FINAL REPORT*:* A pilot study to compare the efficacy of oxalic acid sublimation devices to control *Varroa* mite in Alberta

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Introduction:
Control of the *Varroa* mite is a critical challenge to the Alberta beekeeping industry, in part due to the limited availability of effective treatment options. Apivar® (Amitraz) is an effective miticide against *Varroa*, however, resistance has been reported in some regions, and alternatives are required for the continued and sustainable treatment of *Varroa*-infested honey bee colonies. In addition, many beekeepers prefer to manage their colonies without the use of "hard" chemicals such as amitraz, coumaphos, or fluvalinate, instead relying on cultural controls and/or organic acids.

Oxalic acid (OA) is one such option, and has proven to be effective against *Varroa* mites. The efficacy ranges from 90% to 98% in controlling *Varroa* mites when applied once to a broodless honey bee colony (Charrière et al. 2001; Nanetti et al. 2003; Radedzki et al. 2001). Oxalic acid dihydrate is currently registered in Canada as a *Varroa* mite control product (Registration numbers 29575, 29576 under the authority of the Pest Control Products Act and Regulations). There are three main methods of application: the drip/trickle method, the sublimation / evaporation method, and the spray method; however, the spray method is not registered in Canada. Rademacher and Harz (2006) reviewed OA applications against the *Varroa* mite and showed that geographic area, climate, adult bee and brood population, *Varroa* mite infestation level, application method, and beekeeping practices all affect the efficacy of OA. It is therefore essential that currently available methods of OA application are assessed for efficacy under conditions in Alberta and recommendations for use are developed. While broader studies on mite control using OA are required, we have carried out a pilot study on the vaporization method to guide future research. We intend to use the results of this study and a literature review to design a larger study of OA mite control methods in Alberta.

Objectives:
The objective of this pilot study was to compare different models of OA vaporizers on *Varroa*-infested colonies in late fall (November–December 2018) in Alberta. In this study, changes in *Varroa* mite infestation level, OA efficiency, negative effects of treatments on the honey bee, and the time spent per treatment were assessed.

Methods:
This project was designed to address effectiveness of OA vaporizers against mites and bee health in Alberta. The pilot study took place from October 30th to November 29th, 2018 at the Crop Diversification Centre North, Edmonton Alberta. The colonies (n= 21) in the experiment had average 8.23% mite infestation level with minimal brood. Queen status and bee population

were evaluated in all colonies pre- and post-trial. The bee population area in all experimental colonies was visually inspected by estimating the percentage of bees covering each side of the frames. Colonies were randomly assigned into three treatments with seven replicates for each treatment. Sticky boards (30 X 43 cm) were placed under experimental colonies 3 days before treatment to determine the natural mite drop. Colonies were treated four times in total, November 2, 9, 15 and 22, 2018 (seven days between treatment applications) using the ProVap 110 (sideliner/Commercial vaporizer, OxoVap LLC, SC, USA), Varroa Blaster (designed and made by Terry Greidanus), and the Varroa Cannon (Varroa Cannon, USA). Each device was used according to the manufacturer's protocol. Additional equipment needed to operate the Varroa Blaster and Varroa Cannon include an air compressor (minimum 1 horsepower), a generator (minimum 2000 watts, continuous), a compression hose (minimum ¼ inch), and an extension cord (Varroa Cannon, 2017). The ProVap only requires a generator and extension cord to operate. During treatment, operators used the Personal Protective Equipment (respiratory mask, nitrile gloves, earplugs, Tyvek coverall and safety shoes).

To monitor daily mite mortality during the pilot study, a piece of sticky board (30X43 cm) was placed in the tray of the screened bottom board of each colony to collect dying mites that fell through the screen. Sticky traps were replaced at days 1, 3, 5 and 7 days post-treatment. The dead mites on the sticky boards were counted and daily mite mortality was calculated in each test colony. Temperature of the OA clouds and nuzzle of devices were measured at the time of application using visual infrared thermometer (Flir TG165).

Apivar was applied on November 29, 2018 as a finishing treatment to kill the remaining mites in the colonies, and determine the efficacy of the OA treatment. A sample of 250-300 worker bees were taken from each colony before and after the experiment, and before each application of oxalic acid to evaluate the phoretic mite population. The bee samples, which were stored in 70% ethanol, were shaken in an orbital shaker (300 rpm) for 15 minutes and washed to determine the mite level in samples.

Results:

In this pilot trial three vaporizers were tested:

1-ProVap 110 (sideliner/Commercial vaporizer, OxoVap LLC, SC, USA):

The vaporizer took 2-5 min time to reach to the setting temperature (230 °C / 446 °F) depending on the ambient temperature. One gram of OA crystal (Oxalic acid dehydrate 99.6%, Medivet, AB, Canada) was loaded in Teflon lid using a plastic spoon (2.5 ml) and then the device was inserted in the hive entrance. Colonies were treated for 20s until there was no longer any OA vapor coming out of the machine (Fig. 1). The temperature of device body and OA cloud was 62.5 °C and 16.1 °C, respectively (Fig. 2)

2-Varroa Blaster (designed and made by Terry Greidanus):

Varroa blaster took 5-15 min to reach to the setting temperature (218 °C / 425 °F) depending on the ambient temperature. Approximately 10-12 grams of OA crystal was loaded in vaporizer using a baster and the treatment colonies were exposed to the OA cloud for 10 s (Fig. 3).

3-Varroa Cannon (Varroa cannon, USA)

This vaporizer took 5-15 min to reach to the setting temperature (343 °C / 650 °F) depending on the ambient temperature. Approximately 10-12 grams of OA crystal was loaded in vaporizer using a baster and then OA vapor was applied to treatment colonies for 10 s (Fig. 4). The temperature of device and OA cloud was 192°C and 10.5 °C, respectively (Fig. 2).

Daily mite mortality was significantly different among different time periods, with the highest mite mortality observed for the time points following treatment ($F= 3.31$; $df= 41, 210$; $p< 0.0001$) (Fig. 5). However, there was no difference among treatments in cumulative mite mortality at the end of the experiment ($F= 1.67$; $df= 2, 17$; $p= 0.2182$) (Fig. 6). The cumulative reduction in mite infestation levels of OA (efficiency) was 98%, 94% and 96% in colonies treated with ProVap, Varroa Blaster and Varroa Cannon, respectively. Across all application methods, after four treatments, OA treatment decreased the average abundance of mites from 8.23 ± 0.55% to 0.32 ± 0.55% in all treated colonies ($F= 37.99$; $df= 5, 14$; $p< 0.0001$) (Fig. 7), with no differences observed among the application methods after four treatments.

Our results showed that the ProVap and Varroa Cannon each reduced mite levels to under 1% after the second oxalic acid application, but treatments applied using the Varroa Blaster reduced the mite level to the same point after the 4th application ($F_{application\ x\ date}= 14.22$; $df= 14, 62$; $p< 0.0001$) (Fig. 8). All colonies experienced a decline in bee population during the trials (Oct 30th to Nov 29th) (Fig. 9); this decrease is not believed to be related to the effect of oxalic acid, but rather to natural seasonal declines in population and tighter bee clustering in colder weather. Adult populations were reduced from 10.11 ± 0.99 to 2.18 ± 1.02 frames covered with adult bees ($F= 154.22$; $df= 1, 16$; $p< 0.0001$). In addition, two colonies were recorded as dead at the end of treatment period (Nov 29th), one in each of the ProVap and Varroa Cannon treatments due to having weak populations at the beginning of trials that were unrelated to their OA treatment.

Discussion and Conclusion:

These results demonstrate that oxalic acid is an effective treatment to control *Varroa* mites, and that application using the ProVap, Varroa Blaster and Varroa Cannon is effective. Previous published investigations on vaporizers have shown up to 99% efficacy for Varrox, (Radezki 2000; Ferrero et al. 2004), Varrex (Imdorf et al. 2002; Liebig and Hampel 2002), Varrogaz, Isenring, and Kruso (Imdorf et al. 2004), however, there were no studies on the efficacy of ProVap, Varroa Blaster, or Varroa Cannon.

Our results indicated that the vaporizers used in our pilot study provided a high acaricidal effectiveness, with 98%, 94% and 96% reductions in *Varroa* mite levels in the late fall when using the ProVap, Varroa Blaster and Varroa Cannon, respectively. Importantly, the *Varroa* infestation levels dropped below the recommended fall treatment threshold after a single application of OA using the ProVap and the Varroa Cannon; however, it took three applications of OA using the Varroa Blaster to obtain similar results. This indicates that OA application using any of the three devices is able to reduce mite level under the economic threshold, and can be effective at controlling *Varroa* mite populations in late fall under the broodless or minimal brood conditions. A single administration of oxalic acid using ProVap or Varroa Cannon

vaporizers can effectively control Varroa mites under certain conditions, including broodless phase. Although the bee population was smaller at the end of the trial, this was due to low ambient temperature at the time of evaluation and the natural decline is bee population over the winter season, rather than the application of OA.

All three vaporizers had an efficiency >90%, however, the ProVap uses less OA per colony and the operator is able to control precisely how much OA is used. In addition, the ProVap has the shortest warm-up time, is ready to re-use faster than the other machines, and only requires a generator to power the device. The ProVap treats one colony per OA load compared to the Varroa Cannon and Varroa Blaster, which can treat up to four colonies per OA load. The Varroa Cannon allows for the controlled release of OA through a trigger mechanism on the device. This mechanism is absent on the Varroa Blaster and could potentially lead to the loss of OA if the nozzle is not placed directly in the hive entrance when OA is applied. Using the Varroa Cannon and Varroa Blaster could reduce labor costs to the producer; however, we found both devices required at least two people to run smoothly (one person holding the device and the other loading the OA), compared to the ProVap that requires one operator. The Varroa Cannon and the Varroa Blaster used in this pilot study had modified nozzles to fit into hive entrances, and both require a generator and air compressor to run.

Results of this pilot study indicated a high effectiveness of OA sublimation method to control *Varroa* mites in the late fall. Nevertheless, broader studies on mite control using OA are required to find the effective dose, treatment timing, OA residue in the bee products, and negative effects of OA vapors on the immature and mature honey bees.

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Literature Cited

Charrière, J. D., 2001. Optimisation of the oxalic acid trickling method and bee tolerability of different winter treatments: trials in Liebefeld during the last 3 years. *In* Meet. Eur. Gr. Integr. Varroa Control. York.

Ferrero R., Ferrazzi P., Nanetti A., 2004. Lotta contro Varroa destructor (Anderson and Trueman) con acido ossalico somministrato mediante sublimazi- one o per gocciolamento di soluzioni zuccherine, Apoidea 1, 66–71.

Imdorf A., Charrière J.D., Feuz A., Kuhn R., 2002. Oxalsäureverdampfung-Vergleich verschiedener Verdampfungsgeräte, unpublished data (available on request to the author at Swiss Bee Research Centre, FAM Liebefel d, Bern, Switzerland, http://www.apis.admin.ch). Liebig G., Hampel K., 2002. Zur Anwendung von Oxalsäure durch Verdampfen? Dtsch. Bienenj. 2, 17–18.

Liebig G., Hampel K. (2002) Zur Anwendung von Oxalsäure durch Verdampfen? Dtsch. Bienenj. 2, 17–18.

Nanetti, A., R. Büchler, J. Charrière, I. Friesd, S. Helland, A. Imdorf, S. Korpela, and P. Kristiansen, 2003. Oxalic Acid Treatments for Varroa Control (Review). Apiacta. 38: 81–87.

Rademacher, E., M. Harz, 2006. Oxalic acid for the control of varroosis in honey bee colonies–a review. Apidologie. 37: 98–120.

Radetzki T., 2000. Varroa control with oxalic acid a new application. Field experiments winter 1999/2000, Meeting of the European Group for Integrated Varroa Control, Bern, unpublished data (available on request to the author at Swiss Bee Research Centre, FAM Liebefeld, Bern, Switzerland, http://www.apis.admin.ch).

Radetzki, T., 2001. Varroa control with oxalic acid evaporation. Assessment of the impact for the bees. *In* Meet. Eur. Gr. Integr. Varroa Control. Swiss Bee Research Centre, FAM Liebefeld, Bern, Switzerland, York.

Figure 1. ProVap 110 Oxalic acid vaporizer. Each colony was treated for 20 seconds with oxalic acid vapors through the bottom entrance.

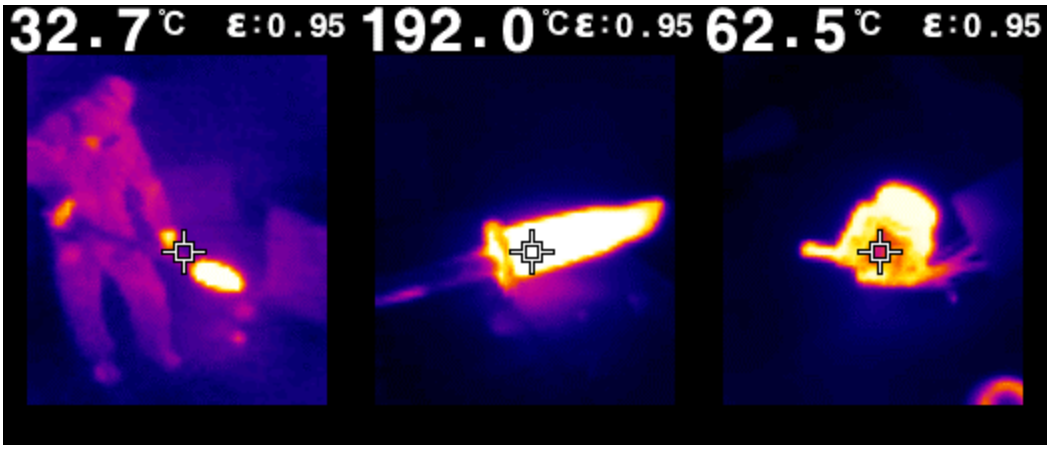

Figure 2. Infrared photos of Varroa Cannon (right and middle) and ProVap (left) vaporizers.

Figure 3. Varroa Blaster Oxalic acid vaporizer. Each colony was treated for 10 seconds with oxalic acid vapors through the bottom entrance.

Figure 4. Varroa Cannon Oxalic acid vaporizer. Each colony was treated for 10 seconds with oxalic acid vapors through the bottom entrance.

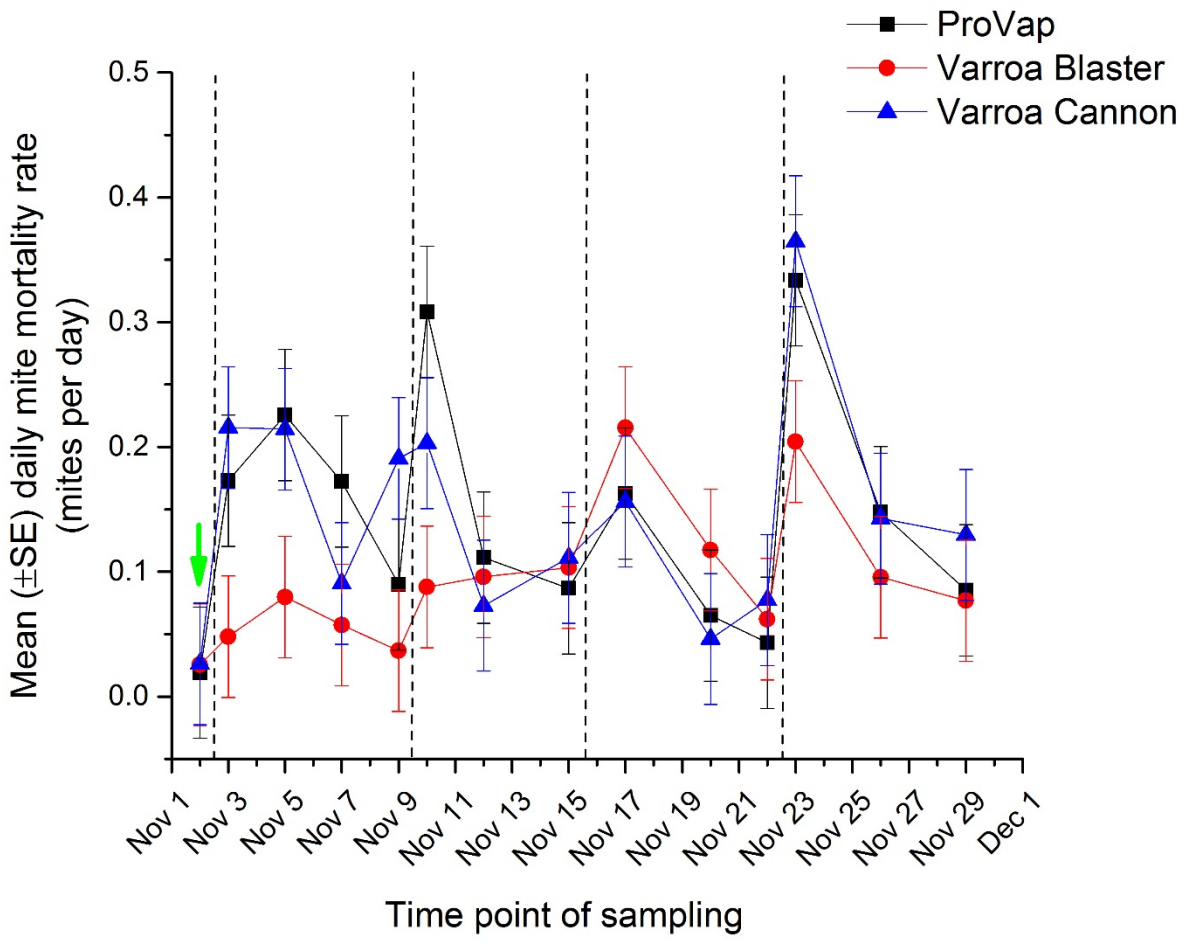

Figure 5. Mean (±SE) daily *Varroa* mite mortality (mite drop per day per colony) in treated colonies with oxalic acid using ProVap, Varroa Blaster and Varroa Cannon vaporizers. Vertical bars on each point indicate ± standard error (SE). Dashed lines indicate the time point of treatment applications (2-Nov-2018, 9-Nov-2018, 15-Nov-2018 and 22-Nov-2018). Green arrow shows the natural mite drop before OA treatment.

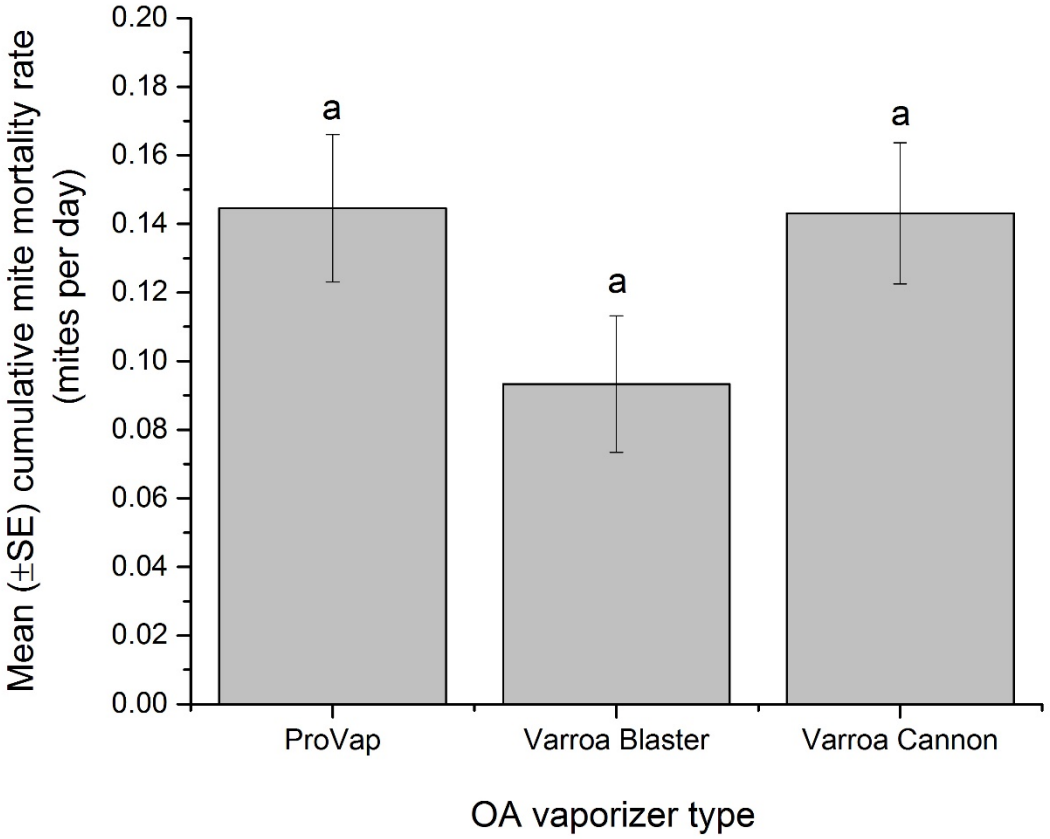

Figure 6. Mean (±SE) cumulative *Varroa* mite mortality rate (proportion of total mites in the colony that died daily over experimental period) in honey bee colonies that received a late fall oxalic acid vapour treatment using one of ProVap, Varroa Blaster or Varroa Cannon application methods. Vertical bars on each column indicate ± standard error (SE). Means followed by the same letters are not significantly different among treatments ($p < 0.05$), indicating there was no difference among application methods after 4 treatments.

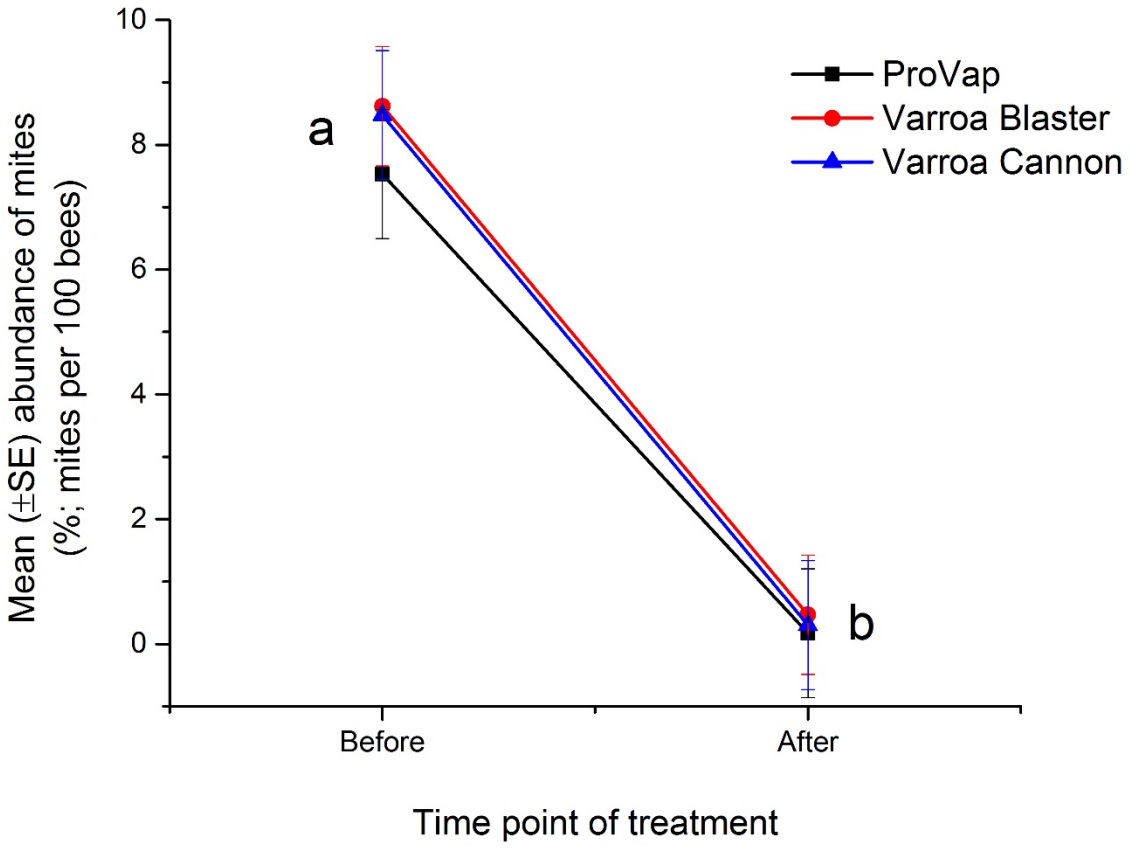

Figure 7. Mean (±SE) abundance of *Varroa* mites (% infested) in honey bee colonies that received a late fall treatment of oxalic acid vapour before (30-Oct-2018) and after (29-Nov-2018) treatment. Vertical bars on each point indicate ± standard error (SE). Means followed by the different letters are significantly different between times ($p < 0.05$), indicating that there was a lower infestation rate after treatment.

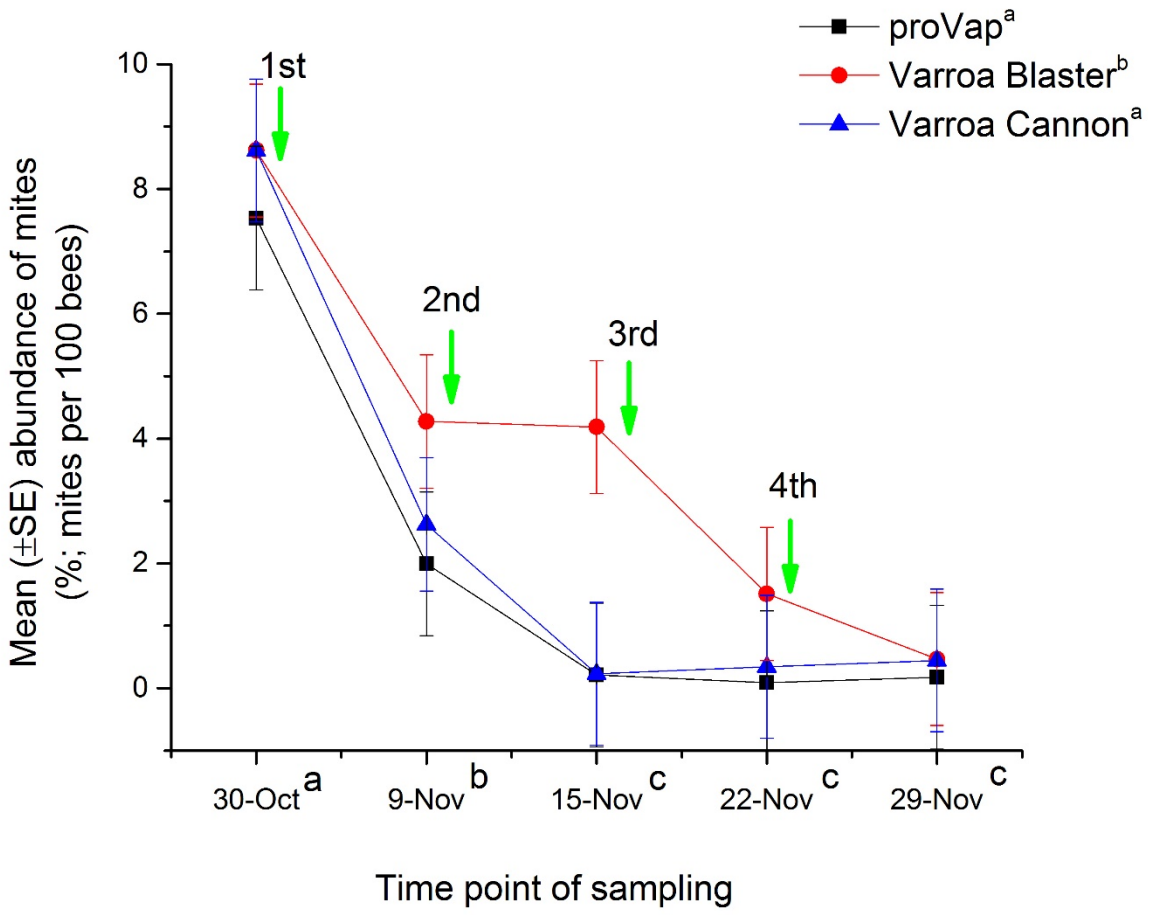

Figure 8. Mean (±SE) abundance of *Varroa* mites in colonies that received a late fall treatment with Oxalic acid. Colonies were treated four times using different vaporizers: ProVap, Varroa Blaster or Varroa Cannon at 2-Nov-2018, 9-Nov-2018, 15-Nov-2018 and 22-Nov-2018. Green arrows represent treatment applications. Means followed by the different letters are significantly different among treatments and times ($p < 0.05$).

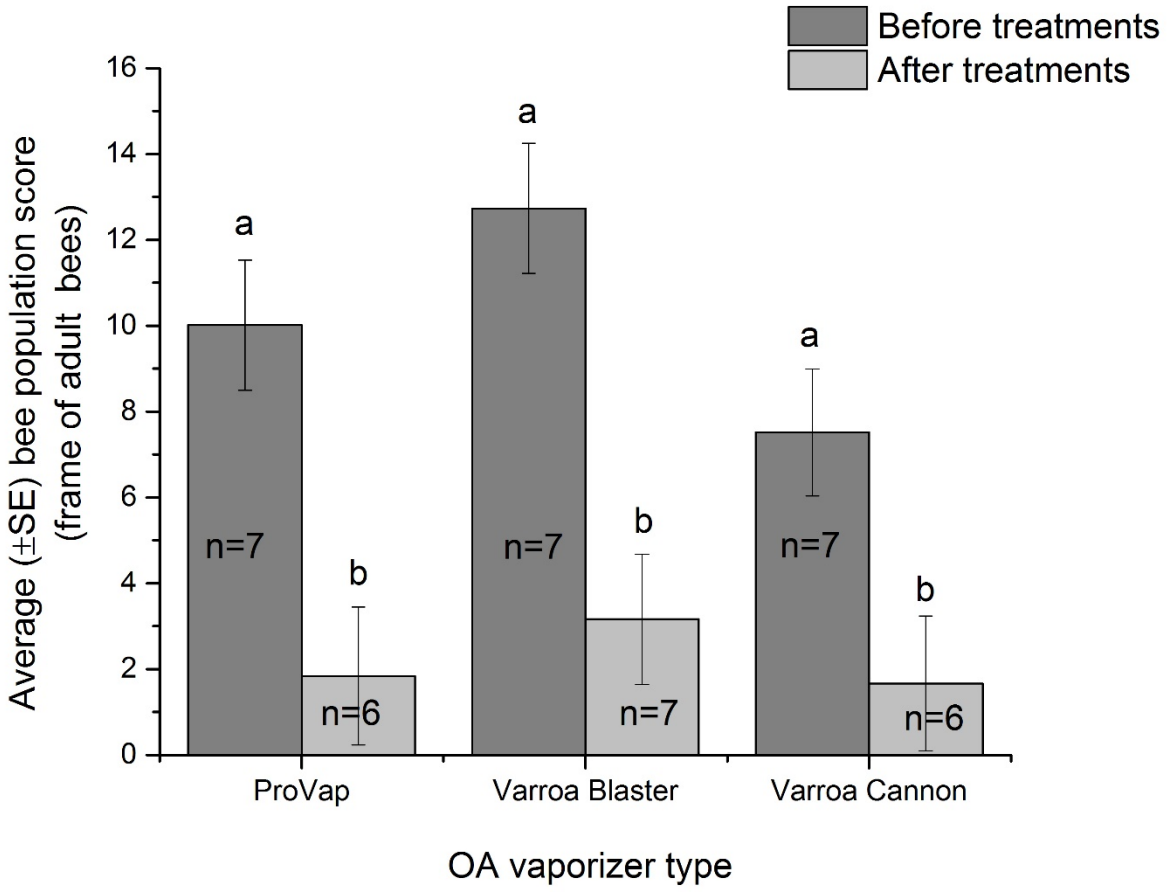

Figure 9. Average (±SE) bee population (number of frames covered with adult bees) in colonies treated with sublimated oxalic acid using different vaporizers (ProVap, Varroa Blaster and Varroa Cannon) before (Oct 30th) and after (Nov 29th) multiple treatments. *n* represents the number of colonies within each treatment. Vertical bars on each column indicate ± standard error (SE). Means followed by the different letters indicate significant differences within treatments ($p < 0.05$) (i.e. smaller cluster scores at the time of the second assessment)