Effects of *Varroa destructor* Infestation on Honey Bee Queen Introduction

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Summary

The varroa mite (*Varroa destructor*) is very detrimental to honey bee, *Apis mellifera,* colonies that are not genetically resistant. Italian colonies are known to be susceptible to mites, and queen introduction has been reported to be more difficult in Italian colonies in recent years. This study compares supersedure rates in Italian colonies to infestation levels of *V. destructor.* Sixty-one Italian colonies divided into groups with comparatively high and low levels of infestation were observed for any queen changes during six weeks following the introduction of a mated queen. Colonies that had supersedure queens were smaller and had higher rates of mite infestation than colonies that retained their original queen. Supersedure and colony deaths were greater in colonies that were more highly infested.

Apis mellifera / supersedure / Varroa destructor

Introduction

The varroa mite (*Varroa destructor*) is an external parasite of the honey bee (*Apis mellifera*) that feeds on the hemolymph of immature and adult bees (Harbo and Harris, 2001). This specialized blood-feeding mite species reproduces within sealed brood, showing a strong preference for drone brood over worker brood (Martin, 2001). Parasitism causes weight loss, wing deformities and sometimes loss of appendages of the emerging bee (De Jong et al., 1982b). High infestation of V. *destructor,* in colonies that lack innate mite suppression characteristics, ultimately leads to the death of that colony (Harbo and Harris, 2001). Mites from a collapsed colony can be dispersed to other colonies on adult bees through behaviors such as robbing, drifting and absconding (Martin, 2001). This parasite has spread rapidly and now infests most of the world's *Apis mellifera* causing much concern to beekeepers (Rinderer at al., 2001; Sanford, 2001).

Colonies naturally rear new queens (supersedure) when old queens are lost or failing and prior to swarming. Beekeepers take advantage of the queen replacement process to create colonies having queens of selected stock. Original queens are removed from colonies and, as the queen replacement process continues, new queens of selected stock are placed or "introduced" into the colonies. Most introductions of new queens are initially successful. However, some introduced queens remain in the colonies only a short time before they are superseded. There are several reasons, beyond the scope of this study, which could cause queen introductions to fail including queen age at introduction, climate, availability of nectar and pollen, general foraging conditions, worker bee behavior toward the new queen, season of queen introduction and general hive conditions (Rhodes et al., 2004; Mangum, 1997). Introduction difficulties are a concern to beekeepers since queens are costly and colonies that fail to accept new queens either develop more slowly or are lost (Rhodes et al., 2004).

There have been reports that rates of supersedure soon after introducing new queens appear to be higher than in past years, especially for Italian honey bees (Guzman-Novoa et al. 1998; Pettis et al., 2004). Italian honey bees are known to be very susceptible to infestation by varroa mites (Rinderer at al., 2001). We suspected that high mite populations may cause an increase in queen supersedure rates. Little or no data are available on the relationship of mite populations to supersedure rates; therefore we conducted a study to determine if higher levels of varroa mites in Italian colonies are associated with queen introduction failure and higher supersedure rates.

Materials and Methods

Sixty-one queenless colonies were prepared by splitting

established Italian colonies. Prior to splitting, rates of mite infestation for adult bees had been estimated in the established colonies from samples of adult bees (200 to 400 adult bees). We chose six colonies with low infestations ($\bar{x}$ = 1% ± 0.2) and five colonies with high infestations (x = 16% ± 0.7). We divided these colonies to produce 34 queenless units considered to have high infestations and 27 queenless colonies considered to have low infestations. All colonies had three 2.5–3 frames of brood on 16.5 cm Langstroth frames and 0.5 to 0.75 Kg of worker bees. The 61 colonies were moved to a new apiary location and arranged randomly by coin toss.

Italian queens were purchased from one California queen breeder for this experiment. Queen's were installed into each colony using a "California mini-cage" with candy release. Queens were both marked with paint and wing clipped for later identification. Eight days after introduction, colonies were inspected for the presence of the marked queens. At that time all queens were seen and considered to be initially successfully introduced. Three weeks later, colonies were inspected for the presence of marked queens and the hives were expanded with an additional box of comb as necessary to prevent swarming.

Six weeks after queen introduction the colonies were again examined. Their queen status was determined. Colonies either had a laying introduced queen, a laying supersedure queen, a virgin supersedure queen or queen cells. Colonies in these last two groups were considered to be in the process of superseding and were classified as supersedure colonies along with those having a laying supersedure queen.

As well as determining the status of the queens, colonies were evaluated for size and mite infestation using the procedures of Rinderer et al. (2001). Estimates of mites in colonies and the sizes of colonies were obtained from: (a) counts of mites in 200 sealed worker brood cells (50 from each side of two combs), (b) counts of mites in 50 sealed drone brood cells (25 from each side of one comb or from several nest areas when drone brood was scattered), (c) the percentage of adult bees infested as determined from washes (in a soap and water solution (Rinderer et al. (2004)) of a sample of 300 to 600 adult bees, in which the number of bees and mites in the sample were counted, (d) comb by comb estimates (to the nearest 5%) of the numbers of sealed worker and drone brood cells in the hive, and (e) comb by comb estimates of the numbers of bees (to the nearest 5%) which make up the colony (Rinderer et al., 2001). The mite counts from brood sampling were obtained by opening cells through the center of the brood pattern and identifying the number of adult female mites within each cell.

The effect of mite levels on colony death rates and supersedure rates were evaluated with Fisher's Exact Tests. A third Fisher's Exact Test (SAS version 8.2, SAS institute, 2001) was used to compare long term success of queen introductions between the two infestation groups. Unsuccessful longevity of introduced queens included supersedures and colony deaths.

A series of two-way ANOVAs with infestation level and supersedure classification (colonies that did and colonies that did not supersede) used as fixed effects (SAS version 8.2, SAS institute, 2001) were calculated. Three measures of numbers of mites (number of mites infesting brood, number of mites infesting adults, and total colony mites), three measures of the rates of infestation (percentage of brood infested, percentage of adults infested and the percentage of infestation for the entire colony population of brood plus adults) and three measures of colony size (numbers of brood, numbers of adult bees, and the total colony population of brood plus adults) were analyzed.

Since the variables related to numbers of mites and percentages of infestation had significant or nearly significant interactions between the fixed effects of infestation level and supersedure, a series of t-tests were conducted to better understand the nature of the interactions.

Results

Initial levels of mite infestation estimated from samples of adult bees were classified as high or low. Five of the colonies with high infestations died within the first three weeks of the study indicating that the queen introduction failed without a successful supersedure queen being produced. A comparison of the death rate between highly infested and less infested colonies was statistically significant (Fisher's Exact Test P = 0.05) (Fig. 1a). After three weeks, nine of the more infested colonies had lost the introduced queen and were in the process of producing a supersedure queen. One of the less infested colonies was superseding its queen. This difference in supersedure rate is statistically significant (Fisher's Exact Test P = 0.007.) (Fig. 1b)

After six weeks, of the 29 colonies in the highly infested group, 15 produced supersedure queens or were in the process of superseding (Fig. 1b), and of the 27 colonies in the low infestation group, 10 produced supersedure queens or were in the process of superseding. The difference after six weeks between the supersedure rates in the highly infested colonies compared to the less infested colonies approaches significance (Fisher's Exact Test P =0.11) (Fig. 2a). We also compared requeening failures between the highly infested and less infested colonies. Both colony death after queen installation and supersedures were considered requeening failures. Of the 34 colonies in the highly infested group, 20 experienced queen loss and of the 27 colonies in the low infestation group, 10 experienced queen loss. The difference in the queen failure rate between highly infested and less infested colonies is significant (Fisher's Exact Test P =0.04 (Fig. 2b).

ANOVAs for all of the colony size measures (combs of brood, numbers of brood, combs of adult bees, numbers of adult bees, and overall colony size) (Table) showed insignificant differences between colonies originally having high or low levels of infestation. Generally, colonies originally classified as having lower levels of infestation had numerically larger amounts of brood and adult bees. The percentages of numerical size differences varied: numbers of brood, 16% larger; numbers of adult bees, 6% larger and total colony size, 13%. For the comparison of colonies that had superseded to those that had not superseded, the colonies with supersedure queens were significantly smaller for all measures of colony size (numbers of brood, P = 0.004; numbers of adult bees, P = 0.002 and overall colony size, P = 0.0008). These measures were 42%, 61% and 48%, respectively, larger in those colonies that had not superseded.

The ANOVAs of absolute measures of varroa mites (mites in worker brood, mites on adult bees and total colony mites) (Table) indicated that no significant differences occurred either for the comparison of highly infested colonies and less infested colonies or for the comparison of colonies that had superseded or colonies that had not superseded. Numerically, colonies designated as having lower infestations had a slightly lower number of mites in brood (2%); a higher number of mites on adults (17%), and a higher number of overall total colony mites (2 %). Colonies that superseded had numerically lower numbers of mites on worker brood (17%), on adult bees (4%), and overall total colony mites (14%). However, the

analyses of the three measures (mites in brood, mites on adults and total mites) all had interaction terms that were either significant or approached significance (mites in brood, $P = 0.09$; mites on adults, $P = 0.008$; total colony mites, $P = 0.04$).

T-tests used to examine the nature of the interactions revealed that colonies designated as less infested that did not supersede had numerically fewer mites (mites in brood, 25% (Fig. 3a); mites on adults, 63% (Fig. 3b) and total mites 44% (Fig. 3c) than more infested colonies that did not supersede. However, colonies designated as less infested that did supersede numerically had more mites in brood 66% (Fig. 3a), and total mites 80% (Fig. 3c). Less infested colonies that had supersedure queens had significantly ($P = 0.05$) more mites on adults (170%) (Fig. 3b). These directionally contrasting differences in mite numbers between the groups that superseded and the groups that did not supersede probably resulted in the interactions identified by the ANOVAs.

Similar results were observed in the ANOVAs for proportional measures of varroa mite infestation (Table). No significant differences were detected for the comparison of colonies with high and low infestation levels although numerically, colonies originally designated as having lower infestation levels had a higher percentage of infested brood (11%), a higher percentage of infested adults (12%) and a higher overall infestation (11%). Significant differences or differences approaching significance were detected for the comparison of colonies that superseded and those that did not. Colonies that superseded had higher rates of infestation of brood (33%) ($P = 0.17$), of adults (60%) ($P = 0.02$) and overall (55%) ($P = 0.05$). The analyses of the three measures (percentage of brood infested, percentage of adults infested, and overall percentage of infestation) all had interaction terms that were either significant or approached significance (percentage of brood infestation, $P = 0.11$; percentage of adults infested, $P = 0.04$ and overall percentage of infestation $P = 0.06$).

T-tests used to examine the nature of the interactions revealed that colonies designated as less infested that did not supersede had numerically lower percentages of infestation in comparison to the colonies designated as having higher infestations that did not supersede (percentage of brood infested, 17% (Fig. 4a); percentage of adults infested, 27% (Fig. 4b) and overall percentage infestation 22% Fig. 4c). However, colonies designated as less infested that did supersede numerically had higher rates of infestation than colonies designated as more highly infested (percentage of brood infested, 57% (Fig. 4a), and percentage of adults infested 82% (Fig. 4b) and overall percentage of infestation 89% (Fig. 4c). These directionally contrasting differences in infestation rates between the groups that superseded and the groups that did not supersede probably resulted in the significant interactions identified by the ANOVAs.

Discussion

Increased difficulties with requeening colonies that have

Fig. 1a

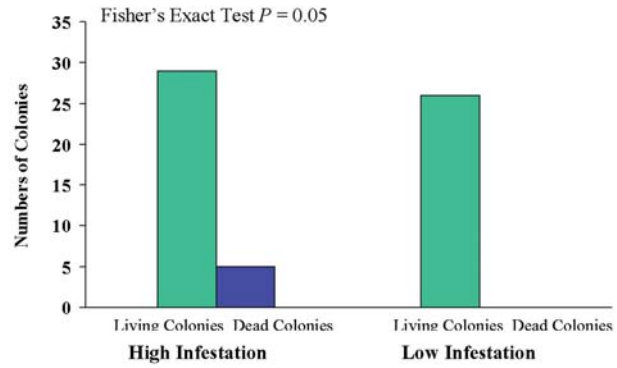

Fig. 1b

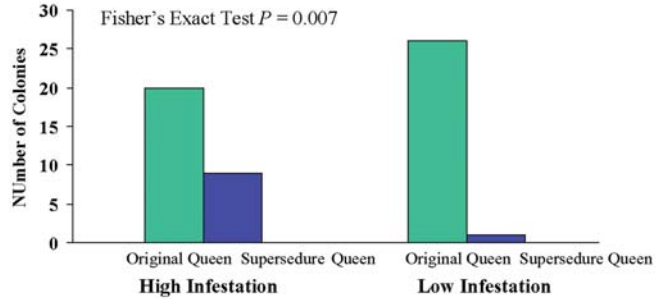

Figure 1*. For colonies with high and low infestations three weeks after the introduction of mated queens to queenless colonies, comparisons of: a) numbers of surviving and dead colonies and b) surviving colonies having original or supersedure queens.*

Fig 2a.

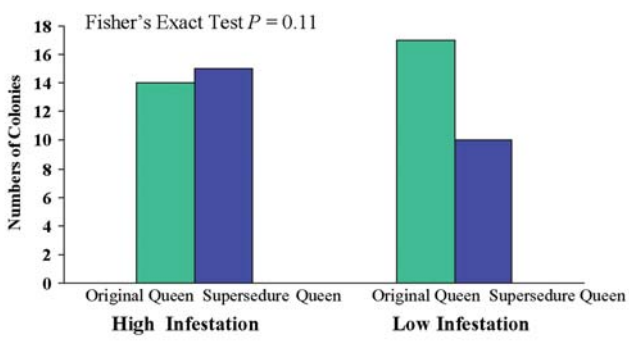

Fig 2a.

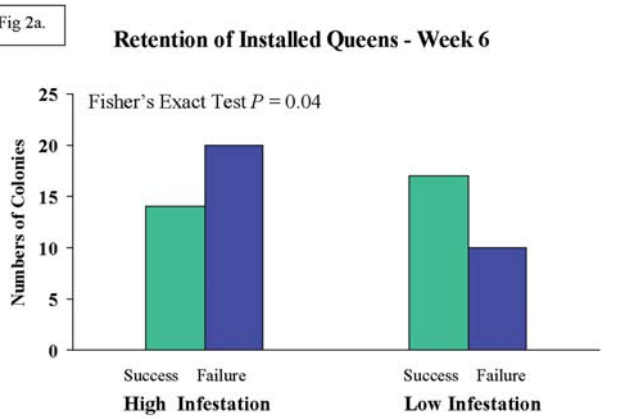

Figure 2. *For colonies with high and low infestations six weeks after the introduction of mated queens to queenless colonies, comparisons of: a) numbers of surviving colonies with original and supersedure queens and b) numbers of colonies with requeening successes and failures (dead colonies and colonies with supersedure queens).*

been reported in the past decade could have multiple nonexclusive causes. Known causes such as poor mating and unfavorable field conditions (Laidlaw, 1979) probably continue to cause problems as they have in the past. However, increased difficulties likely are caused by conditions that are either new or have increased in intensity in the past decade or so. The presence of varroa mites in colonies has become ubiquitous in the United States. Varroa is becoming more difficult to control as mites develop resistance to widely used miticides (Sanford, 2001). Although this study does not extend to secondary effects such as queens having poorer mating success in commercial settings that have elevated levels of varroa, such effects are also possible.

Colonies were reduced in size as a consequence of supersedure. This is an expected result of a colony having a break in brood production especially when the period of observation is only six weeks. After a longer period, colonies with supersedure queens may grow to be equal in size to colonies in the same apiary that did not supersede. Also, supersedure is a natural re-queening process in honey bee colonies which provides colonies with young vigorous queens. Supersedure queens in honey production or pollination apiaries have an excellent chance of long-term success. However, commercial colonies that supersede the queens installed by beekeepers have a temporally reduced size that makes them less likely to meet timely commercial honey production and pollination goals.

The original assignments of colonies to high or low infestation groups appear to be accurate. First, the colonies designated less infested had numerically more brood and bees than colonies designated as more highly infested. This is consistent with mites debilitating colonies prior to the colonies showing easily measurable deleterious effects. Second, of the colonies that did not supersede, the colonies designated as less infested had numerically fewer numbers of mites infesting brood and adults. Variable amounts of different age classes of brood placed into the experimental hives may have resulted in variable mite reproductive rates within colonies which may have blurred but did not eliminate the differences between the high or low infested groups.

The t-tests that were used to examine the significant and approaching significant interactions between the infestation classification and the supersedure classification all had similar

Colony size measures					Infestation		Supersedure	
		df	F	P	High	Low	Yes	No
Sealed	Infestation	1	1.12	0.30	11097	12886	9477	13507
Worker	Supersedure	1	8.94	0.004	±849	±983	±1101	±689
Brood	IxS	1	0.02	0.88				
Numbers	Infestation	1	0.10	0.75	5294	5635	3966	6390
of Adult	Supersedure	1	10.56	0.002	±597	±433	±501	±448
Bees	IxS	1	1.15	0.29				
Total	Infestation	1	0.84	0.36	16391	18521	13444	19897
Colony	Supersedure	1	12.67	0.001	±	±	±1570	±875
Size	IxS	1	0.31	0.58	1242	1326		
Absolute number of mites in colonies		df	F	P	Infestation High	Low	Supercedure Yes	No
Mites in	Infestation	1	0.05	0.83	1171	1149	1032	1241
Worker	Supersedure	1	0.48	0.49	±177	±156	±186	±153
Brood	IxS	1	2.92	0.09				
Mites on	Infestation	1	1.70	0.20	270	315	285	296
Adult	Supersedure	1	0.05	0.83	±47	±52	±72	±36
Bees	IxS	1	7.57	0.008				
Total	Infestation	1	0.26	0.61	1441	1464	1317	1537
Colony	Supersedure	1	0.28	0.60	±214	±189	±236	±179
Mites	IxS	1	4.38	0.04				
Percentages of Infestation		df	F	P	Infestation High	Low	Supersedure Yes	No
Percent	Infestation	1	0.89	0.35	6.2	6.9	7.8	5.9
Infested	Supersedure	1	1.97	0.17	±0.7	±1.4	±1.7	±0.7
Brood	IxS	1	2.52	0.11				
Percent	Infestation	1	1.52	0.22	5.7	6.4	8.0	5.0
Infested	Supersedure	1	6.19	0.02	±1.0	±1.2	±1.7	±0.5
Adult	IxS	1	4.20	0.04				
Bees								
Total	Infestation	1	1.23	0.27	9.2	10.2	12.4	8.0
Colony	Supersedure	1	4.15	0.05	±1.2	±2.2	±2.8	±0.9
Infested	IxS	1	3.46	0.06				

***Table**. ANOVA's comparing measures of colony size (numbers of sealed worker brood, numbers of adult bees and total colony size), numbers of mites in colonies (mites in worker brood, mites on adult bees and total colony mites), and percentages of infestation (percentage of brood infestation, percentage of adults infested and overall percentage of colony members infested) for colonies established with comparatively high and low levels of varroa infestation which retained their original queen or had a supersedure queen six weeks after queen introduction.*

trends. Of the colonies that did not supersede, those designated as having lower levels of infestation did have numerically lower levels of infestation. However, the colonies that did supersede that were designated as having lower levels of infestation, had numerically higher levels of infestation. The cause of this interaction is unclear. If we erred in the original classification of some colonies and the wrongly classified low infestation colonies actually had high infestations, then those high infestations were associated with supersedure. It is more likely that the original classification was more or less correct and that some biological cause resulted in the higher levels of infestation in the superseded colonies that had previously had lower infestations. Those colonies generally had supersedures that started later than the supersedures in the colonies designated as more highly infested. Mites may have reproduced on brood from the original queen prior to supersedure and produced a larger population which later infested the progeny of the supersedure queen. In the more highly infested group, a comparatively quick rejection of the original queen followed by the lack of brood production attending the supersedure process may have resulted in a lessened opportunity for mites to reproduce in these colonies.

While the differences underlying the interaction make analysis and interpretation more complex, the infestations rates between the colonies that superseded and those that did not remains clear. Considering absolute numbers, there were no differences between the colonies that superseded and those that did not. However, since the colonies that superseded were smaller, the rates of infestation of both adults and the overall colony were significantly higher. The rate was numerically higher for brood infestation in colonies with supersedure queens. As these colonies with supersedure queens grow it is reasonable to expect that the mite populations will reach damaging levels more quickly than they would in the colonies that did not supersede.

Conclusion and Recommendations

The data presented here provide strong evidence that elevated levels of varroa in colonies increase reqeening difficulties. Both supersedure rates and colony death following requeening efforts were elevated as direct effects of higher infestation rates. Perhaps any varroa infestation enhances supersedure rates.

Also, supersedure increases rates of varroa infestation. The break in brood production during the supersedure process causes decreased colony size which results in colonies with supersedure queens having higher rates of varroa infestation.

It might be productive for beekeepers to treat colonies they intend to re-queen or divide prior to queen introduction. Also, it might be productive to target colonies that have supersedure queens for an additional or an earlier treatment for mites.

Acknowledgments

Our thanks to Gary Delatte, Tony Stelzer and Joshua Wales for their fine technical assistance in helping set up this experiment.

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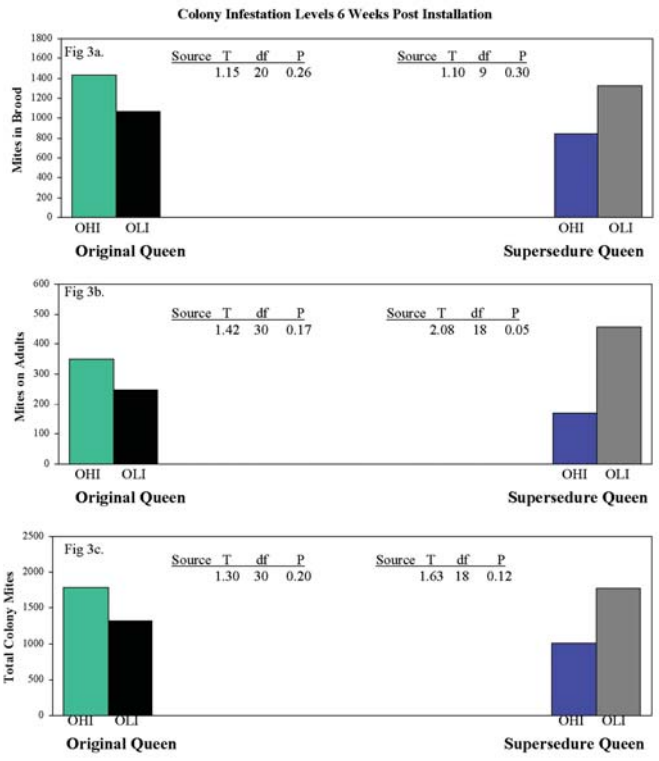

Figure 3. T-tests comparing the numbers of: a) mites in sealed worker brood cells, b) mites on adult bees and c) total mites in colonies established with comparatively high and low levels of varroa infestations in colonies that retained their introduced queens or had a supersedure queen six weeks after queen introduction. (OHI = originally highly infested; OLI = originally less infested)

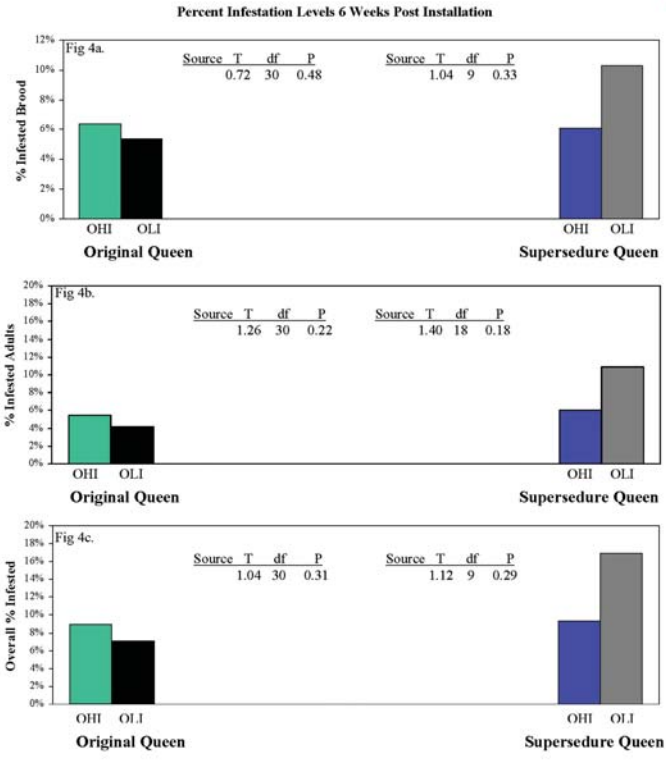

Figure 4. T-tests comparing the percentages of: a) sealed worker brood cells infested with varroa mites, b) varroa mites on adult bees with mites c) overall varroa mite infestation in colonies established with comparatively high and low levels of varroa infestations in colonies that retained their introduced queens or had a supersedure queen six weeks after queen introduction. (OHI = originally highly infested; OLI = originally less infested)

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