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Article

A chemical and overwintering honey bee apiary field study comparing new and expired amitraz miticide

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SUMMARY

The ectoparasitic mite *Varroa destructor* is a significant driver of colony collapse in *Apis mellifera*, the European honey bee. *A. mellifera* is a keystone species that serves an essential role in pollination, particularly for large-scale agriculture. Apiculturists commonly combat *V. destructor* through synthetic acaricides such as amitraz, sold commercially as Apivar® strips. Issues with Apivar strips include their limited shelf life, costliness, and burden to the environment from disposal of unused strips. The purpose of our research was twofold: to determine the difference in levels of amitraz content in plastic Apivar strips from different years and to determine the overwintering success of amitraz-treated hives compared to hives treated with expired amitraz. We expected the amount of amitraz to be significantly reduced in older and expired strips. We extracted amitraz from Apivar strips using chloroform and quantified the concentration of amitraz using high performance liquid chromatography. We also expected that if expired amitraz strips were used to control for *V. destructor* then overwinter survivorship of the honey bee colonies would be significantly reduced. Our findings reveal that there was no statistical difference in the microgram (µg) amount of amitraz in Apivar strips and that expired Apivar does not impact overwintering survivorship. This demonstrates that the shelf life of amitraz is at least 36 months, in contrast to the 24-month expiration label on the product. Our data shows that using expired Apivar may be an economically viable and environmentally-friendly option for beekeepers.

INTRODUCTION

The western honey bee, *Apis mellifera,* is a species of flying insect that is essential for the global pollination of plants and crops. In the United States alone, it is estimated that honey bee colonies produce about 68 million kilograms of honey each year and generate over $400 million in revenue for their role in pollinating crops such as blueberries, apples, and almonds (1). Therefore it is an understatement that humans rely on honey bees for survival; in the absence of honey bees the agricultural industry would suffer reduced crop yields. This could pose an economic strain on mankind because with increasing global population comes the demand for more crops to feed that growing population.

Despite their agricultural importance, honey bee colony survival through the winter is on the decline. Previous studies characterize the overwintering state of a honey bee colony as a reversal of spring and summer time colony behavior; individual worker bees reduce foraging and brood rearing activities while forming a cluster that will help maintain enough warmth to sustain the colony through the winter cold (2). Overwintering occurs in regions that experience seasonal winters and ends as the weather warms and the bees start foraging for resources again (3). Both backyard and commercial beekeepers have recently reported lowered overwinter survival rates, mainly because the colonies in their apiary are too unhealthy to survive into the spring and appear to collapse overnight (4).

The primary cause for reduced overwintering success in the western honey bee is a result of a parasite known as the Varroa mite (*Varroa destructor*) (5). It is a type of bee mite that has geographically migrated from the Asian honey bee (*Apis cerana*) to the European honey bee (*A. mellifera*) (6). *V. destructor* is a specific type of parasitic mite that latches onto and feeds on bee body hemolymph and tissue (6). A reduction in body hemolymph and tissue prevents the honey bee from optimally distributing nutrients and relying on fat stores for energy during the overwintering process (7). *V. destructor* can also transmit deadly viruses to the bees, which further weaken the colony and contribute to overwintering colony collapse (8). Taken together, weakened colonies are not as likely to survive into the next year as a result of the physiological strains caused by Varroa mite infestations.

Despite having mites, beekeepers who want their honey bee hives to overwinter successfully pay close attention to the mite count in their hives throughout the year because the Varroa mite population in an infested honey bee colony differs by the season. Previous studies report the honey bee population is greatest in the fall to winter and that a high fall Varroa mite infestation makes it difficult for fall bees to care for additional emerging young and sustain the entire colony throughout the winter months (6). Locally, the Chester County Beekeepers Association (CCBA) in Chester County, PA advises that hives are at risk of mite damage and becoming unhealthy when the mite count increases to 3% (roughly 3 mites/100 honey bees). In addition, a four-year study involving over 1200 bee colonies from about 120 apiaries reported that higher mite counts of 25% were associated with 50% overwintering mortality (9). Therefore, commercial and backyard beekeepers alike often adopt integrated pest management (IPM) mite treatment strategies in response to increasing and dangerous levels of mite counts.

IPM is a sustainable method of controlling mite counts in bee hives due to its focus on proactive and preventative measures that take into account the current conditions of each individual hive, including the prevalence of damaging mites such as *V. destructor* (10). Experts indicate "an effective IPM program consists of…monitoring the pest population, performing a suite of preventative techniques, and applying a step-by-step treatment plan depending on need" (4). With IPM, bee hives that remain under the CCBA mite count threshold (<3%) may go untreated in the apiary since previous studies report that lowered mite levels are associated with overwintering success (11).

The use of IPM in an apiary is both economically and environmentally friendly; for example, beekeepers with hives that are below dangerous mite count thresholds may forego the expensive purchase of Apivar, a commonly used miticide in the United States for the chemical control of mite populations in a bee hive (4). This opens an opportunity for the beekeeper to spend money on alternative products that help improve the quality of an apiary. When there is a need to treat for mites and Apivar is purchased, it arrives as a package of plastic strips containing amitraz miticide. According to the number of hives affected as well as the mite counts within each hive in an apiary, portions of an opened package of Apivar strips may remain unused during the course of a treatment and will be disposed of by the beekeeper. This is likely a result of the reported decrease in response to amitraz as a result of its consistent application as a miticide (12). However, beekeepers that practice IPM and determine that they can forego treating their hives with Apivar strips will not risk damaging the environment with unused and discarded plastic that contain potentially harmful miticides. In addition, beekeepers who do not routinely practice IPM or are unaware of its benefits may use the miticide treatment out of convenience. This can lead to overuse of expensive pesticides that ultimately contribute to the increased pesticide resistance in *V. destructor* (13). As more resistant mite colonies are likely to strain the next generation(s) of bees, they may further contribute to the colony collapse of overwintering hives. As a result, beekeepers may not be able to sustain enough bee hives for the agricultural pollination of crops due to overwintering failure, contributing to more expensive produce for the economy.

As for the beekeepers who do use IPM and proceed to treat their hives with Apivar, this poses an environmental concern when leftover strips from a package are opened and expire. The discarded plastic Apivar strips could be extremely harmful to the environment; according to a publication, as plastic breaks down into microplastics, they pollute the water and disrupt the digestive tracts of many forms of aquatic life such as the seals, whales, and turtles (14). In addition to this disruption in aquatic ecosystems, the same publication also indicates that tiny bits of microplastics are also likely to reside in animals (birds, humans, etc.) as a result of water and food consumption and may eventually result in similar health complications (14). The amitraz drug embedded within the plastic Apivar strips also stands as a threat to the environment. Previous publications have indicated the pharmacologically active drug acts on the central nervous system (CNS) by suppressing the output of sympathetic nervous activity from the CNS (15). Case studies in human ingestion or skin absorption of amitraz have indicated that amitraz poisoning can be life-threatening and may require hospitalization in exposed individuals (16). Our study considers the implications of treating hives that require miticide intervention with the use of expired Apivar. If successful, then beekeepers should be able to reduce IPM treatment costs, the growing miticide resistance of *V. destructor*, and the potential environmental damage associated with discarded Apivar strips.

The manufacturer of Apivar states that sealed Apivar strips expire after 24 months from manufacture date and opened packages only last for 1–2 weeks due to the oxidation of amitraz (17). Once metabolized (amitraz metabolizes 7000x more in *V. destructor* than honey bees), amitraz binds to neuron receptors, causing behavioral changes that inhibit the mites from interacting with bees, thus preventing the reproduction of *V. destructor* (18). We investigated whether levels of amitraz in expired Apivar drop significantly to prevent an effective miticide treatment.

The objective of this study was twofold. First, we sought to determine whether Apivar retains amitraz after the manufacturer's expiration in comparison to non-expired Apivar. Second, we aimed to determine whether expired Apivar mite strips can be effectively used by backyard beekeepers as part of their ongoing IPM strategy in a small apiary and still obtain overwintering success in their honey bee colonies. We expected that expired miticide strips would contain significantly reduced concentrations of amitraz drug and would also decrease overwintering survivorship in honey bee colonies. Our findings reveal that expired Apivar appears to have a shelf life of an additional 12 months beyond the manufacturer's expiration label and that use of expired Apivar strips is not associated with the loss of overwintering honey bee hives that undergo IPM.

RESULTS

Chemical study: HPLC analysis of Apivar strips

We first quantified and compared the amount of amitraz in micrograms (µg) for the manufacturing dates of 12/2019 (expired), 10/2020, and 3/2021 to determine if there was a statistically significant decrease in the amount present in older Apivar strips. Amitraz was first extracted from Apivar strips using chloroform and then analyzed using high performance liquid chromatography (HPLC; **Figure 1**). The amount of Amitraz drug (ug) was then quantified and compared between new, old, and expired Apivar strips (**Table 1**). The expired strips (2019) had an average of 2.2 µg amitraz in 1 µL of sample, and the strips from 2020 and 2021 both had an average of 2.0 µg amitraz in 1 µL of sample. We performed one-way ANOVA statistical analysis, and results supported our null hypothesis that there was no statistically significant difference between the mean scores of amitraz concentrations from the three different years of manufacture ($p = 0.6539$). Alongside sample analysis was a control analysis for percent amitraz recovery (**Appendix**).

Overwintering honey bee colony field study

We determined mite counts for each hive using an alcohol wash in order to determine whether an above threshold mite count hive would receive Apivar treatment (**Figure 2A**) or serve

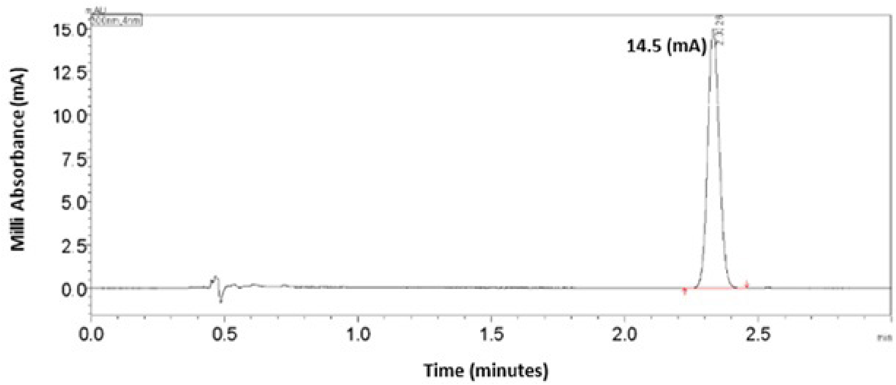

Figure 1: Amitraz detection using high performance liquid chromatography. The output of amitraz sample is detected at 2.34 minutes at a peak concentration measured in milli absorbance units (mA). The concentration of standard stock solution is calculated as area under the peak beginning and ending at the red marks on the horizontal line.

	HPLC Analysis (AUC)				Amitraz concentration (µg/µL)	
Strip	**Disc 1**	**Disc 2**	**Disc 3**	**Average**	**µg / µL**	**Average**
4/2021 a	55195	50198	50479	51957	2.2	2.0
4/2021 b	47571	48284	48540	48132	2.0	
4/2021 c	44411	45255	44913	44860	1.8	
10/20 a	41780	41755	41517	41684	2.0	2.0
10/20 b	49032	49106	49167	49102	2.3	
10/20 c	33195	33293	33128	33205	1.6	
12/19 a	41482	40325	41559	41122	1.9	2.2
12/19 b	46284	46552	46359	46398	2.2	
12/19 c	50356	50347	50471	50391	2.4	
Average amitraz concentration of 3 years (2019, 2020, 2021)						2.1

Table 1: HPLC analysis of amitraz samples from Apivar discs. Three different Apivar strips from the same year of manufacture were analyzed for each year for a total of nine disc samples. The interpreted area under the curve (AUC) values represent similar µg/mL amitraz concentration in each disc sample in each year. A one-way ANOVA analysis indicates that there is no statistically significant difference in concentration between disc samples from different strips of the same year and between newly manufactured and expired Apivar miticide strips.

as a control group (**Figure 2B**). We placed expired Apivar strips in half of the hives that qualified for mite treatment and placed recently manufactured (non-expired) Apivar strips in the other half of the hives that also qualified for mite treatment. We did not treat the control hive with Apivar as it maintained lowered threshold mite counts.

We recorded pre- and post-treatment mite counts for each

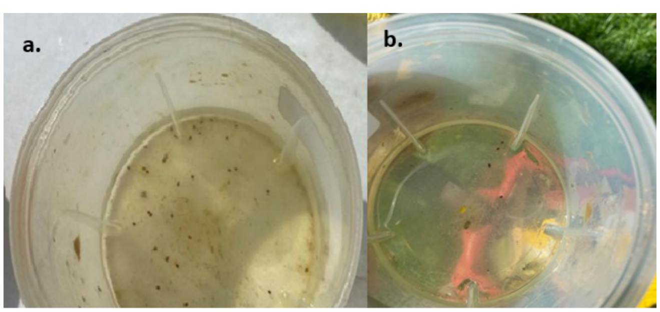

Figure 2: High vs. Low Mite Counts from two hives pre-miticide treatment. Mite count samples from two hives using an alcohol wash. Little Jerry Experimental Hive (a) with a high mite count and the Honeycomb Hive control (b) with a low mite count. Samples taken on September 11th, 2021.

of the five hives. Results of the field experiment indicated that treatment with expired Apivar miticide strips was not as effective in dropping the mite count compared to the treatment with recently manufactured Apivar (**Figure 3A**). There was a 2.5% increase in the average mite count in hives that received old Apivar treatment. The use of newly-manufactured Apivar appeared to be more effective as a miticide, since the average mite count in hives treated with new Apivar strips resulted in a 1.8% decrease in mite counts (**Figure 3B**).

We also monitored overwintering colony survival or collapse for each hive over the beekeeping year, ending on April 1st, 2022, which is the definitive segway into the spring beekeeping season for the region (**Table 2**). The observed efficacy of the different treatments showed that colonies treated with expired Apivar miticide strips overwintered successfully whereas one hive (Little Jerry) treated with new Apivar missed the overwintering cutoff date and died two weeks before April 1st. The visuals provided of the dead cluster of bees with their queen (**Figure 4A**) and the evidence of high mite infestation in the Little Jerry hive provided visual evidence of a collapsed honey bee colony (**Figure 4B**).

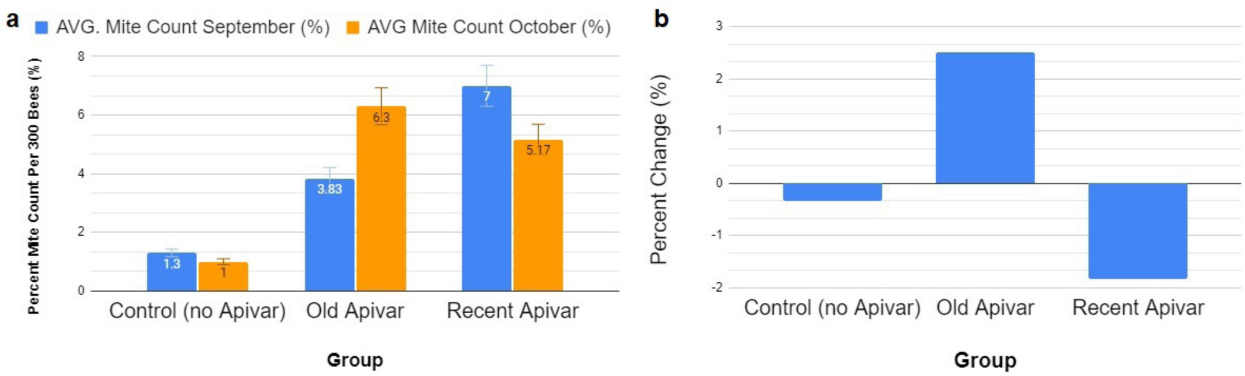

Figure 3: Average percentage mite counts and mite count percent change. Pre (September) and post (October) mite treatment results from control, old (expired) Apivar, and recently manufactured Apivar. The error bars represent a +/-10% range based on each group's average mite counts (a). Percent change in mite count post Apivar miticide treatment. Mite counts taken 49 days post treatment in hive groups and the percent decrease/increase in mite counts is reflected by each bar graph (b).

Name of Bee Hive	Initial Mite Count (%)	Overwinter Survival	Type of Apivar Strips
Honeycomb (Control)	1.33	+	None
Tom	3.33	+	New
Little Jerry	10.67	-	New
Black Beauty	4.33	+	Expired
Little Lemon	3.33	+	Expired

Table 2: 2021–2022 Overwintering honey bee hive survival (+) and collapse (-). Initial mite count taken on September 11th, 2021, and hive survival determined by a hive inspection on April 1st, 2022. Survival or collapse of each hive was determined through a hive inspection with a positive identification of the queen and the presence of hive workers, drones, and brood of all stages.

DISCUSSION

The purpose of this study was to determine whether expired Apivar mite strips can be effectively used by backyard beekeepers as part of their ongoing IPM strategy in a small apiary and still obtain overwintering success in their honey bee colonies. We compared expired versus newly-manufactured Apivar miticide strips for drug presence and potency. Ultimately, we wanted to determine if beekeepers can assume desired levels of amitraz concentrations in expired Apivar strips and then use them as an effective miticide for IPM in their apiaries. In the chemical study of this experiment, we found that the concentrations of amitraz in Apivar strips did not decrease multiple years after the expiration date. After the expiration date, which is 24 months after the manufacture date, our samples (24 months and 36 months past their expiration dates) still had similar amitraz concentrations to new strips. The control analysis run alongside these results accounted for discrepancy between known amitraz concentration and amitraz concentration recovered after being subjected to extraction treatment (**Table 3**). Our data suggests that there is not a significant difference in efficacy between new or expired Apivar strips observed in this study.

For the honey bee field study, we measured both average fall mite counts pre- (September 2021) and post-treatment (October 2021) with Apivar (**Figure 3A**). The control group, which maintained consistently low mite counts (<3%), served to demonstrate the trend in mite counts in hives with below threshold mite counts and which did not qualify to receive a mite treatment. As the control hive remained below mite count threshold without Apivar, it supports the importance for backyard beekeepers to monitor mite counts and avoid potentially unnecessarily treatment of hives that fall below threshold in mite count. The remaining four hives had mite counts >3% and were selected for either recent or expired Apivar treatment. The two hives treated with expired Apivar strips displayed an average increase of 2.5% in mite count during the course of the Apivar treatment window. The two hives that received newly manufactured Apivar strips had an average decrease of 1.8% in mite count (**Figure 3B**). Although the expired pesticide did not lower mite counts, it most likely impeded a more rapid increase in mite counts that would render the hive less healthy as the adult honey bees enter the winter season. As a result, the honey bees present could more

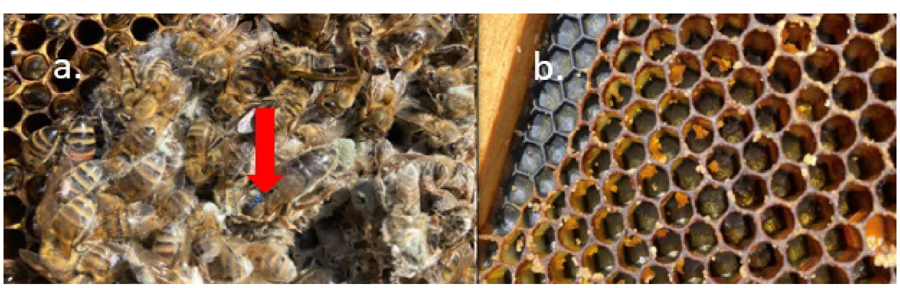

Figure 4: Colony collapse with presence of mite damage in the Little Jerry Hive. Colony collapse of clustered worker bee attendants and their queen (marked by a red arrow) in the Little Jerry Hive (a) as well as evidence of high mite infestation (b) in March, 2022 when the hive collapsed. The Little Jerry Hive had the highest pre-treatment mite count and was treated with newly manufactured Apivar.

	Concentration ug/uL	Area 1	Area 2	Area 3	Average Area	Concentration Recovered (ug/uL)	Percent Recovered (%)
Control 1	6.9	132674	132218	127098	130663	6.1	88.4
Control 2	7.1	134550	135457	135194	135067	6.3	88.7
Average	7					6.2	88.6

Table 3: Control analysis for percent amitraz recovery in samples exposed to the extraction treatment with known concentrations of amitraz. This accounted for discrepancy between known amitraz concentration and amitraz concentration recovered after being subjected to extraction treatment.

effectively sustain the health of the hive by practicing standard hygienic behavior, which is the natural grabbing and biting (i.e., killing) of the mites. It is possible that the expired Apivar strips do not release amitraz as effectively as newly manufactured Apivar but impeding the rapid increase in the hive's overall mite count appears to be a factor in overwintering survivorship. Both the control hive and the expired Apivar-treated hives overwintered successfully (**Table 2**). The only overwintering colony collapse that occurred was in the Little Jerry hive which we treated with new Apivar strips. We conclude that although the expired Apivar is not as effective as newly manufactured Apivar strips for miticide treatment, the use of old Apivar was not associated with overwintering death of honey bee colonies.

A question that remains is what ultimately influenced the colony collapse in the hive that was treated with the new Apivar strips in the Fall of 2021 ("Little Jerry"). This hive was alive as of mid-March 2022 and only missed the overwintering cutoff by two weeks. Perhaps the answer can be found by considering the level of mite infestation in the early Fall. We found that the Little Jerry hive had the highest infestation of mites in September (before Apivar treatment), with a mite count well above the 3% threshold (10.5%) which contributed to the higher average percent mite count of 7% for the recent Apivar treatment group (**Figure 3A**). The visual confirmation of the Little Jerry hive's high mite count pre-treatment is also shown alongside the Honeycomb control hive to reflect the early detection of mite infestation (**Figure 2**). The collapsed Little Jerry hive shows the dead queen, with a small cluster of dead worker bees on a frame that did not contain any honey or pollen resources (**Figure 4A**). There was also evidence of capped worker brood, which indicates that the hive was recently thriving before the April 1st overwintering cutoff date. An area of concern was the evidence of a significant mite infestation in the form of mite crystallized feces throughout the brood chamber comb of the Little Jerry hive (**Figure 4B**). Finally, the month of March in West Chester, PA presented itself with a series of warm vs. cold snaps in temperature. Since previous studies have demonstrated that mite infestations result in honey bee colony health problems that impede overwintering success (e.g. viruses and lowered fatty tissue reserves), the Little Jerry hive was most likely significantly weakened earlier than the other hives in the same apiary (5, 7, 8). We believe a combination of using up the stored honey and pollen resources to sustain the mite-weakened hive along with a mercurial weather pattern of warm and freezing temperatures is likely what caused this hive to collapse before April 1st, 2022.

A factor that served as a limitation in this work was the number of colonies sampled for the field study. A large sampling of colonies is ideal for data collection and analysis; however, the apiary that was used only had a total of five hives. We recognize that it would not be environmentally sustainable to overcrowd an apiary with a large number of hives for the sake of generating more data. The overcrowding would result in competition for resources (nectar, pollen, etc.) between honey bee colonies, as well as between honey bees and the native pollinators in the West Chester, PA area. This competition could strain the hives and influence overwintering colony collapse. We suggest a partnership with additional apiaries for future studies with beekeepers who are willing to subject a portion of their hives to both new and expired Apivar strips. Ideally, all apiaries would be situated in the same area (not the same apiary) so that they are exposed to similar topography and weather patterns. For the chemical study, it would be useful to analyze expired Apivar strips that have expired further away from the 24-month manufacturer's expiration for amitraz concentrations. In doing so, we can determine whether the Apivar strips actually expire. For the field study, we recommend separating the new Apivar and expired Apivar experimental groups by ensuring that each group enters the study with similar ranges of mite counts instead of randomly assigning treatment type to hives. By controlling this variable, future studies can consider the effect of various degrees of mite counts going into their Fall Apivar treatment and whether hives with extreme mite counts before treatment are also significantly more at risk of collapse.

This experiment shows that the use of expired Apivar strips can be an economically and environmentally sustainable option for beekeepers as part of their IPM program for their apiaries. First, a 10-strip pack of Apivar costs about $37 USD and can provide a several-week treatment for roughly two commonly used bee hives in North America known as Langstroth hives. Any unopened, sealed Apivar packs during the time of treatment are likely to exceed the use-by dates for the next round of treatment since it may be up to one year until the beekeeper revisits this medication again as part of their IPM. Our study provides experimental evidence that beekeepers may be able to use expired Apivar as an effective miticide treatment with the potential savings of up to $75–185 USD for a small apiary of 4–10 hives. Second, environmental problems, such as agricultural run-off, are a result of pesticide overuse. Beekeepers that consider sealed, expired Apivar as part of their IPM are less likely to discard old Apivar as waste, reducing the risk of amitraz leaching into the ground and contributing to water source contamination and environmental dead zones.

We believe that this experiment accurately represents how some backyard beekeepers use IPM to determine the course of mite treatment for individual hives located in the same apiary. We also believe that this experiment, though small in field sample size, serves as a starting point to further the knowledge of using miticides, like Apivar, in a more environmentally and economically sustainable way as part of

IPM. Although additional studies involving levels of amitraz in expired Apivar as well as their use in IPM would enhance the understanding of overwintering success in honey bee colonies, one conclusion that can be drawn from this experiment is that expired Apivar use with early fall mite counts (around or slightly above threshold) is not associated with colony collapse and may be a treatment option for beekeepers.

MATERIALS AND METHODS

Chemical extraction of amitraz from Apivar strips

Disc-shaped portions were punched out from Apivar strips (Veto-Pharma batch #210167 manufactured date 03/08/2021, batch #201487 manufactured date 10/16/2020, and batch #191809 manufactured date 12/16/2019), using a hole-puncher and massed in a glass, screw-capped vial (OHAUS Pioneer analytical balance (#PA214). A total of 10 mL of chloroform (Flinn Lot #483505) was added and each vial was placed in hot water at 70°C for 20 minutes to allow the amitraz to dissolve off of the plastic discs. After cooling to room temperature, the solutions were centrifuged at 3300 RPM for 60 seconds (LW Scientific Model E8 3300 RPM). A total of 100 µL supernatant (20–100 µL micropipette Bio Rad) was placed in an uncapped clean glass vial and evaporated to dryness overnight in a fume hood. A total of 10 ml of HPLC-grade acetonitrile (Chemsavers Lot #ACTNR111121) were added to the (invisible) residue (19).

Analysis of amitraz extract using HPLC

A Shimadzu Nexera X2 UHPLC with a Photodiode Array (PDA) (LabSolutions software Columbia, MD) detector set at 300 nm was used. The column was a HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm (Advanced Materials Technology, Wilmington, DE). The column oven was set to 35 °C. The amitraz extract (1 µL) was injected in 100% acetonitrile. The calibration curves were calculated between area under the peak and concentration using the external standard method. A one-way ANOVA statistical analysis was used to compare the mean concentrations of the three amitraz disk samples within each individual year and the overall mean between the three years. Degrees of freedom between the three years was two, and within each of the individual years it was six.

External standard preparation of analytical amitraz

A standard stock solution (1 mg/mL) of amitraz (Sigma-Aldrich Batch BCCB5043) was prepared in 13.4 mL of acetonitrile. Working standard solutions (1, 2, 5, 10, 50 µg/mL) were prepared by dilution with acetonitrile. The mobile phase used for HPLC was 20:80 water: acetonitrile. Amitraz content reported on the Apivar label was 500 mg per strip, equal to 3.33% (w/w) considering each strip was 15 g.

IPM practices

In this experiment, the IPM for *V. destructor* used in the fall was a combination of Apivar® treatment (early Fall) followed by oxalic acid vapor (late Fall) using a Duracell Jump Starter 900 Portable 12V Car Battery Charger to power the Varrox 12V Oxalic Acid Vaporizer and a ¼ plastic teaspoon to deliver 1 g teaspoon of oxalic acid wood bleach from a 16oz. container (Mann Lake Ltd.). An ice bucket of tap water was used to cool the wand between hive treatments and hand towels were used to keep the oxalic acid vapor inside of the hive throughout the treatment duration. Decision to treat with Apivar in early Fall rested on a hive falling above the 3% mite threshold level.

In order to investigate the efficacy of amitraz, the drug found in Apivar miticide strips, we conducted a controlled field experiment with two experimental groups and one control. We provided five established hives located at Smitty's Honeybees apiary West Chester, PA, with the same IPM management strategy throughout the beekeeping year (April 1, 2021–March 31, 2022). Treatments were randomly assigned to each hive. One experimental group of hives (Little Jerry and Tom) received newly manufactured Apivar 10pk Strips (Batch no. 200849 MFG. Date 1-24-2020) as their miticide treatment and the other experimental group of hives (Black Beauty and Little Lemon) received expired Apivar 10pk Strips (Batch no. 14115 MFG. Date 7-29-2014) as their miticide treatment. A control group (Honeycomb Hive) that met mite threshold levels did not receive treatment with Apivar strips. The Apivar treatment for all hives occurred on September 11th, 2021, and remained in the hive for the manufacturer-recommended minimum of 42 days. After 49 days, the Apivar strips were removed from the hive and mite counts for each colony were recorded. The average percent mite counts before and after treatment were quantified and overwintering colony survival to April 1st, 2022, were recorded. No further mite treatment was administered during the month of October.

After obtaining mite counts on 300 honey bees per hive before treatment (September 2021) and after treatment (October 2021), these results were then quantified as a percent. The control and each experimental group's percent mite counts were averaged together before and also after Apivar treatment.

The same oxalic acid vaporization treatments on all hives were then performed on November 27th, 2021, as part of IPM. Specifically, 2 g (½ tsp) oxalic acid crystals were placed on the reaction plate of the vaporizer. This plate rests on the oxalic acid wand that was then inserted into the bottom of the hive. After this, three towels were used to plug up the main entrance of the hive. The oxalic acid wand was connected to a 12 V car battery and allowed to vaporize for five minutes. Then the car battery was turned off and the wand remained in the hive for another five minutes. Finally, the wand was removed, but the hand towels were immediately replaced along the hive entrance for an additional ten minutes for vapor permeation of the hive. An alcohol wash was not performed after this treatment due to the low temperatures that could affect the brood chamber and compromise colony survival.

Mite level monitoring

During routine hive inspections in the spring, summer, and fall months, one queenless frame of mostly worker eggs/larvae and some capped brood was selected for obtaining a mite count. The frame of brood with the adult bees was firmly shaken 2–3 times into a plastic tub. From there, ½ cup of bees (~300 bees) was scooped from the tub and then placed into a Varroa Easy Check apparatus containing 91% isopropyl alcohol. The bees were gently hand-mixed in the alcohol-filled apparatus for 45 seconds. The apparatus sat for another 15 seconds to allow for the dislodged mites to drop. A mite count was recorded from a picture taken from the bottom of the apparatus where the mites dropped. The percentage of mites per 300 bees was then determined to be above or below acceptable the 3% mite level threshold. It is possible that more than one mite can latch onto a bee at a time as well as dislodge from the bee during the alcohol check; therefore, the percent of mites on bees using the alcohol check is an estimate of mite count within an entire 300 bee sample and not reflective of the exact number of mites on any given bee found within that sample.

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REFERENCES

1. Stokstad, E. "Scientists breed honey bees to fight deadly parasite." *Science*. 7 April 2022. doi:10.1126/science.abq4348. Accessed 21 Apr. 2022.
2. Doke, M. *et al*. "Overwintering Honey Bees: Biology and Management". *Current Opinion in Insect Science*, vol. 10, June 2015, pp. 185-193. doi:10.1016/j.cois.2015.05.014.
3. Maes, P., *et al*. "Overwintering Honey Bee Colonies: Effect of Worker Age and Climate on the Hindgut Microbiota". *Insects*, vol. 12, no. 3, March 2021, doi:10.3390/insects12030224.
4. Jack, C. and Ellis, J. "Integrated Pest Management Control of *Varroa* destructor (Acari, Varroidae), the Most Damaging Pest of *Apis mellifera* Colonies." *Journal of Insect Science,* vol. 21, no. 5, September 2021. doi:10.1093/jisesa/ieab058.
5. Guzmán-Novoa. E. *et al*. "Varroa Destructor is the Main Culprit for the Death and Reduced Populations of Overwintered Honey Bee (*Apis mellifera*) Colonies in Ontario, Canada." *Apidologie*, vol. 41, July 2010, pp. 443-450. doi:10.1051/apido/2009076.
6. Bloom, E. *et al*. "Responding to the US National Pollinator Plan: A Case Study in Michigan." *Frontiers in Ecology and the Environment.* vol. 20, no. 2, March 2022, pp.84-92. doi:10.1002/fee.2430.
7. Sarwar, M. (2016). "Mites- The Tiny Killers to Push Honey bee Colonies into Collapse and Integrated Pest Management." *International Journal for Research in Applied and Natural Science*, vol.*2*, no. 7, pp. 21–30. doi:10.53555/ans.v2i7.90.
8. Noël, Amélie, el al. "Varroa destructor: how does it harm Apis mellifera honey bees and what can be done about it?" *Emerging Topics in Life Sciences*, vol. 4, no. 1, June 2020, pp. 45-57. doi:10.1042/ETLS20190125.
9. Genersch, E. *et al*. "The German Bee Monitoring Project: A Long-term Study to Understand the Periodically High Winter Losses of Honey Bee Colonies". *Apidologie*, vol. 41, no. 3, May-June 2010, pp.332-352. doi:10.1051/apido/2010014.
10. Charriére, Jean-Daniel, *et al*. "The Removal of Capped Drone Brood: An Effective Means of Reducing the Infestation of Varroa in Honey Bee Colonies." *Bee World*, Jan. 2003, pp. 117-24, doi:10.1080/0005772X.2003.11099587.
11. Steinmann, N. *et al*. "Overwintering is Associated with Reduced Expression of Immune Genes and Higher Susceptibility to Virus Infection in Honey Bees". *PLoS ONE*, vol. 10, no. 6, June 2015, doi:10.1371/journal.pone.0129956.
12. Adjlane, N., *et al*. "Evaluation of the Efficacy of Different Acaricides Against *Varroa Destructor* on *Apis Mellifera Intermissa* in Algeria." *Acarina,* vol. 21, no. 2, 2013, pp. 141-146.
13. Cameron, J., *et al*. "Evaluating the Efficacy of Oxalic Acid Vaporization and Brood Interruption in Controlling the Honey Bee Pest Varroa destructor (Acari: Varroidae)." *Journal of Economic Entomology*, vol. 113, no. 2, Apr. 2020, pp. 582-88, doi:10.1093/jee/toz358.
14. Natt, N. "Plastics, the Death of Sea Turtles and American Businessmen: The Value in American Business in Adhering to and International Standard of Sustainability." *Journal of International Business and Law*, Vol. 20, Iss 1, Article 7, 6 June 2020, p. 4
15. Veale D.J., C.A. Wium, and G.J. Muller, "Amitraz poisoning in South Africa: A Two-Year Survey (2008-2009)." *Clinical Toxicology (Phila)*, vol. 49, January 2011, pp.40-44. doi:10.3109/15563650.2010.542159.
16. Mwita, C. "Amitraz Poisoning in a Patient from Rural Africa." *Journal of Case Reports*, vol. 2, July 2012. pp.39-41. doi:10.17659/01.2012.0014.
17. Posada-Florez, F. *et al.* "Deformed wing virus type A, a major honey bee pathogen, is vectored by the mite Varroa destructor in a non-propagative manner." *Scientific Reports*, vol. 9, no. 12445, August 2019. doi:10.1038/s41598-019-47447-3.
18. Staveley, Jane *et al*. "A Causal Analysis of Observed Declines in Managed Honey Bees (*Apis mellifera*). Human and ecological risk assessment". *Human and Ecological Risk Assessment.* vol. 20, no. 2, November 2013, pp. 566-591. doi:10.1080/10807039.2013.831263.
19. de Villers, Melgardt, et. al. "The Relationship Between Surface Adsorption and the Hydrolysis of Amitraz in Anionic Surfactant Solutions". *Journal of Environmental Science and Health*, vo. 40, February 2007, pp. 2 – 5. doi:10.1081/PFC-200045540.

Appendix

Concentration of Stock = 1mg/mL				
Micrograms on column	**Area 1**	**Area 2**	**Area 3**	**Average Area**
1	20500	20635	20649	20595
2	38271	37706	37981	37986
5	106102	105601	105885	105863
10	219538	218770	219615	219308
50	1068323	1067176	1067411	1067637

Table S1. HPLC analysis of internal standard for calibration curve.