Control of *Varroa destructor* development in Africanized *Apis mellifera* honeybees using Aluen Cap (Oxalic Acid formulation)

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Abstract

Aluen Cap is an oxalic acid (OA) formulation ~~that Aluen Cap is an oxalic acid (OA) formulation~~ that shows promise as a control method for *Varroa destructor*. This work explores the use and efficacy of Aluen Cap against *V. destructor* in Africanized honeybees in Veracruz Mexico, where capped brood is always present across the year and acaricide resistance was a problem. Twenty-four beehives were assessed over 42 days, a period of time associated with the liberation of OA from Aluen Cap. The acaricide formulation shows significantly higher mean efficacy (92.1 %) than control hives (36.5 %.). This test suggests the high value of the new oxalic acid formulation in

AHBs despite the high temperatures and presence of brood, avoiding the necessity of multiple applications of OA, hence simplifying ~~the~~ colony management.

Keywords: *Varroa destructor*, Mite, Africanized Honey Bees, Oxalic Acid, Aluen Cap.

Introduction

Varroa destructor (Acari: Varroidae), is an obligate ectoparasitic mite of the honey bee, *Apis mellifera* (Hymenoptera: Apidae), that feeds primarily on the fat tissue of adults, larvae, and even pupae (Ramsey et al. 2019). This parasite constitutes one of the most severe threats to beekeeping all around the globe (De Jong 1997; Oldroyd 1999; Sammataro et al. 2000). However, it seems that this damage varies with climate, bee~~’s~~ ecotype, and *V. destructor* haplotype (e.g. Carneiro et al. 2007). Bee~~’s~~ ecotype is linked with variation in features such as hygienic behaviour, the development time of bee brood, and grooming behaviour, which are all related to the levels of *V. destructor* virulence in *A. mellifera* (Locke 2016). In mites, the Korean haplotype is predominant in unbalanced host-parasite systems, whereas the Japan haplotype is apparently less virulent, as seen in some regions of Brazil (Carneriro et al. 2007). Without periodic treatment, most of the honeybee colonies in temperate climates parasitized by the Korean haplotype collapse within a 2–3 year period (Rosenkratz et al. 2010).

The misuse of synthetic acaricides has led to a decrease in mite susceptibility with respect to the specific compounds (Maggi et al. 2010a, 2011), with *Varroa* resistance being detected around the world (Sammataro et al. 2005). In Mexico, Africanized honeybees were detected for the first time in 1986 (e.g. Clarke et al. 2002), while the first *V. destructor* infestation was in 1992 (Rodríguez et al. 1992). Although honeybees in Mexico display different degrees of tolerance to *V. destructor*, Mexican beekeepers treat colonies with synthetic acaricides in order to improve honey yield. Nonetheless, resistance to amitraz and flumethrin was detected in Mexico, thus the serious global problem brought by the resistance phenomena is also affecting Mexican apiculture. In this direction, there is an increasing interest in alternative treatments in Mexico (e.g. Rodríguez-Dehaibes et al. 2005; Rodriguez-Dehaibes et al. 2011). Elsewhere, essential oils and organic acids, have been explored as natural acaricides (e.g. Eguaras et al. 2001, 2003; Maggi et al. 2010b; Ruffinengo et al. 2014). Oxalic acid (OA) generally exhibits high efficacy (>90 %) against *V. destructor* and lowers the risk of hive contamination (Gregorc and Planinc 2001, 2002; Nanetti et al. 2003; Marinelli et al. 2006; Rademacher and Harz 2006; Bacandritsos et al. 2007). Additionally, mites have never developed resistance to OA (Maggi et al. 2017). However, the method of OA application constrains its efficacy, reaching as low as 66% when brood is present (Charrière 1997; Rademacher and Harz 2006). Thus, the acaricidal power of OA is limited during long brood seasons typical of warm climates. In this scenario, Aluen Cap is an alternative OA formulation, whose application exhibits high levels of efficacy even when brood is present, and lacks residual contamination (Maggi et al. 2016). This formulation has never been tested on AHBs under tropical climates. In this context, this work explores the use and efficacy of an acaricide formulation based on oxalic acid (Aluen Cap) against *Varroa destructor* in Mexican AHBs, where capped brood was always present across the year; taking into consideration the usefulness and means of treating *V. destructor* in AHBs.

Materials and Methods

Location of the study. The apiary was situated in Tejería, Veracruz, Mexico, Latitude:19.173225 N and Longitude 96.21114 W. This area presents a humid tropical climate throughout the year, with a minimum average temperature of about 26°C and a maximum of about 38°C, and an average ~~of~~ precipitation of about 1500 mm. This study was conducted from June 29^{th} to August 30^{th} in 2016, coinciding with the warmer and more humid period of the year. The maximum average temperature value oscillates from 31 to 32°C, and the minimum average from 23 to 24°C. The average ~~of~~ precipitation during the rainy season oscillates between 214-293 mm per month (see https://smn.cna.gob.mx/es/).

Field trial. Twenty-four beehives were used to assess Aluen Cap efficacy. This product was made by Cooperativa de Trabajo Apícola Pampero Ltda and contains oxalic acid as the main acaricide molecule. Colonies were divided in 2 groups, a control group consisting of 5 colonies and a treated group of 19 colonies. The experiment ran for 42 days, a period of time associated with the OA liberation of Aluen Cap (Maggi et al. 2016). All colonies used during trials were previously equalized with respect to bee population, brood area, honey, and pollen stores (Table 2). Also, hive floors were standardized for collection of dead mites. The apiary was selected based on geographic isolation to avoid re-infestation phenomena (Allsopp 2006).

The composition and nature of Aluen Cap was described by Maggi et al. (2016). Briefly, this acaricide treatment consists in a U-shaped strip with a matrix mainly composed of cellulose (45 cm × 3 cm × 1.5 mm) which contains 10g of OA mixed with 20mL of glycerine. Each strip was placed on frames 2, 4, 6, and 8 of the brood chamber (Maggi et al. 2016, fig. 1). Fallen mites were counted daily. To standardize percentage of efficacy of Aluen Cap, after the day number 42, all strips were removed and colonies received flumethrin treatment according to the instructions of the manufacturer (registered trade name: Flumevar®, supplied by APILAB SA, Argentina). Flumethrin was chosen because no prior resistance had been detected in the apiary (personal communication Rodriguez-Dehaibes). The flumethrin was left for 40 days to ensure the death of all mites. Thus, mean efficacy of Aluen Cap was expressed as a percentage of dead mites killed by this product, considering as the 100% the dead mites of both treatments: ([number of dead mites during oxalic acid treatment]/[number of dead mites collected during the treatment with OA plus flumethrin])×100.

The cumulative mite fall after oxalic acid and flumethrin treatment was assumed to be 100 %. Mean efficacy obtained in different treatments was used to compare results. Then, the efficacy obtained was corrected taking into account the natural mite mortality according to the Abbott's formula (Abbott 1925; European working group CA3686, 2002), calculated as: ([C% = (Cs–Ts)/Cs]) where

Cs and Ts are the percent of surviving mites in control and treated hives respectively. The percentage of surviving mites was calculated as: 100 [If / (If + It)] where If = total number of mites at the bottom of the hive during the treatment period and It = the residual mites collected after the final treatments.

Population features considered. To standardize the colonies for the a~~e~~ssay (Table 2), we established the number of combs fully covered with adult bees, and brood areas either in open or sealed cells (Maggi et al. 2013; Negri et al. 2015; Maggi et al. 2016). Hence, the effect of the treatment on colony strength is expressed by~~throughout~~ the number of combs full of adult bees and brood at the start and at the end of the treatment. All colonies were monitored to guarantee regular queen behaviour. Efficacy was obtained during a period where the brood was~~is~~ present in high quantity. Data were analyzed comparing means or medians as was explained for the efficacy test.

Test of normal distribution was~~ always~~ assessed according to the Shapiro-Wilk index with 0,05 of significance and, as data was found to be not normally distributed, the Mann Whitney test was used for comparing means.

3. Results

3.1. *Efficacy*. (Table 1)~~ The efficacy for Aluen Cap was not normally distributed in the treated colonies~~. Aluen Cap mean efficacy was 92.1 %, with an Abbott corrected efficacy of 87.8%, which was much greater than control hives with just 36.5 % mortality (Mann Whitney, $U=0$, $Z=3.34$, $p=0.0008$).

Effect on colony strength. There were no significant differences in initial and final strength values between treated and control colonies either for adult or brood values (all Mann and Whitney $p>0.05$) (Table II). Aluen Cap treatment finished the period assessed with 7-9 combs full of adult bees and 3-4 of brood areas. Dead queens or bee brood were not detected during or after treatment.

Varroa population dynamics during treatment. Most mites died within the first 21 days in both the treatment and control hives,~~. However, this first 21 days indicates the greatest mites mortality under Aluen Cap treatment~~ but mortality was more evenly spread in the control. For example, the greatest number of fallen mites occurred within the first seven days of the treatment, but for only two of the five control hives (Figure 1; Table 1).

4. Discussion

Our test of efficacy showed a great effect of Aluen Cap against *Varroa destructor* (87.8%), proving the high value of the new oxalic acid formulation in AHBs under the conditions present in Veracruz (Table 1). ~~Treated colonies remained consistently strong after 42 days of treatment (Table 2), indicating that Aluen Cap helps to sustain colony strength during a *V. destructor* infestation due to a lack of negative effects of the acaricide formulation on bee populations.~~ As found in~~According to~~ Maggi et al. (2016), the majority of the fallen mites using Aluen Cap ~~had been registered~~occurred within the first 21 days ~~and our data supports that pattern (Figure 1~~(Table 1). Indeed, significant mortality caused by Aluen Cap occurred quickly in this study and continued to be effective for at least three weeks (Figure 1). Conversely, control hives exhibited lower and more spread mortality during the trial. However, colony strength before and after the trial was similar either for control or treated colonies (Table 2), perhaps *Varroa* had no impact on colony strength during the trial since to see any potential decay in colonies might require one or two years of monitoring (see Maggi et al. 2016). It is important to note that we estimated efficacy with a synergistic effect where regular mite mortality (probably due to the grooming behaviour of AHBs) is camouflaged by the killing activity of the treatment. Thus, the proportion of killed mites due to Aluen Cap is exhibited (Table 1) as a corrected mean efficacy according to Abbott's formula (European working group CA3686, 2002).

Oxalic acid has been widely used to treat colonies infested by *Varroa* (Charrière and Imdorf 2002; Marinelli et al. 2006). Different strategies of application and different formulations have been tested to improve the effectiveness of oxalic acid (e.g. Rademacher and Harz 2006). Before Aluen Cap, the treatment success depended on bee development, because the *Varroa* that were settled inside sealed cells avoid contact with the acaricide long enough to withstand the OA effect (Imdorf et al. 2003). Furthermore, climatic seasons affects the OA efficacy. In summer, the liquid OA efficacy is reduced due to an increase of product evaporation, enhancing the problem of the mites that were inside sealed brood cells during application (Eguaras et al. 2003). In this way, Aluen Cap maintains the OA concentration and availability inside the colony for 42 days after its application due to its glycerine matrix (Eguaras et al. 2003) increasing its ~~killing mites~~ efficacy, even for those that emerge from brood cells several days after the initial application. This avoids the necessity of multiple applications as was suggested by Rademacher and Harz (2006), simplifying the management of the colony.

No detrimental effect was registered on colony development, however, in 2 of our colonies, the AHBs had bitten the glycerine strip likely reducing its effect, and thus leading to some variable results (Table 1, A8, A9 colonies). Observing certain ecotypes of Africanized honeybees with high tolerance to *V. destructor* (e.g. Martin and Medina 2004; Carneiro et al. 2007; Calderón et al. 2010; Medina Flores et al. 2014) and the complex system among honeybee ecotypes, climate, and mites haplotype (Garrido et al. 2003; Martin and Medina 2004; Mondragón et al. 2005), further investigation should ~~be~~ focus~~ed~~ on the potential use of Aluen Cap in Africanized honeybees but regarding each ecotype across the continent, in order to develop regional strategies of care and management both in bee conservation and commercial activities.

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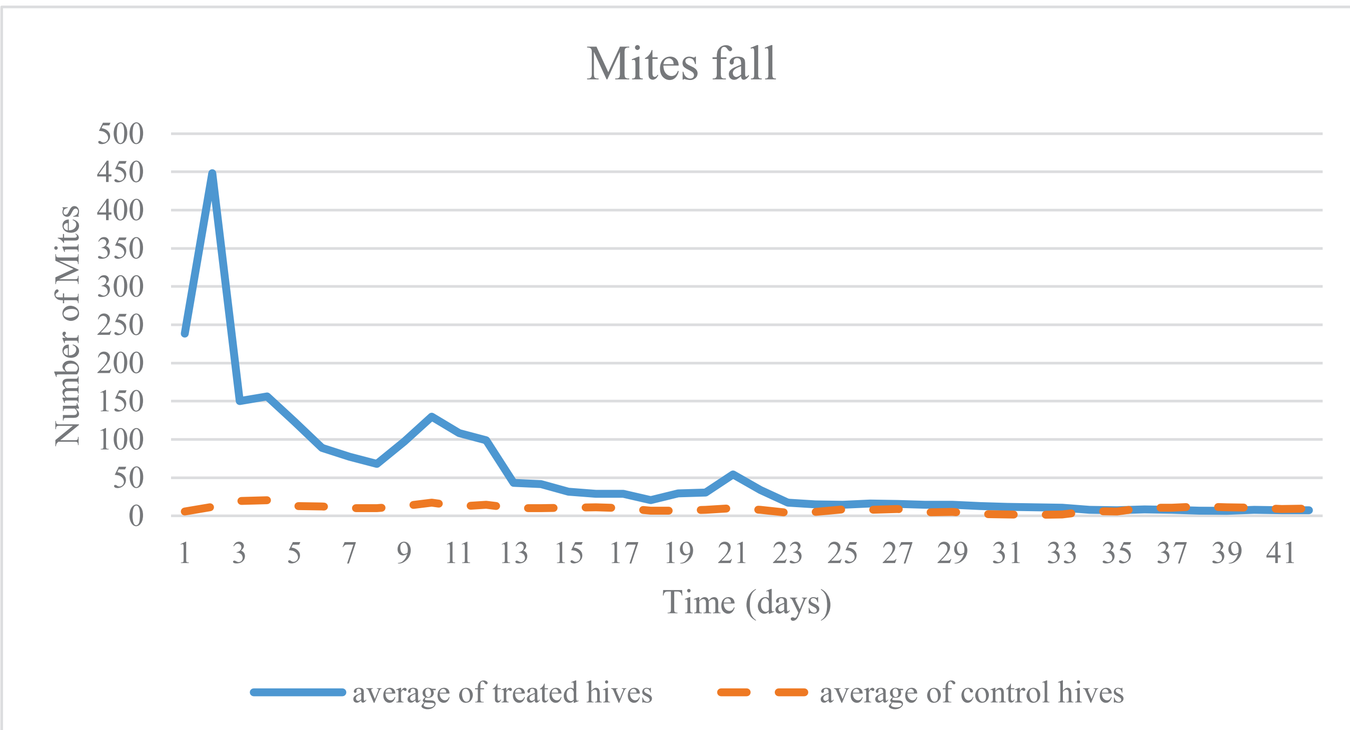
Table 1. Total number of fall<u>en</u>~~ing~~ mites in each colony ~~every 7 days per~~<u>after each week in Aluen Cap</u> treatment<u> and control hives</u>. Colony ~~M~~<u>m</u>ortality, ~~C~~<u>c</u>olony efficacy and its corrected value ~~all~~<u>are presented</u> as percentage values~~, for Veracruz summer trials~~.

Table 2. Colony population parameters<u> in control hives</u> before and after treatment<u> with Aluen Cap</u>~~s~~.

Figure 1. Average mortality of *Varroa* mites in the Aluen Cap treatment and control hives, as estimated by daily counts of fallen mites.

Figure 1. Mites fall over time during the trials. Bold line represent treated hives and discontinuous line control hives.



Trial	Treatment	Colony	Number of fallen mites at day						total	Number of fallen mites with shock treatment	Mortality (%)	Mean efficacy of formulation (%)	Mean efficacy corrected (%) (Abbott's formula)
			7	14	21	28	35	42					
summer trial Veracruz 2016	Oxalic acid formulation (Aulen Cap)	A1	2559	1740	439	109	113	86	5046	252	95,2	92,1	87,8
		A2	3008	2515	1299	842	601	109	8374	119	98,6		
		A3	550	314	131	86	64	42	1187	267	81,6		
		A4	1835	1269	272	202	120	102	3800	327	92,1		
		A5	2143	940	223	238	133	116	3793	223	94,4		
		A6	2524	1699	473	289	72	173	5230	429	92,4		
		A7	2010	661	245	249	143	304	3612	817	81,6		
		A8	250	249	90	82	59	64	794	428	65,0		
		A9	551	541	98	106	52	64	1412	650	68,5		
		A10	171	35	26	11	6	4	253	16	94,1		
		A11	897	129	66	4	0	1	1097	4	99,6		
		A12	345	146	70	7	1	2	571	1	99,8		
		A13	800	11	12	3	2	3	831	1	99,9		
		A14	3208	642	439	76	39	24	4428	48	98,9		
		A15	703	44	115	13	17	5	897	13	98,6		
		A16	661	28	67	10	9	7	782	12	98,5		
		A17	519	27	21	8	4	18	597	5	99,2		
		A18	960	104	132	38	5	12	1251	18	98,6		
		A19	670	38	13	31	10	2	764	55	93,3		
	Control	C1	75	29	26	25	23	23	201	499	28,7	36,5	
		C2	292	365	192	137	64	353	1403	1102	56,0		
		C3	49	24	22	12	3	4	114	239	32,3		
		C4	27	17	57	43	23	19	186	369	33,5		
		C5	18	2	19	15	5	7	66	141	31,9		

Table 2.

Number of combs	Treatment	Number of hives	Before treatment	After treatment
Adult bees	Aluen Cap	19	6,675±1	6,8±0,83
	Control	5	6,87±0,83	6,525±1,11
Brood areas	Aluen Cap	19	3,3375±0,25	3,4±0,42
	Control	5	3,435±0,42	3,26±0,56