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Understanding the trends in prevalence and abundance of *Acarapis dorsalis* and *Acarapis externus* in *Apis mellifera* colonies

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ABSTRACT

Acarapis dorsalis and *Acarapis externus* are parasites of adult honey bees in the United States since the 1930s. Here, we present historical and current data on their prevalence and abundance. In the late 1980s to early 2000, these two *Acarapis* species were frequently detected with *A. externus* being found at higher levels than *A. dorsalis*. The abundance of *A. externus* over *A. dorsalis* may be due to the lack of host age preference by *A. externus* as their prevalence and intensity remained high on bees up to 35 days old. In contrast, infestation rate and mite load of *A. dorsalis* decreased as bees become older. By examining 16,515 worker bees from 2007 to 2019, *A. dorsalis* was detected yearly while *A. externus* infestation was sporadic. The higher frequency of detecting *A. dorsalis* over *A. externus* may be due to their differences in colonization ability. *A. dorsalis* was faster in establishing their population in mite-free colonies than *A. externus* and was also successful in invading *A. externus*-infested colonies. The introduction of 50 *A. dorsalis* in mite-free colonies was sufficient to found a population while 500 *A. externus* may be too small to establish a population. Variation in responses to parasitic mites by different honey bee stocks also influenced *Acarapis* population. *A. dorsalis* was most prevalent in the Hastings stock while the levels of *A. externus* were higher on the ARS-Y-C-1, Hastings x ARS-Y-C-1 hybrid and Louisiana stocks. The Russian honey bees also had higher levels of *A. dorsalis* than the Italian honey bees. However, both stocks' responses to *A. externus* were inconsistent. Nonetheless, both ARS-Y-C-1 and Russian honey bees are known to be resistant to another *Acarapis* species, *A. woodi*, which is known to be a more serious parasite of honey bees than these two external *Acarapis*. The potential role of external *Acarapis* in virus transmission especially in *Varroa*-infested colonies needs to be studied.

KEYWORDS: external *Acarapis*, *Acarapis dorsalis*, *Acarapis externus*, colonization ability, mite resistance, honey bee stocks.

1. INTRODUCTION

Loss of honey bee colonies or reduced colony productivity is the costliest economic effect of mite parasitism. Aside from *Varroa* and *Tropilaelaps*, three *Acarapis* species are known to be parasitic on honey bees (*Apis mellifera* L.). One species (*Acarapis woodi* Rennie) infests inside the tracheae while two species (*Acarapis dorsalis* Morgenthaler and *Acarapis externus* Morgenthaler) live and reproduce outside the body of adult honey bees. These three *Acarapis* species [1] and *Varroa* mites can co-exist in a single colony [2]. While the concurrent infestation by the two external *Acarapis* is common especially

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in highly infested colonies [3], infestations by these three *Acarapis* species on a single bee are rarely observed. With the ubiquitous presence of *Varroa* in colonies, bees that are previously infested as pupae by *Varroa* may subsequently incur one or two of these *Acarapis* species.

The two external *Acarapis* species are known to be widely distributed. *A. dorsalis* was first detected in Canada in 1926 and in the United States in 1930 [4]. In 1936, both *A. dorsalis* and *A. externus* were observed infesting bee samples from 21 countries including Canada and the US, and were rediscovered in both countries in 1959 [4]. In the late 1980s, both species were frequently collected in Oregon with *A. externus* being more abundant than *A. dorsalis* at colony and individual levels [3, 5]. *A. externus* is also more prevalent than *A. dorsalis* in British Columbia [6] and New Zealand [7]. A new haplotype of *A. externus* has recently been identified in New Zealand [8]. In contrast, *A. dorsalis* is more prevalent than *A. externus* in Britain [9]. These two *Acarapis* species have also been observed in Iran in the 1990s [10]. A recent survey in South Korea showed about 32% of the colonies examined was infested with *A. dorsalis*, 9% with *A. externus* and only 1% with *A. woodi* [11].

In 2006, the term colony collapse disorder (CCD) was coined to describe the rapid disappearance of adult workers ultimately leading to the death of the colony [12, 13]. This loss of colonies having CCD-like symptoms continued for years [12, 14]. CCD is caused by a myriad of factors including parasitic mites [14]. Worldwide, *Varroa* mites and the viruses they vector remained the number one problem of honey bees. In addition, *A. woodi* had caused significant losses of colonies in the UK, Canada, and US [15-20]. In contrast, the two external *Acarapis* species are considered harmless to honey bees. Much like any other parasitic mites, external *Acarapis* also obtain nourishment from their honey bee hosts. While feeding by *Varroa* or *Tropilaelaps* on honey bee hosts activates virus replication [21-24], the contribution by *Acarapis* mites in the transmission or replication of honey bee viruses on infested honey bees has not been studied. In fact, external *Acarapis* mites have been totally ignored by researchers in recent years. Hence, current knowledge on their existence or degree of infestation in honey bee colonies in the US is generally lacking. By presenting historical data and examining historical and recently collected honey bee samples from different locations, we assessed the patterns of prevalence and abundance of these two external *Acarapis* species from the late 1980s to the present.

2. MATERIALS AND METHODS

To assess the changes in relative prevalence and abundance of *A. dorsalis* and *A. externus*, we used unpublished historical data from 1988 and 1990-1992, and examined historical and recently collected honey bee samples from 2001 to 2002, and from 2007 to the present. No historical samples were available for 2012 and 2015.

2.1. Prevalence of *A. dorsalis* and *A. externus* (2007 to 2019)

From 2007-2019, a total of 554 colonies (n = 16,515 worker bees) were examined. Samples were collected from stationary colonies in Kansas (n = 88 colonies), Arkansas (n = 88 colonies), Florida (n = 35 colonies) and Louisiana (n = 286 colonies). In addition, migratory colonies based in Louisiana (n = 27 colonies) and Mississippi (n = 30 colonies) were also sampled. For each colony, 30 bees were individually examined for the presence or absence of the two external *Acarapis* species. The dorsal groove of the thorax was inspected for the presence of *A. dorsalis*, and the neck and tentorial pits for *A. externus* (Figure 1).

2.2. Prevalence and intensity of *A. dorsalis* and *A. externus* in different stocks of honey bees (1990-1992)

For 1990 to 1992, data were obtained from a larger experiment that evaluated the potential tolerance of honey bees imported from Yugoslavia (later named as ARS-Y-C-1) towards *Varroa* and tracheal mites in Florida [2, 25]. Four types of honey bees were evaluated: ARS-Y-C-1, Hastings from Northern Saskatchewan, hybrid between ARS-Y-C-1 and Hastings, and bees from Louisiana served as control. Eighty colonies (20 colonies per bee type) were used for Trial 1 (1990-1992), and 40 colonies (10 colonies per bee type) for Trial 2 (1991-1992). For each colony, external *Acarapis* infestations were estimated by subsampling

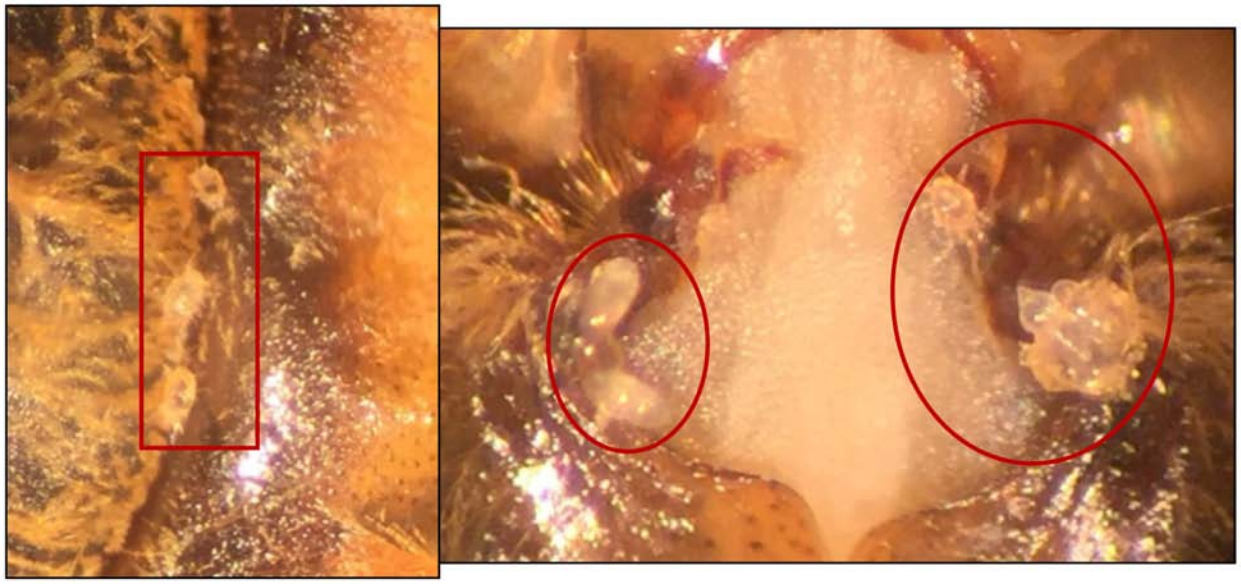

Figure 1. (a) *Acarapis dorsalis* adults on the dorsal groove of the thorax, and (b) different stages of *Acarapis externus* glued with a mucus-like substance on the neck of an adult honey bee.

30 worker bees from a sample of about 300-500 bees per colony. Data on prevalence and mite intensity were subjected to analysis of variance (ANOVA) for repeated measures using the Mixed Procedure (SAS Institute, Inc. 1992). Before analysis, data for the proportion of bees infested and mite intensity were transformed using the arcsine and square root transformation, respectively.

2.3. Prevalence and intensity of *A. dorsalis* and *A. externus* in mixed- and single-stock apiaries (2001-2002)

Honey bee colonies used for *Varroa* research [26] were examined for external *Acarapis* infestations in 2001 to 2002. In two apiaries with a mixture of Russian (n = 20) and Italian (n = 21) colonies, samples were collected in October 2001 and then in October 2002. Apiaries with single stocks were also monitored in May 2002 and October 2002: two apiaries having only Russian colonies (n = 54) and two apiaries having only Italian colonies (n = 45). Mite infestation parameters were estimated as described above. Data for mixed-stock and single-stock apiaries were analyzed separately. To better approximate normality, arcsine square-root transformation was used to transform data on prevalence, and square-root transformation was used for data on mite intensity. First, a two-factor mixed measures analysis was performed to determine the effects of honey bee type and date of observation. Where interactions occurred, post-hoc *t*-tests were conducted to determine significant differences [27].

2.4. Prevalence and mite intensity according to honey bee age (1988)

Newly emerged bees (<24 h old) were paint-marked and introduced into two host colonies. One host colony was infested with *A. dorsalis* and the other was predominantly infested with *A. externus*. From each host colony, 50 bees were sampled every five days and examined under a dissecting microscope for the presence of the two external *Acarapis* for up to 40 days. Prevalence and mite intensity of both *Acarapis* species were recorded. Data on infestation were analyzed using frequency tables to compare the infestation trends throughout the age of the host bees (PROC FREQ).

2.5. Mite population growth (1988)

To follow population growth, known mite populations were introduced into mite-free nucleus colonies. This was an attempt to establish a founding population that would enable *Acarapis*

mites to grow larger populations in colonies. Nucleus colonies are more manageable units than standard colonies and were kept small by removing 1-2 frames of brood every week to prevent swarming. Since brood is not infested by *Acarapis* mites, brood combs of emerging bees were reared in an incubator to obtain the worker populations of the nucleus colonies (n = 36) used in this study. For each nucleus colony, two frames with honey and pollen and two brood frames without adult bees were used. Newly mated, uninfested queens were installed.

Acarapis mites are microscopic and are difficult to introduce into a colony. Therefore, infested bees (2-3 days old) with known mite populations were used as inoculum. These infested bees were obtained by marking newly emerged bees and introducing them into an infested colony. After 48-72 h, marked bees were recovered and examined under a dissecting microscope. Since both external *Acarapis* species are found on the wings during migration and sometimes during reproduction, mites within the axillaries, on the wings, and on the bees' body were discarded to prevent possible species confusion. Mites on the scutoscutellar groove of the thorax were considered as *A. dorsalis* and those on the neck region were *A. externus*. Due to the paucity of adult mites, eggs also were used to attain the required number of mites per treatment. Inoculum bees were placed in a screen cage, introduced into the colonies and then released after 2 days. For each species, 12 nucleus colonies (six received 50 mites and six with 500 mites) were used. Population development was also observed in nucleus colonies (n = 6 per treatment) deliberately infested with equal numbers of both species (25 *A. dorsalis* + 25 *A. externus* or 250 *A. dorsalis* + 250 *A. externus*). Sampling was done after 10-15 days to give ample time for the mites to reproduce. After arcsine transformation, a two-way factorial ANOVA was used to analyze the percentages of infestation.

3. RESULTS

3.1. Prevalence of *A. dorsalis* and *A. externus* (2007 to 2019)

Of the 554 colonies (n = 16,515 worker bees) examined, about 50% (279 colonies) were infested with *A. dorsalis* and only ~2% (10 colonies) were infested with *A. externus* (Table 1). On average, prevalence of the two external *Acarapis* species

Table 1. Number of colonies sampled from different locations, and the number of colonies infested with *Acarapis dorsalis* or *Acarapis externus*. LA - Louisiana, KS - Kansas, AR - Arkansas, MS - Mississippi, FL - Florida.

Year	Place of collection	# colonies examined	*A. dorsalis*				*A. externus*			
			0	1-10	11-20	≥21	0	1-10	11-20	≥21
2007	LA	32	7	15	5	5	32	0	0	0
2008	LA	27	2	16	6	3	24	1	0	2
2009	LA	25	7	9	4	5	21	2	0	2
2010	LA	23	11	10	1	1	22	1	0	0
2011	KS & LA	90	28	57	5	0	90	0	0	0
2013	AR & LA	67	47	20	0	0	65	2	0	0
2014	LA	23	20	3	0	0	23	0	0	0
2016	LA	30	14	13	1	2	30	0	0	0
2017	LA	50	37	8	4	1	50	0	0	0
2018	MS, LA & FL	104	79	25	0	0	104	0	0	0
2019	AR & LA	83	23	37	17	6	83	0	0	0
	TOTAL	**554**	**275**	**213**	**43**	**23**	**544**	**6**	**0**	**4**

were low (Figure 2). *A. dorsalis* was detected every year with the highest infestation of about 10% observed in 2007 to 2009 (the maximum infestations were 60% and 53%, respectively). The proportion of colonies infested with *A. dorsalis* in 2019 parallelled that of 2009 with prevalence approaching the same level. *A. externus* was only recorded in 2008-2010, and 2013 with the highest level of 6.8% observed in 2009 (two colonies of which had 77% and 87% infestations).

3.2. Prevalence and intensity in four stocks of honey bees (1990 to 1992)

3.2.1. Prevalence of *Acarapis dorsalis*

There was a significant interaction between bee type and sampling month on the levels of *A. dorsalis* infestation for trial 1 (P = 0.028) (Figure 3a). Before test queens were introduced into the colonies in June 1990, comparably low levels of *A. dorsalis* infestation of the colonies were observed (P = 0.826). By August 1990, a distinct drop in *A. dorsalis* infestation was recorded. This decrease was probably due to the acaricide treatment applied for *Varroa* control in June 1990. Rates of *A. dorsalis* infestation in the Hastings stock apparently grew faster than in any of the other test stocks. A clear trend was observed in both years of observation. Infestations in Hastings stock started to increase in October with the highest infestation recorded in December and February. The lowest infestation was observed in August. In the F_1 hybrid, ARS-Y-C-1 and Louisiana stocks, *A. dorsalis* infestations remained well below 5% infestation levels throughout the study.

For trial 2, a significant interaction between stock and sampling month was also detected (P = 0.038) (Figure 3b). The same trend was observed. Hastings stocks had the highest infestations throughout the study with a peak of about 17% observed in October 1991. Similarly, the three other stocks maintained less than 5% levels of *A. dorsalis* infestation throughout the experimental period.

3.2.2. Prevalence of *Acarapis externus*

A significant interaction between honey bee stocks and sampling month was also observed for the proportion of bees infested with *A. externus* for trial 1 (P = 0.0004) (Figure 4a). The initial infestation of this *Acarapis* species did not differ significantly among the stocks (P = 0.983), which ranged from 11 ± 5% to 14 ± 5%. For the first year of evaluation, infestation of *A. externus* was maintained at the comparably low levels (below 10%) for all the stocks. In August 1991, infestations gradually increased in the hybrid colonies and Louisiana stocks. All stocks increased in infestations in October before the last colony representing Louisiana stock died because of *Varroa* infestation. Peaks of *A. externus* infestations were observed in December for the F_1 hybrid and ARS-Y-C-1 stocks. A distinct decrease in infestation levels was observed in February in the surviving colonies and remained low until the end of experiment.

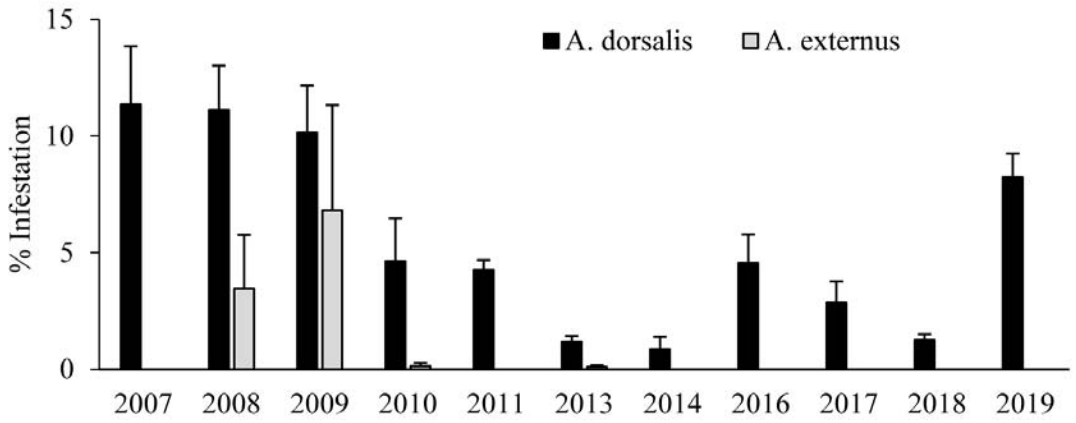

Figure 2. Prevalence of *A. dorsalis* and *A. externus* in *Apis mellifera* colonies collected from different locations in 2007 to present.

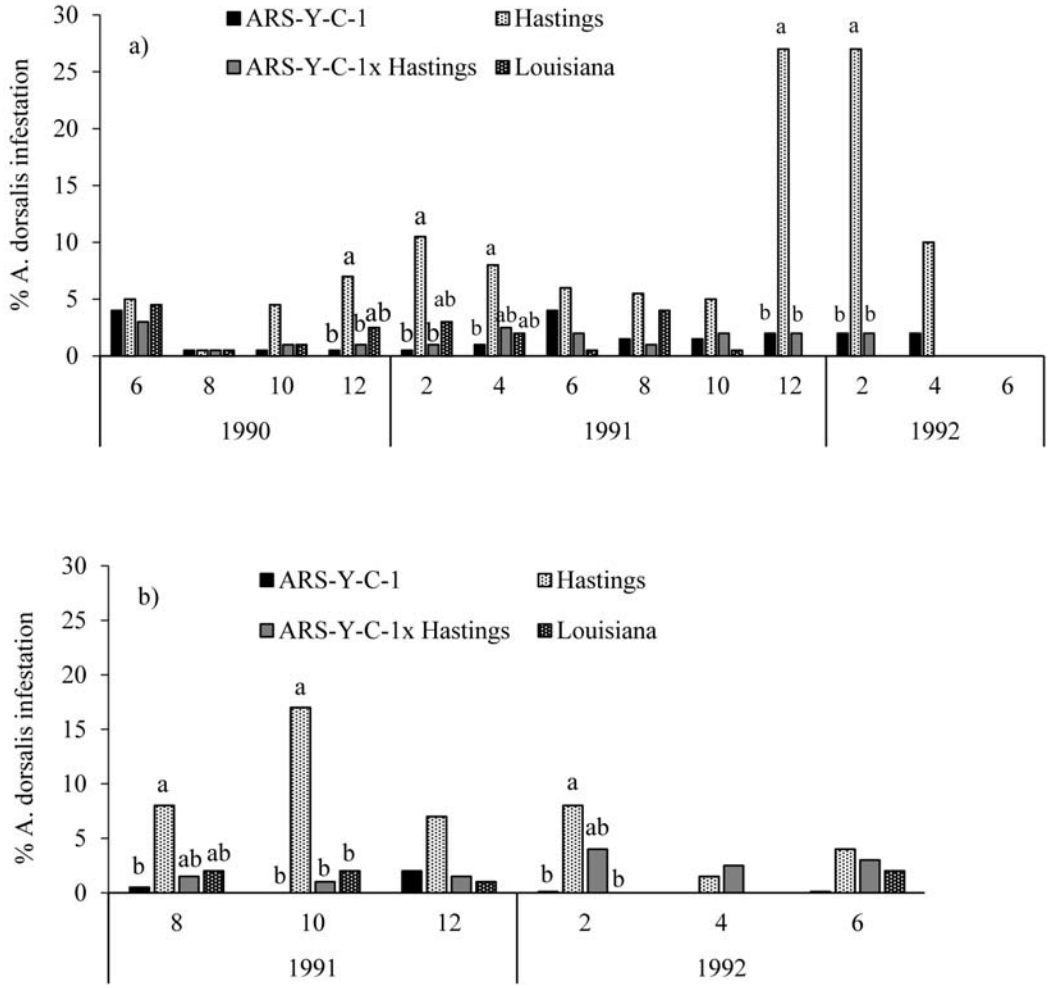

Figure 3. Prevalence of *A. dorsalis* in four stocks of honey bees for (a) trial 1 (June 1990-1992) and (b) trial 2 (August 1991-1992). For each month, bars with different letters are significantly different; unlabeled groups of stocks do not differ significantly ($P > 0.05$).

A. externus in the Hastings stock maintained the lowest infestation (about 2%) throughout the experimental period except in October 1991.

In trial 2, no significant interaction between stocks and month was detected ($P = 0.348$) (Figure 4b). Stock effect also showed no significant differences ($P = 0.923$).

3.2.3. Intensity of *Acarapis dorsalis* and *A. externus*

Mite intensity was monitored from August 1990 to August 1991 (trial 1 only). No significant interaction ($P = 0.14$) and no significant differences in the numbers of *A. dorsalis* per infested bee were observed among honey stocks ($P = 0.156$) (Figure 5a). Likewise, initial numbers of *A. dorsalis* did not differ among stocks ($P = 0.382$). Worker bees from any stock infested with this mite species sustained about 1-3 mites through time.

For *A. externus*, a significant interaction between stock and sampling month was detected ($P = 0.0003$) (Figure 5b). The initial number of *A. externus* per infested bee did not differ significantly ($P = 0.272$) among the stocks, which ranged from 2.59 ± 0.35% to 3.47 ± 0.31%. For the first 10 months (until April), mite load in all the test stocks was limited to about 1 mite only. However, a sharp increase to about 2-3 mites in June and August was observed in the F_1 hybrid colonies.

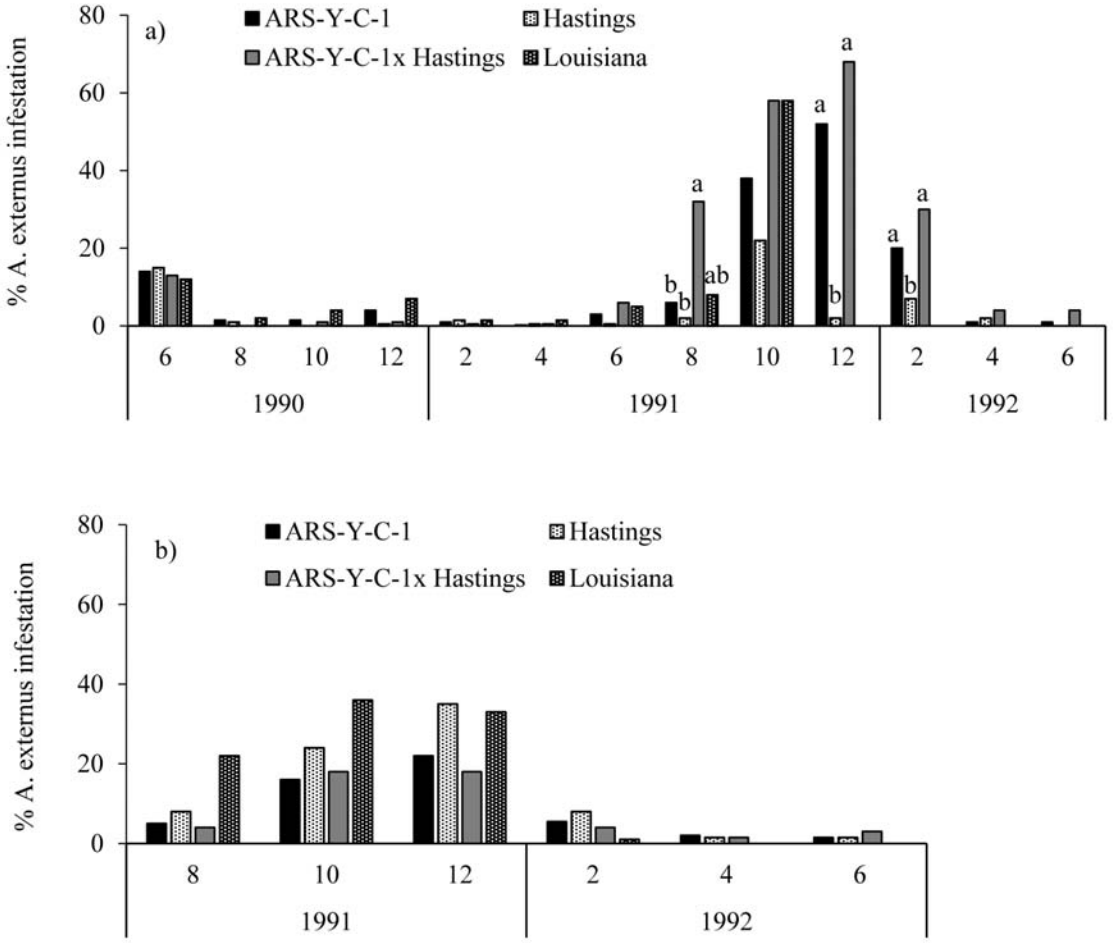

Figure 4. Prevalence of *A. externus* in four stocks of honey bees for (a) trial 1 (June 1990-1992) and (b) trial 2 (August 1991-1992). For each month, bars with different letters are significantly different; unlabeled groups of stocks do not differ significantly ($P > 0.05$).

3.3. Prevalence and intensity in mixed- and single-stock apiaries (2001 to 2002)

3.3.1. Mixed stock apiaries

Overall, the prevalence of *A. dorsalis* was lower (below 20%) than that of *A. externus* in apiaries having a mixture of Italian and Russian honey bee colonies. For the prevalence of *A. dorsalis*, ANOVA revealed no two-way interaction ($F = 0.28$, $P = 0.599$), and no influence of sampling time ($F = 3.44$, $P = 0.068$) (Figure 6a). However, a significant influence of honey bee type was detected with the Italian colonies (7.1 ±1.0%) supporting lower *A. dorsalis* infestation than the Russian honey bees (13.4 ± 2.4%) ($F = 4.8$, $P = 0.031$). For *A. externus*, no two-way interaction was observed ($F = 1.78$, $P = 0.187$) (Figure 6b). In contrast, the Russian honey bees had lower *A. externus* infestation (35.3 ± 4.5%) than the Italian bees (49.4 ± 4.0%) ($F = 6.94$, $P = 0.010$). A significant increase in *A. externus* infestation was recorded through time: from 31.9 ± 4.3% in October 2001 to 53.3 ± 3.8% in October 2002.

For the number of *A. dorsalis* per infested bee, no two-way interaction ($F = 0.10$, $P = 0.754$), and no effects of honey bee type ($F = 1.25$, $P = 0.268$) or sampling time ($F = 3.40$, $P = 0.070$) were observed (Figure 6c). For the intensity of *A. externus*, no significant interaction between honey bee type and sampling time was detected ($F = 0.44$, $P = 0.508$) (Figure 6d). However, we found that infested Italian bees had higher numbers of *A. externus* (4.06 ± 0.15) than Russian bees (3.45 ± 0.19)

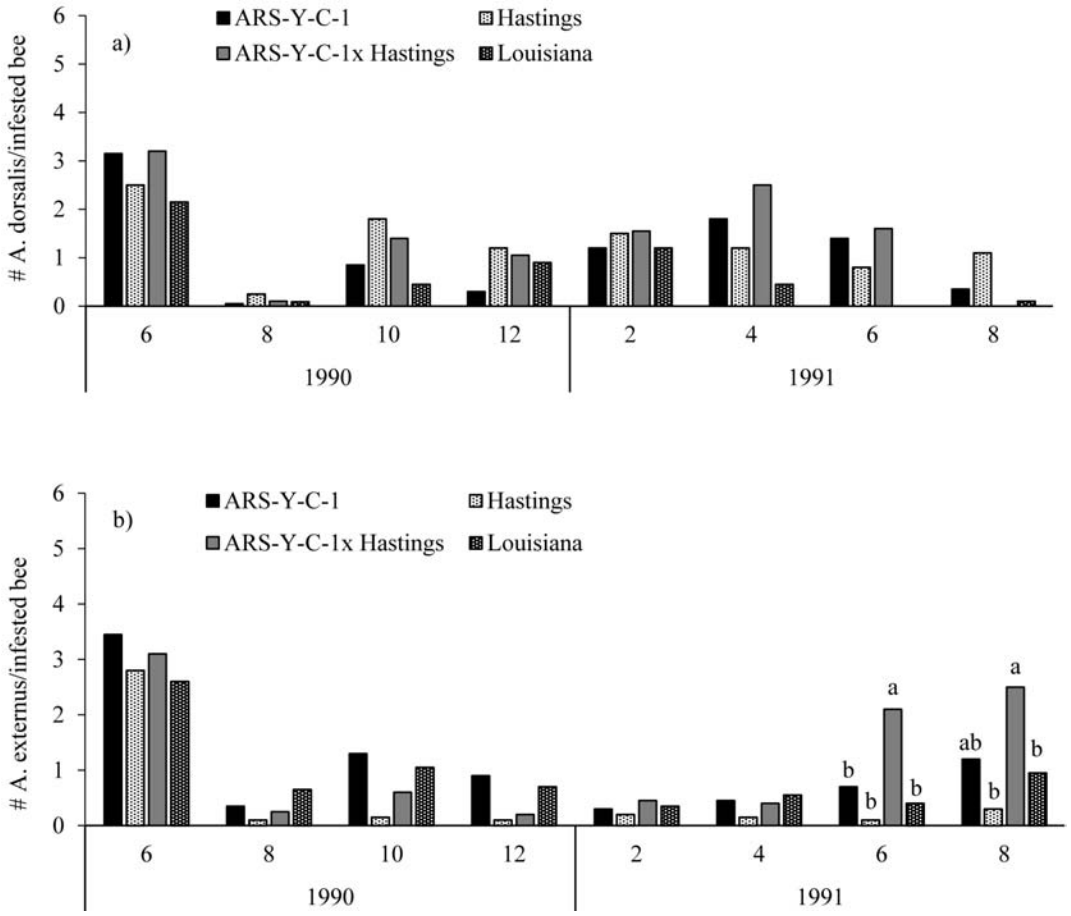

Figure 5. Intensity of (a) *A. dorsalis* and (b) *A. externus* in four stocks of honey bees for June 1990 to 1991. For each month, unlabeled groups of stocks do not differ significantly ($P > 0.05$).

(F = 7.23, P = 0.009). Overall, the number *A. externus* per infested bee decreased at the end of the experiment (from 4.32 ± 0.15 to 3.35 ± 0.16) (F = 20.94, $P < 0.0001$).

3.3.2. Single stock apiaries

Infestations by *A. dorsalis* were also lower than *A. externus* even when colonies of each honey bee type were isolated from each other. ANOVA revealed no two-way interaction (F = 0.01, P = 0.907) for the prevalence of *A. dorsalis* (Figure 7a). However, honey bee type (F = 5.07, P = 0.025) influenced *A. dorsalis* infestation with the Russian honey bees (8.6 ± 0.9%) having a higher prevalence than the Italian bees (6.1 ± 0.8%). *A. dorsalis* infestation was lower in October 2001 (5.7 ± 0.8%) than in October 2002 (9.2 ± 0.9%) (F = 13.54, P = 0.0003). A similar trend was observed for the prevalence of *A. externus* (Figure 7b). Russian colonies (27.3 ± 2.7%) had higher *A. externus* infestation than the Italian bees (17.6 ± 2.3%) (F = 15.21, P = 0.0001). Infestations also increased significantly at the end of the experiment (from 5.1 ± 1.1 to 40.7 ± 2.4%) (F = 245.8, $P < 0.0001$).

The intensity of *A. dorsalis* was affected by an interaction between the two variables (F = 11.57, P = 0.0009) (Figure 7c). Both stocks had similar numbers of mites at the beginning of the experiment while the Italian bees sustained higher numbers than the Russian bees at the end of the experiment (3.83 ± 0.48 vs 2.68 ± 0.13 *A. dorsalis*). For *A. externus*, a two-way interaction (F = 17.25, $P < 0.0001$), and significant effect of sampling time (F = 5.67, $P < 0.019$) were observed (Figure 7d). In October 2001, Russian bees supported higher

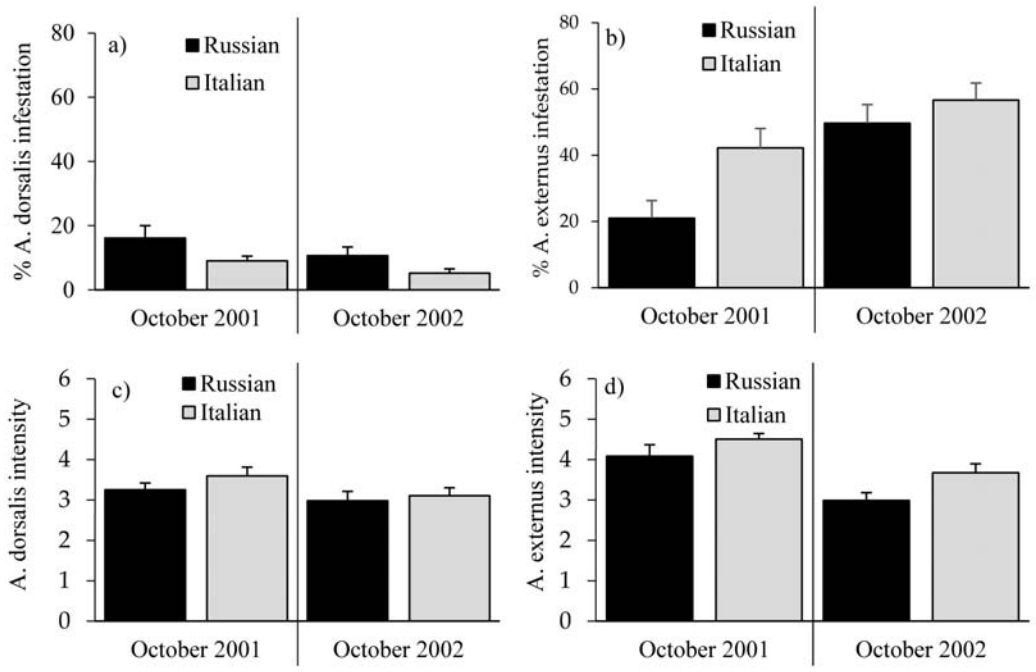

Figure 6. Prevalence and intensity of *A. dorsalis* (a, c) and *A. externus* (b, d) when Russian and Italian colonies were kept in the same apiaries. For each month, unlabeled groups of stocks do not differ significantly ($P > 0.05$).

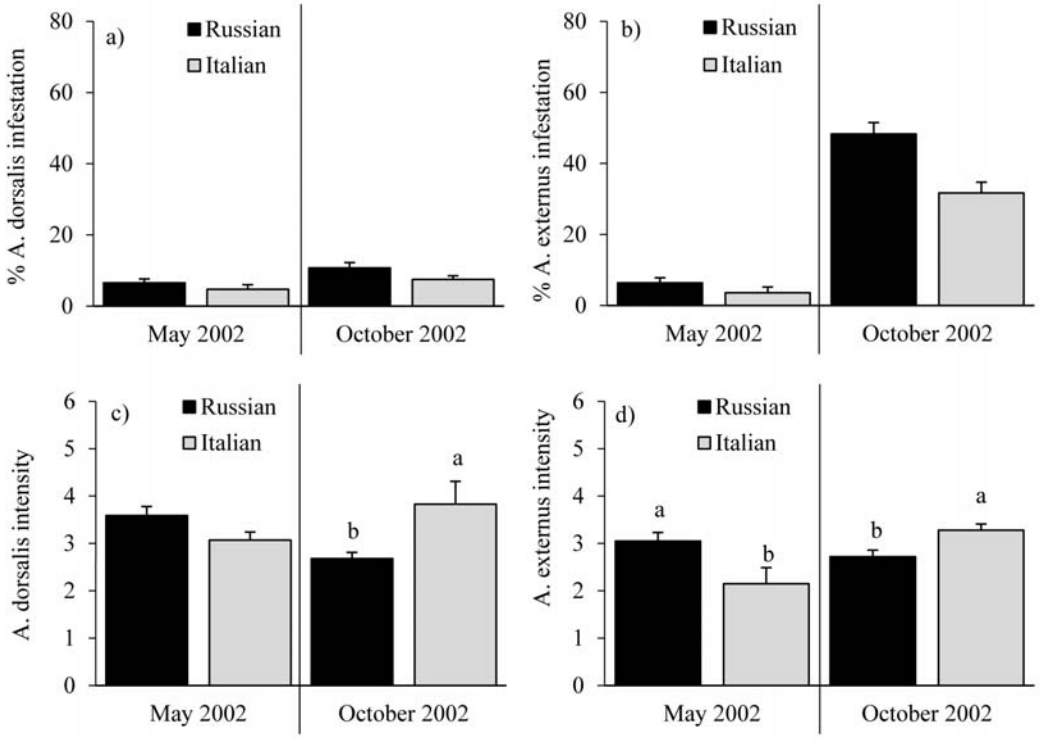

Figure 7. Prevalence and intensity of *A. dorsalis* (a, c) and *A. externus* (b, d) when Russian and Italian colonies were kept in different apiaries. For each month, bars with different letters are significantly different; unlabeled groups of stocks do not differ significantly ($P > 0.05$).

mite numbers than the Italian bees. However, Italian bees had more mites than the Russian bees at the end of the experiment (October 2002). Honey bee type did not affect the intensity of *A. externus* (F = 1.28, *P* = 0.260).

3.4. Prevalence and mite load according to honey bee age (1988)

Overall, infestations of *A. externus* were higher than those of *A. dorsalis* (Figure 8a). Our results showed a decrease in the infestation percentage of *A. dorsalis* on the 25th day (χ^2 = 15.73, *P* = 0.028). Percentage of infestation by *A. externus* remained relatively high for bees 35 days of age and declined thereafter (χ^2 = 40.49, *P* < 0.0001).

Mite load was generally higher for *A. externus* than *A. dorsalis* through time (Figure 8b). Differences were more pronounced when the bees were 15, 20, 30 and 35 days old. While a decline in *A. dorsalis* intensity was observed on the 20th day, the drastic decline in *A. externus* intensity was not observed until the 40th day, when marked bees were approaching the end of their life span. No more marked bees were present in the experimental colonies 40 days post introduction.

3.5. Mite population growth

ANOVA revealed that population growth was significantly affected by *Acarapis* species (*P* < 0.0001), number of mites introduced (*P* < 0.0001) and the combination of the two variables (*P* = 0.026). Infestations by *A. dorsalis* can increase rapidly in a relatively short period of time, reaching 25 to 40% within 9-12 weeks (Figure 9a). In contrast, infestations by *A. externus* only attained 6% in three months (Figure 9b). Regardless of the number of mites introduced, *A. dorsalis* showed a higher rate of infestation having an average of 12.71% as compared to 1.49% for *A. externus*. Overall, introducing 500 mites (*A. dorsalis* = 17.1%; *A. externus* = 2.67%) established a higher rate of mite infestation, which was faster than introducing 50 mites (*A. dorsalis* = 8.31%; *A. externus* = 0.31%). Further, nucleus colonies deliberately infested with only *A. dorsalis* maintained this species as their sole parasite. In contrast, *A. externus*-infested colonies were invaded by *A. dorsalis* within six weeks (Figure 9b).

Higher rates of infestation by *A. dorsalis* were also observed when an equal number of each *Acarapis* species was introduced into mite-free nucleus colonies. A low-level infestation by *A. dorsalis* and no *A. externus* were observed 1½ months after 25 mites of each species were introduced into the mite-free colonies (Figure 9c). At the higher initial mite inoculation, *A. dorsalis* always had a higher infestation rate that *A. externus* in all nucleus colonies observed (Figure 9d).

4. DISCUSSION

This study showed that *A. externus* and *A. dorsalis* infestations were commonly observed in managed *A. mellifera* colonies in the late 1980s to early 2000. However, infestation by *A. externus* was generally higher (up to 100%) than that by *A. dorsalis*. The apparent dominance of *A. externus*

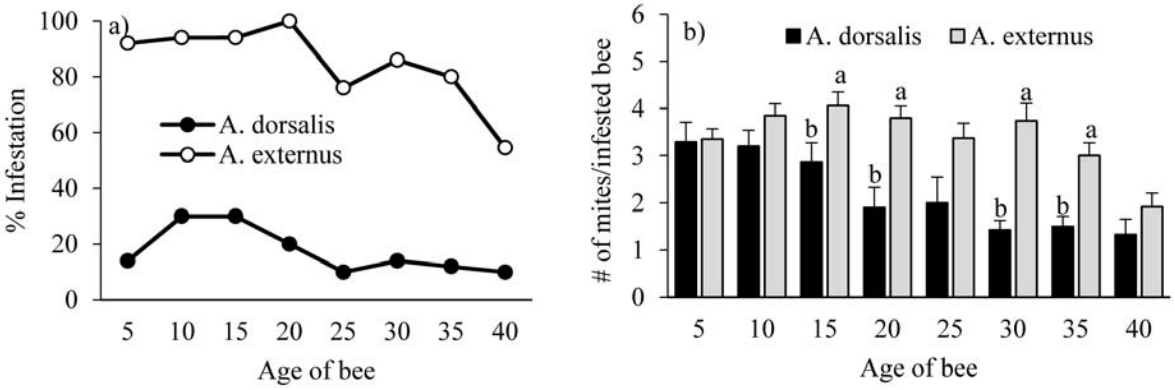

Figure 8. (a) Prevalence and (b) variations in mite intensity of *A. dorsalis* and *A. externus* in honey bees of various age.

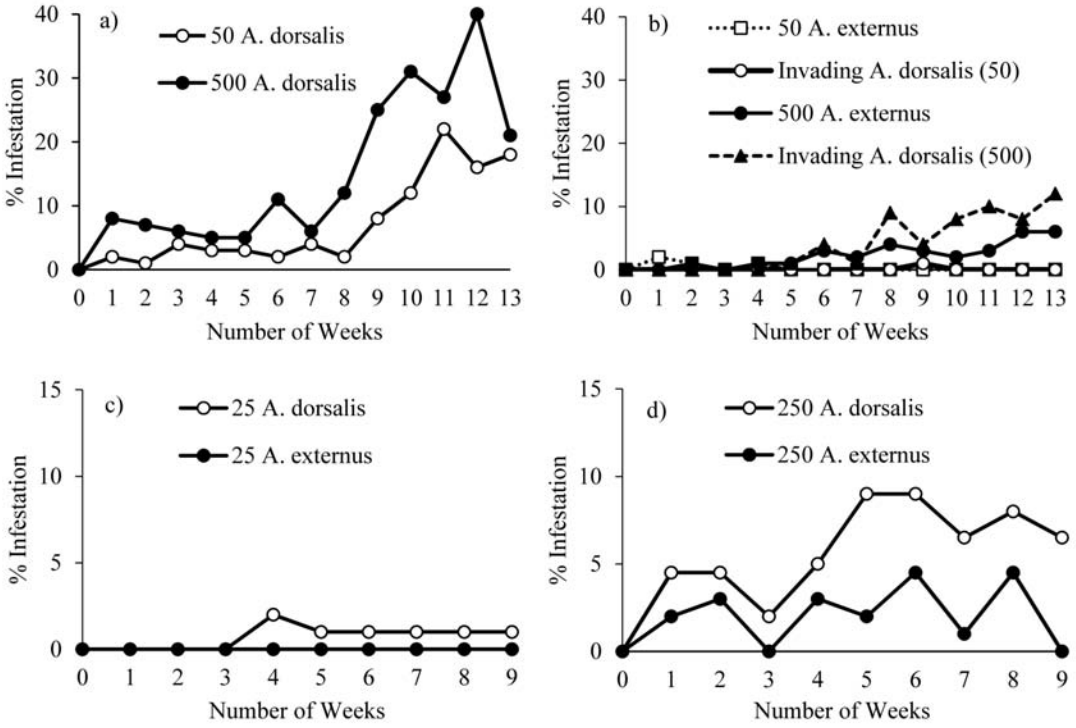

Figure 9. Population growth of external *Acarapis* following introduction of varying number of mites into mite-free nucleus colonies: a) 50 *A. dorsalis* vs 500 *A. dorsalis*, b) 50 *A. externus* vs 500 *A. externus*, c) 25 *A. dorsalis* + 25 *A. externus*, and d) 250 *A. dorsalis* + 250 *A. externus*. Nucleus colonies deliberately infested with *A. externus* only (b) were eventually infested with *A. dorsalis*.

over *A. dorsalis* in colonies with established mite populations may be due to the lack of host age preference by *A. externus*. In marked honey bees introduced into host colonies for infestation, the number of *A. externus* (mite intensity) found on each infested marked bee was usually higher than that of *A. dorsalis*. It appears that *A. externus* is more capable than *A. dorsalis* in surviving on older bees. Relatively high prevalence and mite intensity of both species when hosts are younger could be an indication of mite preference for younger bees. However, this observation could also be attributed to the temporal division of labor among honey bees. While old bees generally leave the hive to forage for nectar and pollen, young worker bees clean, build combs and care for the brood [28-30]. Since the majority of young bees are hive bees, there are potentially more contacts among young bees. This condition could facilitate the transmission of mites from infested to uninfested bees, with the subsequent increase in infestation level and mite load. For *A. dorsalis*, the decline in the rate of infestation and mite load as the bees become older may be advantageous for the mites. At the onset of foraging activity (23 days), bee mortality increased markedly, thereby drastically affecting mite population in the colony. Having high mite load in the older bees for *A. externus* may be advantageous for mite dispersal through robbing or drifting by older foraging adults. Robbing or drifting had been found to accelerate spread of *Varroa* mites from colony to colony [31, 32]. Natural introduction of mites into uninfested colonies is probably caused not only by drifting of older foraging bee adults, but also by lost young bees as they stray into other hives during orientation flights. Additional factors, which should be considered include swarming and management techniques such as division of colonies, joining of weak colonies, and migratory beekeeping.

While we detected *A. dorsalis* yearly from 2007 to 2019 and only four times for *A. externus*, the average levels of infestation were lower than

those observed in the 1980s to early 2000. It is possible that acaricides applied to colonies for *Varroa* and tracheal mite control also killed external *Acarapis* species. However, old and recent samples from Louisiana (2001-2002, 2007-2019) were collected from our experimental or maintenance colonies that never received acaricide treatments. Hence, the decline of external *Acarapis* may be due to the death of mites together with their hosts. The massive die-off of managed *A. mellifera* colonies from CCD in 2006 [12], which continued for years, may be enough to cause the significant decline of *Acarapis* mite population. Both external *Acarapis* mites are specific to honey bees; thus this disruption may be enough to drive these mites to significantly lower levels. Nonetheless, the frequency of *A. dorsalis* detection over *A. externus* may be due to their differences in colonization ability. When introduced either separately or simultaneously into mite-free nucleus colonies, we found that *A. dorsalis* can establish itself more rapidly in a colony than *A. externus*. Further, nucleus colonies deliberately infested with only *A. dorsalis* maintained this species as their sole parasite, whereas those infested with *A. externus* were eventually invaded by *A. dorsalis*. This invasion may be due to drifting of foraging bees from *A. dorsalis*-infested colonies at the apiary. It is also possible that *A. dorsalis* outcompetes *A. externus* in these colonies. However, interaction between these two *Acarapis* species needs further study. There may be a critical mite population required for both species to establish a viable population. The introduction of 500 *A. externus* may be too small an initial population, so that *A. dorsalis* can easily outcompete them. *A. dorsalis* may dominate a host colony at lower mite populations. As noted earlier, where uninfested bees were introduced into a mite-infested colony, *A. externus* appeared to have higher rate of increase that *A. dorsalis*. The reverse seemed to be true for the rate of infestation in initially mite-free nucleus colonies inoculated with infested bees. This observation suggests that a critical population must be attained by *A. externus* to establish itself in a colony. Unless this critical population is reached prior to invasion by *A. dorsalis*, *A. externus* appears to be unable to maintain its population in a hive. The experiments described here did not reveal the critical mite population requirement for either mite species, but from our available data, *A. externus* probably requires an initial population higher than 500 mites. For *A. dorsalis*, introduction of 50 mites was probably sufficient for establishing a viable population in an uninfested colony. This estimate is supported by Figure 8a in which a maximum infestation of 30% is shown for *A. dorsalis*, a level very close to the highest infestation reached by introducing 50 *A. dorsalis* into mite-free nucleus colonies with an average population of 5,193 bees.

Different stocks of honey bees respond differently towards parasitic mites and these responses can profoundly influence their populations. In this study, *A. dorsalis* was most prevalent in the Hastings stock while levels of *A. externus* were higher on the ARS-Y-C-1, hybrid between Hastings and ARS-Y-C-1 and Louisiana stocks. Russian honey bees had higher levels of *A. dorsalis* than the Italian honey bees. However, the stock's response to *A. externus* was inconsistent. Nonetheless, both ARS-Y-C-1 and Russian honey bees are known to be resistant to *A. woodi*, which is a more serious parasite of honey bees than the two external *Acarapis* [33]. Russian honey bees are also resistant to *Varroa* mites [34, 35]. Hence, limited or no use of acaricides in *Varroa* or tracheal mite-resistant stocks may allow the development of external *Acarapis* population in these colonies. Nevertheless, the potential role of external *Acarapis* in pathogen proliferation in these colonies or in colonies infested with *Varroa* or tracheal mites needs to be studied.

5. CONCLUSION

In the US, *A. dorsalis* and *A. externus* were common parasites of adult honey bees in the 1980s to early 2000 when infestations reached up to 100%. Results of our survey from 2007 to 2019 showed that *A. externus* infestation was sporadic with the highest infestation of 87% observed in 2009. In contrast, *A. dorsalis* is frequently detected with the highest infestations of 60% and 53% observed in 2007 and 2019, respectively. Although both mites are considered parasites of adult honey bees, their pest status is largely unknown. With the recent discovery of several types or variants of viruses infecting honey bees,

the potential role of these parasitic mites in virus transmission needs to be studied.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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