UV Light and Parafilm: Methods for inoculation and quantification with agave mites (*Oziella sp.*)[1]

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Abstract

Agave mites can be damaging to ornamental agave, yet little research has been conducted on them. Our goal was to validate basic methods for quantifying and inoculating plants with agave mites. We compared white light to using UV light or washing mites off plants to quantify mite and egg abundance. We also tested using UV light to estimate agave mite abundance without magnification. Finally, we quantified the number of mites on symptomatic agave and compared methods for inoculating uninfected plants. Significantly more agave mites and eggs were found and counting took significantly less time using UV light compared to using white light or washing plants. For aloe mites, white light was more effective than UV light. Lesions caused by agave mite feeding damage correlated to the number of agave mites and eggs present. Symptomatic agave had high variation among plants in abundance of mites and eggs. Wrapping inoculated agave in parafilm significantly increased the number of mites and eggs found on plants compared to unwrapped plants and increased inoculation success rate. Overall, using UV light is an effective way to quantify agave mites and eggs, and parafilm-inoculated plants provide a more consistent abundance of agave mites and eggs.

Species used in this study: *Oziella sp*., *Aceria aloinis* Keifer, 'Blue Glow' agave, *Agave attenuata* × *Agave ocahui*, *Parry's agave*, *Agave parryi* Engelm, *Aloe haworthioides* Baker.

Index words: Agave mite, grease mite, *Oziella*, agave, Blue Glow, succulent, UV light, black light.

Significance to the Horticulture Industry

Agave mites cause significant cosmetic harm to ornamental agave in both commercial production and landscaping. However, almost nothing is known about agave mites. Some of the biggest challenges are determining how to quantify agave mites due to their small size and hidden feeding locations, and how to inoculate plants for future curative experiments. Based on our results, we found that using commercially available UV flashlights (365 nm wavelength) was an effective way to find and quantify agave mites. For researchers, UV light is more reliable for counting both agave mites and eggs than using white light or washing mites off plants using established methods from Monfreda et al. 2007. Lesions from active agave mite feeding can also be seen with the naked eye under UV light as light stippled sections, and researchers can use this either as a metric to determine if agave mites are present, or to estimate agave mite abundance. For growers or pest scouts, looking for lesions with UV light is a useful tool for determining if agave mites are present, and we strongly suggest using UV light to see if preventative treatments are working or when determining if plants may be infested before symptoms appear. Finally, researchers should wrap plants in parafilm when inoculating them to achieve better agave mite abundance and have a reliable source of infested plants for curative studies. Our results provide methods that will be useful to anyone studying this pest or trying to manage it.

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Introduction

Eriophyoid mites are common pests on many different ornamental plant species, including ornamental agave and aloe, often causing significant cosmetic damage to their hosts. Agave mites (*Oziella sp*.) are colloquially called "grease mites" and leave behind greasy streaks as they feed at the base of leaves and in the core of host agave (Maggio 2012, Parker 2018). Aloe mite (*Aceria aloinis* Keifer) feeding causes lesions and tumor-like growths on a variety of aloe species (Deinhart 2011, Keifer 1952). The damage from agave mites and aloe mites can cause plants to become unsightly and unsellable, and both are major pests for commercial growers and home hobbyists alike.

While some research has been conducted on aloe mites and ways to manage them (Villavicencio et al. 2014), very little work has been done on agave mites. Much of what is known about agave mites consists of anecdotes from growers or eriophyoid experts. Furthermore, while standards exist for gathering and quantifying eriophyoids from other plant species (Monfreda et al. 2007, 2010), no published work exists on quantifying agave mites or how to work with them in the lab. This lack of knowledge, combined with agave mites' small size (~1/3 mm) (0.013 in) and hidden feeding locations (Maggio 2012), creates a significant barrier to researchers who want to study or investigate ways to manage this pest.

Agave mites are very difficult to see on their host plants, even after leaves have been separated and are viewed under high magnification. This is largely because the mites and their eggs are a translucent whitish color, and they blend in easily on the whitish core and base of agave leaves where they live. Separating the mites from the host plant may allow them to be more easily visible, and washing eriophyoid mites off plants using a mixture of water, detergent, and bleach has previously been suggested as a way of quantifying their abundance more easily (Monfreda et al. 2007). As a separate method, some eriophyoid mites

such as agave mite weakly fluoresce under UV light (Amrine, personal communication[3]), and UV light could be used to quantify mite abundance. Additionally, small lesions caused by agave mite feeding also fluoresce under UV light, can be seen without a microscope, and appear as light stippled sections (Personal observation). Anecdotally, these stippled lesions seem to correlate to high numbers of living mites (Personal observation). UV light may therefore be a useful tool to identify living agave mites on pieces of agave both with and without magnification.

Testing miticides or other curative treatments against agave mite requires having infested plants with a relatively uniform number of agave mites to start with. However, the variation in agave mite abundance on natural infested, symptomatic agave is currently unknown. Additionally, while agave mites are thought to spread on the wind (Maggio 2012) like other eriophyoid mites (Majer et al. 2021), it is unclear how best to inoculate uninfected agave with a known number of mites to begin an infestation. Placing pieces of agave infested with mites into direct contact with the core or base of leaves of an uninfected plant has been suggested previously, as has wrapping the agave with parafilm after adding mites to keep humidity high and increase the chances of successful infestation (Amrine, personal communication). Understanding how many mites are present on already symptomatic plants and how best to infest uninfected plants are necessary before beginning experiments on treatments to control agave mite.

In this paper, we document basic methods we validated to aid in studying agave mites in lab and field research. Our objectives were to:

1. Compare different methods for counting agave mites and their eggs including washing mites off plants, visually searching for mites with and without a UV light under a dissecting scope, and estimating the number of mites associated with lesions visible to the naked eye.
2. Determine how many agave mites are present in already symptomatic agave found in commercial nurseries and the variability of these populations.
3. Test the efficacy of parafilm to infest uninfected agave plants with mites for further trials.

Materials and Methods

Visual search with and without UV light. Uninfected 'Blue Glow' agave (*Agave attenuata* × *Agave ocahui*) in 9 cm (3.5 in) pots were obtained from a local wholesale grower. Agave were inoculated with agave mites using pieces of mite-infested leaves taken from previously symptomatic 'Blue Glow' agave. Infested agave pieces were visually confirmed to have 20-50 agave mites present on them under a dissecting scope (Nikon SMZ-2T, Nikon, Tokyo, Japan) and were placed in direct contact with the core of the uninfected agave. The surroundings leaves were then wrapped in parafilm (Bemis Company, Neenah, Wisconsin), creating a covering over the core and infested pieces. A total of 19 'Blue Glow' agave were inoculated. The same process was repeated with 9 uninfected 'Parry's' agave (*Agave parryi* var *truncata*) in 10 cm (3.9 in) pots obtained from a separate local wholesale grower. All agave were placed in a greenhouse for 3 weeks to allow mite populations to build. Temperatures inside the greenhouse ranged between 20 - 40 C (68 - 104 F). After 3 weeks, agave were destructively sampled to search for mites.

During destructive sampling, agave were separated from their roots by twisting the plant until it broke free. Leaves were then individually peeled off and set aside, taking care to not disturb or handle the whitish lower section of the leaves where mites tended to congregate. Leaves were removed until no more leaves could be removed without snapping or damaging the remaining core piece. The last 6 leaves removed (those closest to the core) and the remaining core were examined for the presence of agave mites and their eggs.

Each leaf was individually examined under a dissecting scope. The same leaf was viewed under white light (light in the visible spectrum) from a 92.5mm 144 LED ring light (AmScope, Feasterville PA, USA), and then separately with a 365 nm handheld UV flashlight (Weltool M2-BF 365 nm UV Professional Black Light LED Flashlight) (Weltool, Dalian City, Liaoning Province, China). The number of visible agave mites and their eggs per leaf were recorded under both white light and UV light.

The number of visible mites and eggs did not meet assumptions of normality, so paired Wilcox tests with a Benjamini-Hochberg adjustment were used to compare the abundance of both mites and of eggs between white light and UV light. Leaves with no mites or eggs present were removed from the analysis. A total of 101 leaves (74 from 'Blue Glows' and 27 from Parry's agave) had agave mites present, while a total of 91 leaves (68 from 'Blue Glows' and 23 from Parry's agave) had eggs present.

As a comparison with a different species of eriophyoid, we also conducted the same experiment with aloe mites. Ten *Aloe hawarthioides* in 10 cm (4 in) pots showing signs of aloe mite infestation were obtained from a local wholesale grower. The same methodology was used as for agave mites, except all leaves on the aloe plant were examined for mites and eggs. The number of visible aloe mites and their eggs per leaf were recorded under both white light and UV light and were compared to each other using paired Wilcox tests with a Benjamini-Hochberg adjustment. All analyses were conducted in R v4.3.1 (R Core Team, 2024). A total of 40 aloe leaves had aloe mites present, while 7 had eggs present.

Visual search vs. wash and timing methods. A total of 23 'Blue Glow' agave were infested with agave mites as previously described above and were placed in a greenhouse where temperatures ranged between 25 - 40 C for 3 months to allow mite populations to increase. Of these, 9 displaying symptoms of agave mite infestation were selected and used in the experiment.

Agave leaves were separated as described above and the number of mites and eggs counted under a dissecting scope using both white light and with UV light. UV light was used to count mites and eggs first, followed by white light. This was then alternated for each following leaf. The amount of time it took to fully examine each leaf was recorded for both lighting conditions.

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After counting under both lighting conditions, leaves were placed into beakers to be washed to extract mites. If the leaves had over 100 mites, they were individually washed. If they had fewer than 100 mites, multiple leaves were collected in the same beaker until at least 50 mites were present across all leaves in the beaker. Leaves were then washed to extract mites using the methods described in Monfreda et al. 2007. This consisted of submerging leaves in a washing solution of tap water with a 1% concentration of bleach (Clorox 7.5% bleach, The Clorox Company, Oakland CA, USA), and 0.2% dish detergent (Dawn Ultra, Proctor and Gamble Cincinnati OH, USA) and stirring for 5 minutes. The washing solution was then filtered through a series of 4 ASTM stainless steel sieves (ASTM International, West Conshohocken PA, USA) of decreasing mesh size (850 μm, 180 μm, 53 μm, and 25 μm). The last two sieves (53 μm and 25 μm) were rinsed with a handheld squirt bottle and the rinsate collected into a petri dish to examine for mites and eggs under a dissecting scope with white light.

Paired Wilcox tests with a Benjamini-Hochberg adjustment were used to compare the abundance of mites and of eggs between UV light, white light, and washing. Paired Wilcox tests with a Benjamini-Hochberg adjustment were also used to compare the amount of time it took to examine leaves under UV light and white light.

Correlating agave mites to visible lesions. Nineteen 'Blue Glow' agave were infested with agave mites as previously described above and were placed in a greenhouse where temperatures ranged between 25 - 40 C for 4 weeks to allow mite populations to increase. Agave leaves were then separated as described above. Leaves were examined for stippled lesions indicative of agave mite feeding using a UV light without magnification, and the number of lesions per leaf and per agave were recorded. The number of agave mites and eggs were then counted under a dissecting scope with UV light as described above.

The number of agave mites and eggs did not meet assumptions of normality, so generalized linear models with a poisson distribution were used to correlate the number of agave mites and eggs to the number of lesions found per plant. On a per-leaf basis, the number of agave mites and eggs were compared between 0, 1, and 2 lesions per leaf using Kruskal-Wallis tests.

Mite abundance on symptomatic plants. A total of 45 'Blue Glow' agave in 9 cm containers showing significant symptoms of agave mite infestation were collected from a greenhouse operated by a local commercial wholesale succulent grower. Agave were classified as symptomatic if a majority of their leaves had characteristic greasy streaks present and if the entire core appeared greasy and/or was collapsing. Agave were then destructively sampled using the same methods used for inoculated agave and the total number of agave mites and eggs on the core and innermost 6 leaves were counted using a dissecting scope and UV flashlight. The minimum, maximum, mean, and standard deviation of the number of agave mites and their eggs were calculated across all symptomatic agave sampled, and histograms of mite and egg abundance created.

Infesting agave with and without parafilm. Ten mite-free 'Blue Glow' agave and 3 mite-free Parry's agave were obtained from local wholesale growers and inoculated using the same technique as previously described. Another 10 mite-free 'Blue Glow' agave and 3 mite-free Parry's agave were inoculated as previously described but were not wrapped in parafilm at the end as a control treatment.

All 26 plants were placed in a greenhouse with a temperature range of 20 - 40 C for 3 weeks and then destructively sampled in the same manner as previously described. The number of agave mites and eggs did not meet assumptions of normality and were compared between plants with parafilm and plants without parafilm using Kruskal-Wallis tests.

Results and Discussion

Visual search with and without UV light. Agave mites and their eggs were found to weakly fluoresce under UV light (Fig. 1A and B). Significantly more agave mites were found on recently inoculated 'Blue Glow' agave ($p = 1.965e-05$), Parry's agave ($p = 8.867e-05$), and both agave species combined ($p = 1.027e-08$) when searching with a UV light compared to white light (Table 1). Significantly more agave mite eggs were also found on recently inoculated 'Blue Glow' agave ($p = 1.94e-08$), Parry's agave ($p = 0.0002049$), and both agave species combined ($p = 2.285e-11$) when searching with a UV light (Table 1). Significantly fewer aloe mites ($p = 4.574e-05$) were found on *Aloe hawarthioides* when searching with a UV light compared to white light. There was no significant effect of light type on the number of aloe mite eggs found ($p = 0.2918$) (Table 1).

Based on these results, UV light appears to be a useful tool for finding eriophyoids when it leads to increased visual contrast. On *Aloe hawarthioides* leaves, aloe mites were harder to find under UV light because the leaves fluoresced strongly, reducing the contrast between mites and the background leaf. On agave leaves however, UV light increased the contrast between the mites and the background leaf while simultaneously reducing glare, both of which were problems under white light. UV light was a more effective method both on heavily symptomatic agave with far more mites and eggs, and on newly infested agave with fewer mites and eggs. The use of UV light to find eriophyoid mites, and agave mites in particular, should be considered as a viable tool to improve both accuracy and speed under the correct conditions.

Visual search vs. wash and timing. Significantly more agave mites ($p = 7.88e-06$) and their eggs ($p = 1.283e-07$) were found on highly symptomatic inoculated agave when using UV light compared to white light (Table 2). Using UV light to examine leaves for mites and eggs took significantly less time compared to white light ($p = 6.854e-06$) (Table 2).

Significantly more agave mites ($p = 0.001329$) and eggs ($p = 0.0007247$) were found using UV light compared to washing plants, and significantly more agave mites ($p = 0.008253$) and eggs ($p = 0.001359$) were found using white light compared to washing plants (Table 3). As with previous experiments, significantly more mites ($p = 0.002854$) and eggs ($p = 0.0007247$) were found with UV light compared to white light. (Table 3)

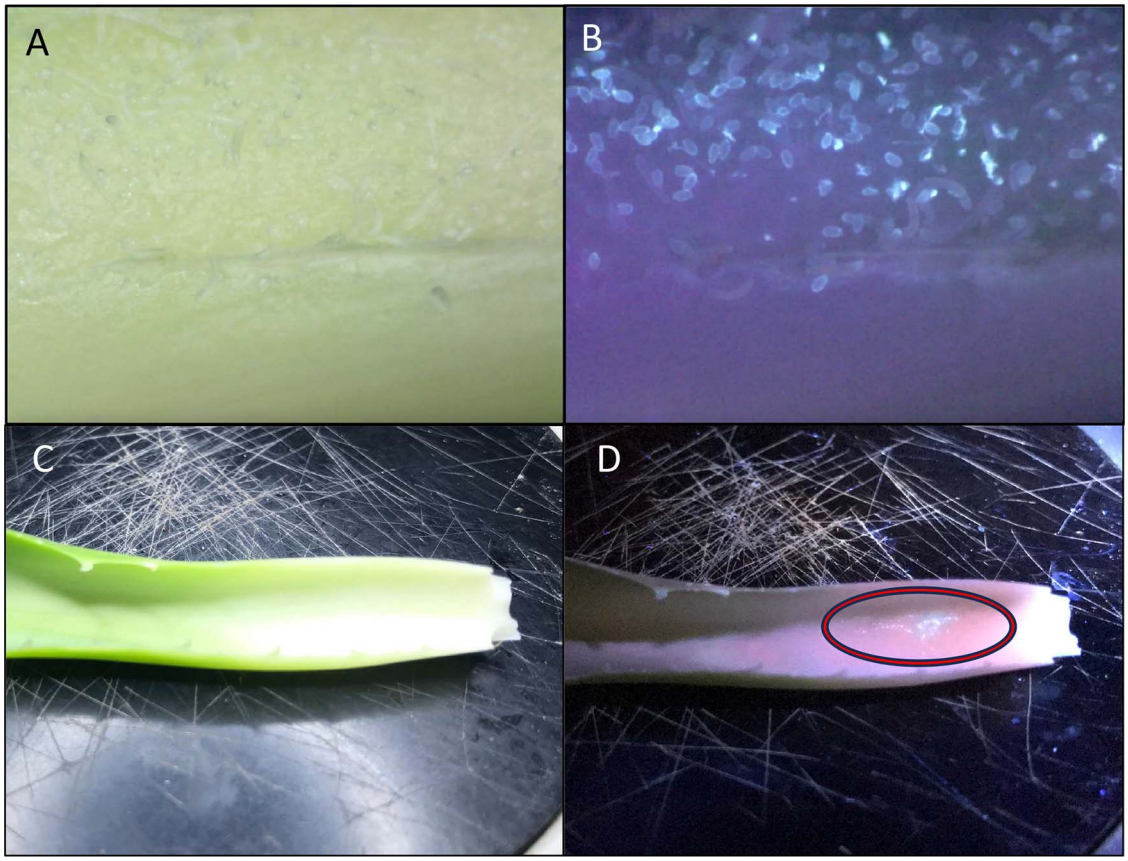

Fig. 1. **A comparison of agave leaves seen under white light vs UV light. A) A close view of an agave leaf with numerous agave mites (*Oziella sp*) and eggs viewed under white light; B) The same leaf viewed under UV light where more agave mites and especially eggs can now be seen. C) A separate mite-infested 'Blue Glow' agave leaf seen under white light; D) The same agave leaf seen under UV light, where a light stippled section (circled) indicative of agave mite feeding can now be seen.**

Unlike Monfreda et al. 2007, we found direct observation with either UV light or white light yielded better results for finding eriophyoid mites compared to washing. This is likely because agave mites are relatively easier to visually locate than the eriophyoid mites Monfreda et al. 2007 studied. In our case, agave mites do not form galls and agave leaves consist of smooth, relatively flat surfaces. This means that while agave mites themselves can be hard to see, there are few places for them to hide once the leaves are peeled off the core, and a thorough visual search will turn up almost all of them. By contrast, Monfreda et al. 2007 investigated a variety of eriophyoid mite species found on much more complex structures like fig leaves, flowers, and buds, grapevines and grape clusters, thistles, and galls formed by mite feeding. Washing has previously been used when eriophyoids are in complex structures like flowers (Solo et al. 2020) that make it harder to visually find mites. Because these additional visual impediments do not exist with agave mites, it follows that the visual searches we conducted would be more effective on agave.

Despite our results, there are times when washing agave plants will likely be a better option than visually searching. If trying to quantify the number of mites on large agave, or across multiple plants at the same time, the washing method will likely be faster even if it is ultimately less accurate. Additionally, Monfreda et al. 2007 describes a separate method for extracting eriophyoid eggs from plant species that we did not test on agave mites, so it remains unknown if it is more accurate than our method.

Table 1. **Mean abundance of visible eriophyoid mites and their eggs per leaf ± 1 standard error. "White light" refers to light in the visible spectrum (LED ring light), and "UV Light" refers to using an UV flashlight for illumination (365 nm UV flashlight). Different letters indicate statistically significant differences (paired Wilcox tests) in mite or egg abundance between white and UV light.**

		Mites		Eggs	
Host Plant	**Mite Species**	**White Light**	**UV Light**	**White Light**	**UV Light**
Agave					
'Blue Glow' *Agave attenuata* × *Agave ocahui*	*Oziella sp.*	2.85 ± 0.60a	3.44 ± 0.67b	6.15 ± 1.05a	9.38 ± 1.51b
Parry's Agave *Agave parryi* var *truncata*	*Oziella sp.*	2.74 ± 0.47a	4.32 ± 0.63b	3.91 ± 1.50a	7.83 ± 1.84b
Total	*Oziellasp.*	3.35 ± 0.46a	4.32 ± 0.52b	5.58 ± 0.87a	8.99 ± 1.22b
Aloe					
Aloe haworthiodes	*Aceria aloninis*	11.3 ± 2.25b	7.38 ± 1.53a	3.14 ± 1.32a	1.57 ± 0.92a

Table 2. **Mean abundance of visible agave mites and their eggs per leaf and the time it took to examine leaves in seconds, ± 1 standard error. "UV Light" refers to using an UV flashlight for illumination (365nm UV flashlight) and "White light" refers to light in the visible spectrum (LED ring light). Different letters indicate statistically significant differences (paired Wilcox tests) in mite abundance, egg abundance, or time to search leaves white and UV light.**

Mite Species	Mites		Eggs		Time (Seconds)	
	UV Light	White Light	UV Light	White Light	UV Light	White Light
Oziella sp.	76.66 ± 18.00a	58.74 ± 14.98b	56.61 ± 14.44a	17.95 ± 5.02a	132.14 ± 13.61b	167.69 ± 15.58a

Taken together, our method of searching for agave mites with a UV light and dissecting scope is a good option when high accuracy of both mites and eggs is desired, and when going through relatively small samples. It is also a cheaper and more accessible option than purchasing the sieves required for washing, or buying equipment needed for other previously proposed methods of quantifying eriophyoids like electrostatic methods (Stone 1981) or using ultrasonic radiation (Gibson 1975). Furthermore, we found using UV light to be the superior method across multiple different experiments and when quantifying agave mites in various situations. However, our method is not likely to be effective if mites are hidden in complex structures, or if UV light does not lead to increased visual contrast between the eriophyoid mites and plant material.

Correlating agave mites to visible lesions. Areas of agave leaves with heavy feeding damage from active agave mites fluoresced a lighter color under UV light compared to the rest of the leaf. These areas appeared as light stippled lesions and although often subtle, could reliably be seen with the naked eye (Fig. 1C and D). On individual leaves, the number of lesions ranged from 0 to 3, and on whole plants, the number of lesions ranged from 0 to 10.

As the overall number of lesions visible under UV light increased on agave plants, so did the number of agave mites ($p < 2e\text{-}16$) and eggs ($p < 2e\text{-}16$) (Fig. 2A and B). Comparing between leaves there were significantly more agave mites on leaves with both 1 and 2 lesions compared to 0 lesions ($p = 6.395e\text{-}15$), and significantly more eggs on leaves with more lesions compared to fewer ($p = 2.626e\text{-}15$) (Table 4).

Even with UV light and a good dissecting scope, it is time consuming and difficult to count agave mites. As an alternative, counting lesions under UV light is a viable way to determine if agave mites are present and to estimate their abundance. It takes a fraction of the time, can be done without magnification, and takes little additional skill, training, or supplies. Growers or scouts should strongly consider counting lesions instead of looking for mites when assessing if preventative treatment options were effective or when checking asymptomatic plants for infestations.

For researchers, searching for lesions has multiple benefits. While we demonstrated that lesions correlated with agave mite and egg abundance on inoculated plants, we anecdotally found lesions on already symptomatic plants also correlated with high agave mite and egg numbers. Seeing lighter stippled lesions became a useful tool for us to quickly assess if a symptomatic plant was likely to have many mites present and enabled us to focus more on those areas when looking for groups of agave mites to inoculate mite-free plants. Researchers should consider using the presence of lesions on symptomatic plants as a guide to find heavy mite infestations and can use the presence of lesions on inoculated plants to quantify mite presence and roughly estimate mite and egg abundance. This could save significant time and effort when estimating mite abundance post-treatment in efficacy trials for agave mite control.

Mite abundance on symptomatic plants. There was large variation in the number of mites found on highly symptomatic agave (Fig. 3A). The mean number of agave mites found on plants was 477.71, the minimum number was 2, the maximum was 2,864, and the standard deviation was 657.96.

There was also a large variation in the number of eggs found on highly symptomatic agave (Fig. 3B). The mean number of eggs found on plants was 566, the minimum was 7, and the maximum was 5,138. The standard deviation was 978.85.

Because agave mite abundance varied so much, it would be difficult to use symptomatic plants to assess the efficacy of curative treatments. While other studies have successfully used already symptomatic plants to study curative treatments against eriophyoids (Villavicencio et al. 2014), it is difficult to draw comparisons between their work and ours. The discrepancy in mite and egg abundance and variance may be from different host and eriophyoid species, aloes displaying symptoms earlier in infestations than agave, or happenstance given we have anecdotally found symptomatic *Aloe hawarthioides* containing hundreds of

Table 3. **Mean abundance of visible agave mites and their eggs ± 1 standard error. "UV Light" refers to using an UV flashlight for illumination (365nm UV flashlight), "White light" refers to light in the visible spectrum (LED ring light), and "Wash" refers to washing mites off agave using techniques described in Monfreda et al. 2007. Different letters indicate statistically significant differences (paired Wilcox tests) in mite or egg abundance between UV light, white light, and washing.**

Mite Species	Mites			Eggs		
	UV Light	White Light	Wash	UV Light	White Light	Wash
Oziella sp.	179.2 ± 30.81a	141 ± 28.54b	93.07 ± 23.26c	153.8 ± 34.99a	42.8 ± 9.76b	0.2 ± 0.2c

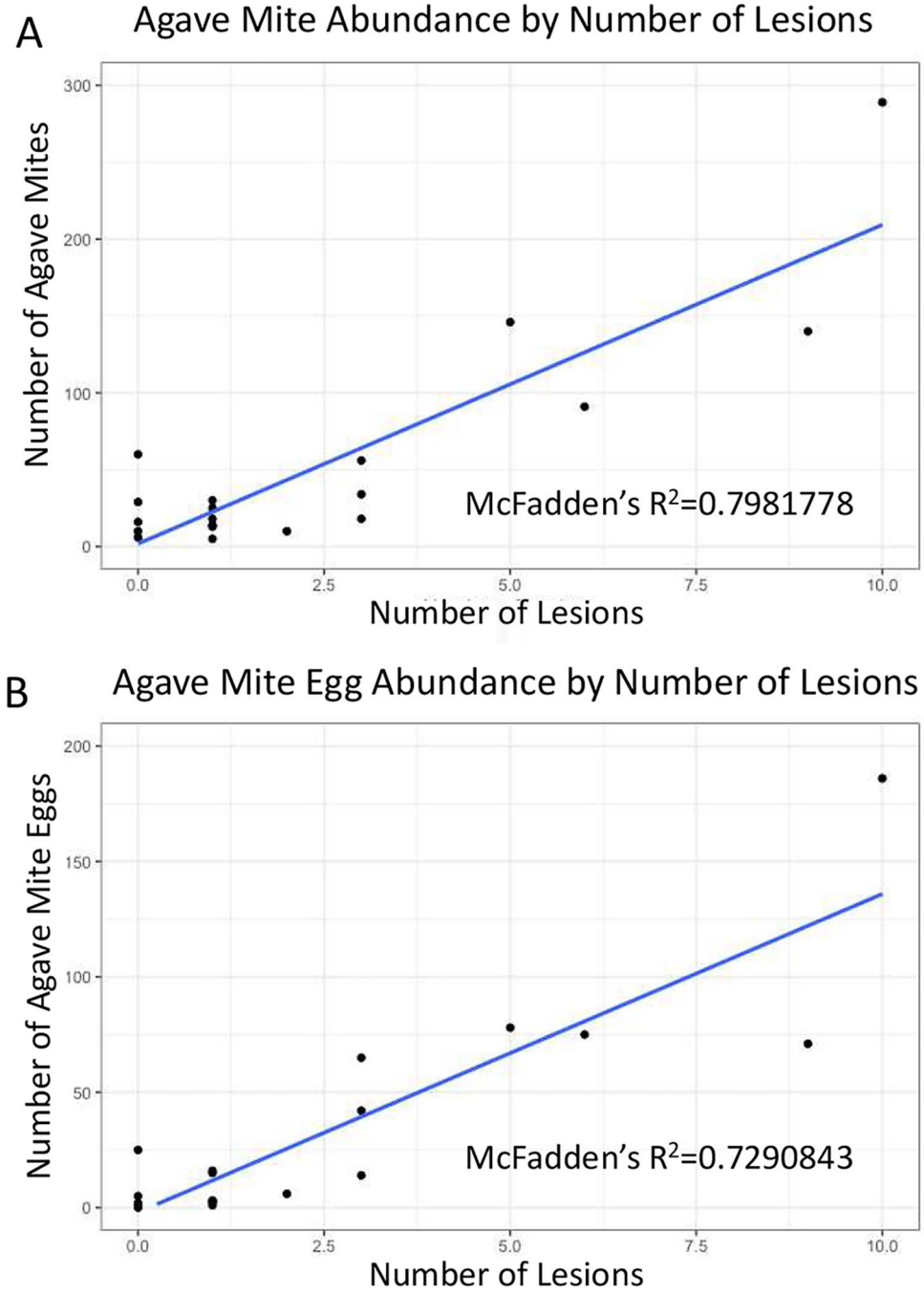

Fig. 2. **Regression plots of the number visible lesions per agave plant and A) Agave mite abundance; and B) Agave mite egg abundance on inoculated 'Blue Glow' agave. Blue lines represent trend lines for data.**

Table 4. **Mean abundance of visible agave mites and their eggs ± 1 standard error. "Lesions" refers to the number of stippled lesions observed with the naked eye under UV light (365 nm UV flashlight). Different letters indicate statistically significant differences (paired Wilcox tests) in mite or egg abundance between lesion numbers.**

Number of Lesions	Agave Mite Abundance	Agave Mite Egg Abundance
0	2.009 ± 0.314a	0.641 ± 0.182a
1	12.125 ± 2.205b	9.083 ± 2.63b
2	43.7 ± 9.202b	31.6 ± 8.957c

aloe mites and eggs. Whatever the reason, our results suggest using already symptomatic agave for curative experiments may be problematic.

Infesting agave with and without parafilm. There was a significant difference both in the number of agave mites ($p = 0.009687$) and the number of eggs ($p = 0.01079$) present comparing between agave with and without parafilm. An average of 24.62 ± 9.89 agave mites and 26.62 ± 9.75 eggs were found on parafilmed plants compared to an average of 3.15 ± 1.51 agave mites and 4.31 ± 2.53 eggs

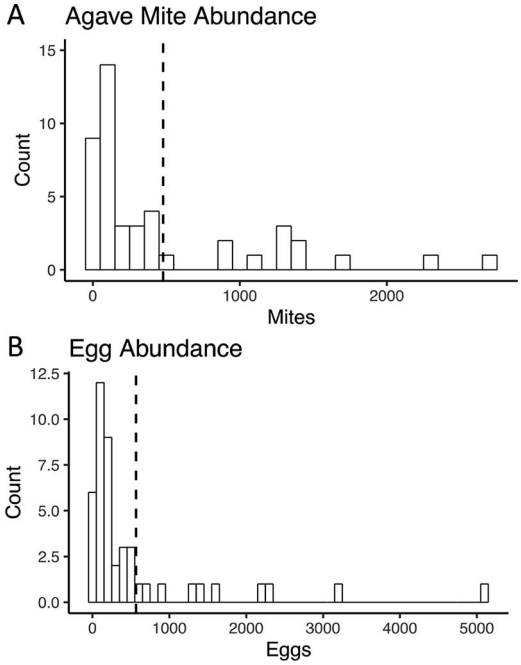

Fig. 3. Histograms of the number of plants with varying A) Agave mite abundance on heavily symptomatic 'Blue Glow' agave; and B) Agave mite egg abundance on heavily symptomatic 'Blue Glow' agave. Dashed lines represent mean abundance of A) mites or B) eggs.

found on non-parafilmed plants. Eleven of the 13 plants with parafilm had mites present, and 10 had eggs present. Four of the 13 plants without parafilm had mites present, and 3 had eggs present.

While studies on other eriophyoids have successfully inoculated hosts with infested plant material without using parafilm (Varia et al. 2022), our results clearly indicate that parafilm helps agave mites establish and increase populations. Using parafilm also led to notably higher mite numbers compared to indirect inoculation like Villavicencio et al. 2014 used in their aloe mite study, where they placed symptomatic plants adjacent to uninfected plants. Indirect inoculation may not be an appropriate method for quantitative studies, since aerial dispersal is not well quantified and is difficult to study (Zhao and Amrine 1997), although it can be achieved under the correct circumstances (Majer et al. 2021). As stated before, parafilm likely increases the humidity in and around the core of the plant (Amrine, personal communication), preventing the mites from desiccating or potentially giving them more time to migrate from the source of inoculum to the core of the agave. Agave inoculated with parafilm still had high variation in mite and egg abundance but did not have the same large differences that symptomatic agave did. Based on our data, using agave inoculated with parafilm will likely provide the best results for most experiments testing curative treatments on whole plants.

In conclusion, agave mites are difficult to study, both because of their biology and a lack of existing research and methodology. Our results begin to fill in these gaps, providing data on methodology that can be used for agave mites. UV light is an effective tool for counting agave mites, takes less time than counting mites under white lighting conditions, and is more accurate than counting mites under white light or washing mites off plants. UV light also allows lesions from active agave mite feeding to be seen without magnification and may serve as an effective proxy to easily and quickly estimate agave mite abundance. The high variation in agave mite and egg abundance in symptomatic plants makes them difficult to use in future experiments, and we recommend they be avoided in most circumstances beyond determining if treatments are 100% effective. Inoculating agave with parafilm appears to be an effective method for establishing relatively even numbers of mites on plants and could be used to study curative treatments in the future.

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