020	NY	JG20RWSM	42	12
2020Aug19	2020	NY	JG20RWSM	42	12
2020Aug20	2020	NY	JG20RWJO	121	19
2020Aug21	2020	NY	JG20RWJO	121	19
2020Aug22	2020	NY	JG20RWJO	121	19
2020Aug25	2020	NC	LC20MDAW	18	4
2020Aug25	2020	NC	LC20MDAW	18	4
2020Aug25	2020	NC	LC20MDAW	18	4
2020Oct6	2020	NC	LC20LTLT	38	43
2020Oct6	2020	NC	LC20LTLT	38	43
2020Oct6	2020	NC	LC20LTLT	38	43
2020Sep28	2020	NC	AL2020PGPG	1	14
2020Sep28	2020	NC	AL2020PGPG	1	14
2020Sep28	2020	NC	AL2020PGPG	1	14
2020Sep29	2021	NC	AL2020PGPG	1	14
2020Sep3	2020	NY	DW20MSMS	15	11
2020Sep3	2020	NY	DW20MSMS	15	11
2020Sep3	2020	NY	DW20MSMS	15	11
2020Sep3	2020	NY	DW20MSMS	15	11
2020Sep30	2020	NC	LC20WEWE	37	50
2020Sep30	2020	NC	LC20WEWE	37	50
2020Sep30	2020	NC	LC20WEWE	37	50
2020Sep30	2020	NC	LC20WEWE	37	50
2020Sep30	2020	NC	LC20WEWE	37	50

2020Sept21	2020	NC	AL2020NGNG	60	86
2020Sept21	2020	NC	AL2020NGNG	60	86
2020Sept21	2020	NC	AL2020NGNG	60	86
2020Sept21	2020	NC	AL2020NGNG	60	86
2021Aug10	2021	NC	LC21HEER	21	3
2021Aug10	2021	NC	LC21HEER	21	3
2021Aug11	2021	NC	LC21MENC	18	11
2021Aug11	2021	NC	LC21MENC	18	11
2021Aug11	2021	NC	LC21MENC	18	11
2021Aug11	2021	NC	LC21MENC	18	11
2021Aug18	2021	NY	JG21WANO	10	5
2021Aug18	2021	NY	JG21WANO	10	5
2021Aug18	2021	NY	JG21WANO	10	5
2021Aug18	2021	NY	JG21WANO	10	5
2021Aug18	2021	NY	JG21WANO	10	5
2021Aug18	2021	NY	JG21WANO	10	5
2021Aug2	2021	AL	GW21AUMOT	18	6
2021Aug2	2021	AL	GW21AUMOT	18	6
2021Aug24	2021	AU	AU-299	32	3
2021Aug24	2021	AU	AU-299	32	3
2021Aug24	2021	AU	AU-299	32	3
2021Aug24	2021	AU	AU-299	32	3
2021Aug24	2021	AU	AU-299	32	3
2021Aug25	2021	AU	AU-14	1	4
2021Aug25	2021	AU	AU-14	1	4
2021Aug31	2021	AU	AU-602	3	7
2021Aug31	2021	AU	AU-602	3	7
2021Aug31	2021	AU	AU-602	3	7
2021Aug31	2021	AU	AU-602	3	7
2021Aug31	2021	AU	AU-602	3	7
2021Aug31	2021	AU	AU-602	3	7
2021Aug5	2021	GA	LB21CLWA	7	3
2021Aug5	2021	GA	LB21CLWA	7	3
2021Aug5	2021	GA	LB21CLWA	7	3
2021Aug7	2021	GA	LB21CLLS	72	1
2021Aug7	2021	GA	LB21CLLS	47	3
2021Aug7	2021	GA	LB21CLLS	47	3
2021Aug7	2021	GA	LB21CLLS	47	3
2021Oct1	2021	MN	SW21BUSW	36	60
2021Oct1	2021	MN	SW21BUSW	36	60
2021Oct1	2021	MN	SW21BUSW	36	60
2021Oct1	2021	MN	SW21BUSW	36	60
2021Oct1	2021	MN	SW21BUSW	36	60
2021Oct1	2021	MN	SW21BUSW	3	9

2021Oct1	2021	MN	SW21BUSW	3	9
2021Oct1	2021	MN	SW21BUSW	3	9
2021Oct1	2021	MN	SW21BUSW	3	9
2021Sept1	2021	NC	DH21NCBB	39	29
2021Sept1	2021	NC	DH21NCBB	39	29
2021Sept1	2021	NC	DH21NCBB	39	29
2021Sept1	2021	NC	DH21NCBB	39	29
2021Sept1	2021	NC	DH21NCBB	39	29
2020Aug13	2020	NJ	JG20MAMA	52	13
2020Aug19	2020	NY	JG20RWSM	42	12
2020Aug19	2020	NY	JG20RWSM	42	12
2020Aug25	2020	NC	LC20MDAW	18	4
2020Aug25	2020	NC	LC20MDAW	18	4
2020Aug25	2020	NC	LC20MDAW	18	4
2020Oct6	2020	NC	LC20LTLT	38	43
2020Oct6	2020	NC	LC20LTLT	38	43
2020Oct6	2020	NC	LC20LTLT	38	43
2020Sep3	2020	NY	DW20MSMS	15	11
2020Sept21	2020	NC	AL2020NGNG	60	86
2021Aug10	2021	NC	LC21HEER	21	3
2021Aug10	2021	NC	LC21HEER	21	3
2021Aug11	2021	NC	LC21MENC	18	11
2021Aug11	2021	NC	LC21MENC	18	11
2021Aug2	2021	AL	GW21AUMOT	18	6
2021Aug5	2021	GA	LB21CLWA	7	3
2021Aug7	2021	GA	LB21CLLS	72	1
2021Aug7	2021	GA	LB21CLLS	72	1
2021Aug7	2021	GA	LB21CLLS	72	1
2021Aug7	2021	GA	LB21CLLS	72	1
2021Aug7	2021	GA	LB21CLLS	47	3
2021Aug7	2021	GA	LB21CLLS	47	3
2021Aug7	2021	GA	LB21CLLS	47	3
2021Mar16	2021	LA	FR21BRRA	34	0
2021Mar16	2021	LA	FR21BRRA	34	0
2021Mar16	2021	LA	FR21BRRA	34	0
2021Mar16	2021	LA	FR21BRRA	34	0
2021Mar16	2021	LA	FR21BRRA	34	0
2021Oct1	2021	MN	SW21BUSW	3	9

e collector so those performing the genotyping were blinded to the amitraz re
the Apivar® efficacy test, and therefore amitraz-susceptible, while those that
ozygous resistant, and H indicating heterozygotes
pe (R)

Bees[12]	Total Varroa[13]	% Efficacy[14]	% Infestation[15]
290	65	80	22
290	65	80	22
390	26	58	7
293	24	88	8
374	24	75	6
374	24	75	6
374	24	75	6
341	35	91	10
275	5	20	2
303	10	70	3
303	10	70	3
502	12	25	2
350	68	57	19
290	65	80	22
290	65	80	22
290	65	80	22
372	140	86	38
371	54	78	15
371	54	78	15
371	54	78	15
371	54	78	15
372	140	86	38
372	140	86	38
372	140	86	38
340	22	82	6
340	22	82	6
340	22	82	6
432	81	47	19
432	81	47	19
432	81	47	19
512	15	7	3
512	15	7	3
512	15	7	3
512	15	7	3
390	26	58	7
390	26	58	7
390	26	58	7
390	26	58	7
342	87	43	25
342	87	43	25
342	87	43	25
342	87	43	25
342	87	43	25

432	146	41	34
432	146	41	34
432	146	41	34
432	146	41	34
293	24	88	8
293	24	88	8
347	29	62	8
347	29	62	8
347	29	62	8
347	29	62	8
310	15	67	5
310	15	67	5
310	15	67	5
310	15	67	5
310	15	67	5
310	15	67	5
374	24	75	6
374	24	75	6
341	35	91	10
341	35	91	10
341	35	91	10
341	35	91	10
341	35	91	10
275	5	20	2
275	5	20	2
303	10	30	3
303	10	30	3
303	10	30	3
303	10	30	3
303	10	30	3
303	10	30	3
303	10	70	3
303	10	70	3
303	10	70	3
332	73	99	22
291	50	94	17
291	50	94	17
291	50	94	17
447	96	38	21
447	96	38	21
447	96	38	21
447	96	38	21
447	96	38	21
502	12	25	2

502	12	25	2
502	12	25	2
502	12	25	2
350	68	57	19
350	68	57	19
350	68	57	19
350	68	57	19
350	68	57	19
290	65	80	22
371	54	78	15
371	54	78	15
340	22	82	6
340	22	82	6
340	22	82	6
432	81	47	19
432	81	47	19
432	81	47	19
390	26	58	7
432	146	41	34
293	24	88	8
293	24	88	8
347	29	62	8
347	29	62	8
374	24	75	6
303	10	70	3
332	73	99	22
332	73	99	22
332	73	99	22
332	73	99	22
291	50	94	17
291	50	94	17
291	50	94	17
372	34	100	9
372	34	100	9
372	34	100	9
372	34	100	9
372	34	100	9
502	12	25	2

sistance status

were collected from the wash were amitraz-resistant. Therefore, amitraz resistar

nt Varroa may be found in samples with high Apivar® efficacy, and vice-versa.