A Test for Interactions Between *Varroa destructor* (Acari: Varroidae) and *Aethina tumida* (Coleoptera: Nitidulidae) in Colonies of Honey Bees (Hymenoptera: Apidae)

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ABSTRACT Field surveys indicate that declining colonies of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), suffer simultaneously from multiple stress factors, raising concern that multiple stressors could be interacting to compound bee stress in an additive or synergistic fashion. We tested two null hypotheses: 1) *Varroa destructor* Anderson & Trueman (Acari: Varroidae) (=varroa) and *Aethina tumida* Murray (Coleoptera: Nitidulidae) do not interact such that the number of one affects the number or density of the other and 2) bee damage from one does not change in response to changing levels of the other. In a split-split plot design replicated in 2 yr and two states, experimental apiaries were established and each manipulated to achieve one of five average ± SE colony adult *A. tumida* populations: 0; 285 ± 6; 721 ± 5; 1,544 ± 14; or 3,175 ± 90. Within each apiary, the population of varroa mites in each colony was manipulated to achieve one of three average ± SE colony mite populations: 763 ± 121; 1,111 ± 155; or 1,856 ± 300. On a one-way basis, there was a predictable increase in measures of bee morbidity with increasing densities of each pest. Colony varroa mite levels decreased as apiary-wide *A. tumida* levels increased. In contrast, colony levels of the honey bee mite, *Acarapis woodi* (Rennie) (Acari: Tarsonemidae), increased as colony varroa levels increased. Concerning measures of bee morbidity, varroa and *A. tumida* did not interact such that damage by one was affected by changing levels of the other. A treatment threshold established for varroa before the arrival of *A. tumida* has not changed during the years since *A. tumida* has become established in the region.

KEY WORDS *Apis mellifera*, varroa mite, *Acarapis woodi*, colony decline

There is evidence that managed honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), are declining in much of North America and Europe (Biesmeijer et al. 2006, National Research Council 2007); and although viruses figure prominently in the list of suspected agents (Johnson et al. 2009), field surveys indicate that declining colonies suffer simultaneously from multiple stress factors (Cox-Foster et al. 2007, vanEngelsdorp et al. 2009), raising concern that multiple stressors could be interacting to compound bee stress in an additive or synergistic manner. At the microorganismal scale, an interaction has been shown between black queen cell virus and the microsporidian *Nosema apis* Zander (Dissociodihaplophasida: Nosematidae) (Bailey et al. 1983). The microsporidian enhances replication of the virus, and co-infected bees die at higher rates than singly infected bees. Interactions also occur between deformed wing virus (DWV) and the macroscopic parasitic bee mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) (=varroa), such that varroa parasitism is linked to high levels of DWV (Yang and Cox-Foster 2007). At the macroorganismal scale, there is cause for concern between varroa and *Aethina tumida* Murray (Coleoptera: Nitidulidae), a nest scavenger and natural associate of African *A. mellifera* introduced to the United States in the mid-1990s. Varroa is associated with a wide range of bee morbidities, including the vectoring or activating of viruses (Sammataro et al. 2000), and *A. tumida* is associated with reduced colony bee populations, brood area, and flight activity (Ellis et al. 2003b). The terminal result of unchecked adult *A. tumida* infestation is colony absconding or death (Ellis et al. 2003a).

Arthropod nest enemies such as varroa and *A. tumida* facilitate an examination of interacting stress factors on honey bees at the macroorganismal scale. Moreover, for each of these pests there are literature guidelines for colony densities that range from innocuous to damaging. For varroa, mite densities are considered nondamaging at levels <3–13 mites per 100 bees (Delaplane and Hood 1997, 1999; Strange and Sheppard 2001), whereas for *A. tumida* average adult numbers <400 do not significantly reduce colony bee

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year							
2004				2005			
state				state			
Georgia		South Carolina		Georgia		South Carolina	
apiary	*A. tumida*[1]	apiary	*A. tumida*	apiary	*A. tumida*	apiary	*A. tumida*
1	0	1	3175	1	285	1	721
2	3175	2	0	2	0	2	285
3	721	3	721	3	1544	3	1544
4	1544	4	1544	4	721	4	3175
5	285	5	285	5	3175	5	0

[1]Apiary *A. tumida* levels (average beetles per colony) randomly assigned within year and state

colony within apiary	colony varroa populations[2]
1	763
2	1111
3	763
4	1856
5	1856
6	1111

[2]Colony varroa populations randomly assigned within apiary (2 colonies per population)

Fig. 1. The experiment was a split-split plot design replicated in 2 yr (split 1: 2004, 2005) and two states (split 2: Georgia, South Carolina). Each apiary within year and state was randomly assigned one of five target colony *A. tumida* populations. Each colony within apiary was randomly assigned one of three target varroa populations.

populations, brood, flight activity, or honey yields in three-frame nucleus colonies (Ellis et al. 2003b). This knowledge is helpful in designing field experiments that bracket a range of realistic pest densities. In a field experiment replicated across 2 yr and two states, we tested two null hypotheses: 1) two honey bee pests do not interact such that the number or density of one affects the number or density of the other, and 2) bee damage from one does not change in response to changing levels of the other. The nest invaders *V. destructor* and *A. tumida* served as model honey bee pests. In a factorial treatment arrangement like this, rejection of null hypothesis 1 requires demonstrating a significant change in pest numbers in response to changing numbers of the other, and rejection of null hypothesis 2 requires demonstrating an interaction between the main effects varroa and *A. tumida* on measures of bee morbidity.

Materials and Methods

The experiment was a split-split plot design replicated in 2 yr (split 1: 2004, 2005) and two states (split 2: Georgia, South Carolina) (Fig. 1). Within each state, in June of each year 30 experimental colonies (five apiaries × six colonies each), each with one Langstroth hive body, a queen excluder, and one food super, were established with nearly equal amounts of bees, brood, and honey. Numbers of *A. tumida* adults were manipulated at the level of apiary because beetles are strong flyers and move easily between colonies. Numbers of varroa were manipulated at the level of colony within apiary because their drift rate is much lower.

Within each state, one of the apiaries was designated an *A. tumida* control apiary and received no inoculated beetles. Each of the remaining four apiaries was inoculated with different numbers of laboratory-reared adult *A. tumida* in June, August, and October. By December of both years, this resulted in apiaries with the following average ± SE sum of beetles added to each colony: 0; 285 ± 6; 721 ± 5; 1,544 ± 14; or 3,175 ± 90. In both years, the ground in front of hives was treated with permethrin (GardStar, Y-Tex Corp., Cody, WY) to kill wandering *A. tumida* larvae and limit local population increase. Within state, no apiary was nearer than 5 km to another known apiary. Although we cannot exclude the possibility of immigrating adult *A. tumida*, trap recovery of flying adults is known to decrease linearly within a range of 0–160 m from release site (Arbogast et al. 2009). Thus, we believe the actualized *A. tumida* levels in our apiaries were the product of our inoculating efforts and not *A. tumida* immigration or reproduction in colonies.

Within apiary, each colony was randomly assigned one of three varroa miticide treatments (two colonies per treatment) to approximate the range of colony varroa populations achieved by Delaplane and Hood

Table 1. Effects of colony varroa treatment on dependent variables

Variable	Miticide repeatedly[a,b]	Miticide in Aug.[a,b]	Miticide in Oct.[a,b]
Adult bee population	14,128 ± 434 (107)a	13,415 ± 517 (104)ab	12,133 ± 652 (92)b
Avg bee mass (mg)	115.6 ± 2.3 (34)a	120.9 ± 3.7 (33)a	112.1 ± 2.2 (28)a
Total brood (cm^2)	3,482 ± 310 (107)a	3,455 ± 320 (101)a	3,195 ± 322 (91)a
Colony varroa mite population	763 ± 121 (120)b	1,111 ± 155 (120)b	1,856 ± 300 (120)a
Mites per 100 bees	5.4 ± 0.8 (120)b	10.1 ± 1.6 (120)b	24.7 ± 5.4 (120)a
Colony wt (kg)	37 ± 1.2 (34)ab	37.6 ± 1.7 (33)a	33.1 ± 1.3 (28)b
% bees positive for *A. woodi*	0.7 ± 0.2 (34)b	1.3 ± 0.3 (33)b	2.7 ± 0.7 (29)a

[a] Values are mean ± SE (*n*).
[b] Row values with the same letter are not different (*t*-test on LSmeans; $\alpha \leq 0.05$).

(1997, 1999): miticide treatment applied repeatedly, applied in August only, or in applied October only. For 2004, the repeatedly treated group received fluvalinate (Apistan, Vita-Europe, Basingstoke, Hants, United Kingdom), whereas for 2005 we switched to thymol-based miticides (Api-Life VAR [Chemicals LAIF, Vigonza, Italy] or Apiguard [Vita-Europe]) over concerns of varroa resistance to Apistan. These manipulations exploit the principle that mite populations can be expected to grow as miticide applications are delayed (Delaplane and Hood 1997). The resulting average ± SE colony mite populations were 763 ± 121 for the repeatedly treated group; 1,111 ± 155 for the August-treated group; and 1,856 ± 300 for the October group (see methods below).

In August, October, and December of both years, we sampled each colony to determine colony adult bee population, total brood (cm^2), colony varroa mite population, and mites per 100 adult bees. Adult bee population and the brood area measures were derived by summing proportions of whole deep frames covered by bees or brood (after Skinner et al. 2001), converting frames of adult bees to bee populations with the regression model of Burgett and Burikam (1985), and converting frames of brood to square centimeters by the observation that one deep Langstroth comb (both sides) = 1,754 cm^2. Realized colony varroa populations were derived from 24-h mite counts with the linear regression model of Delaplane and Hood (1997); levels were determined immediately before scheduled miticide treatments were applied. From colony populations of bees and mites we derived the number of mites per 100 bees. Realized *A. tumida* populations by colony were not determined because we have not been successful at developing a reliable field sampling technique (contra Schäfer et al. 2008). For December only, colonies were weighed (kilograms) and adult bees from each sampled to determine average bee mass (milligrams); and, via dissection, the percentage of bees positive for the parasitic tracheal honey bee mite, *Acarapis woodi* (Rennie) (Acari: Tarsonemidae). Average bee mass was determined by collecting and weighing live bees in preweighed jars.

The combined August, October, and December data for both years were analyzed with mixed models (Proc Mixed, SAS 2002–2003) recognizing colony varroa treatment (V), apiary *A. tumida* level (B), and the interaction of V × B as fixed effects and year (Y), state (S), Y × S, and Y × B as random effects. Y × S and Y × B were later dropped from analyses because they did not explain any variation. Tukey's mean separation test was performed on least square means, but nonadjusted means are reported in tables. Differences were accepted at the $\alpha \leq 0.05$ level.

Results

A significant effect of varroa treatment was detected for adult bee population ($F = 5.6$; df = 2,286; $P = 0.004$), colony varroa mite populations ($F = 7.5$; df = 2,343; $P = 0.0006$), mites per 100 bees ($F = 9.8$; df = 2,343; $P = 0.0001$), colony weight ($F = 3.9$; df = 2,78; $P = 0.02$), and percentage bees positive for *A. woodi* ($F = 5.7$; df = 2,79; $P = 0.005$). Colonies in which varroa treatment had been delayed until October had lower bee populations than the continuously treated group and higher colony mite populations, higher mites per 100 bees, lower colony weights, and higher levels of *A. woodi* than both other groups (Table 1). In spite of the fact that thymol was only used in year 2 and has toxic properties against *A. woodi* (Calderone et al. 1997), the percentage bees positive for *A. woodi* was unaffected by year ($F = 11.2$; df = 1,2; $P = 0.06$) or the interaction of year with varroa treatment ($F = 2.4$; df = 2,69; $P = 0.1$).

A significant effect of apiary *A. tumida* level was detected for average colony varroa mite populations ($F = 3.2$; df = 4,343; $P = 0.01$) and colony weight ($F = 3.8$; df = 4,78; $P = 0.007$). Colonies that had received ≥721 beetles had significantly fewer mites than colonies with 285 beetles. Colonies which had been inoculated with zero beetles had comparatively highest colony weights, and there was a trend for significant and stepwise decline in colony weight as beetle numbers increased (Table 2). In no case was an interaction detected between colony varroa treatment and apiary *A. tumida* level ($0.09 < F < 1.1$; df = 8,78–343; $0.4 < P < 1.0$).

Discussion

With each of our model arthropod nest enemies varroa and *A. tumida*, we demonstrated increasing honey bee morbidity with increasing levels of nest invader. For varroa, this was significantly true of adult bee population and percentage bees positive for *A. woodi* (Table 1), and for *A. tumida* this was

Table 2. Effects of apiary *A. tumida* level on dependent variables

Beetles added to colonies	0	285 ± 6	721 ± 5	1,544 ± 14	3,175 ± 90
Adult bee pop[a,b]	14,335 ± 644 (68)a	12,314 ± 533 (65)a	13,517 ± 724 (65)a	12,573 ± 661 (59)a	13,642 ± 939 (46)a
Avg bee mass (mg)[a,b]	118.6 ± 3.9 (22)a	120.9 ± 3.2 (21)a	118.9 ± 4.8 (20)a	109.6 ± 1.8 (19)a	111.4 ± 3.6 (13)a
Total brood (cm^2)[a,b]	3,164 ± 349 (68)a	3,341 ± 382 (65)a	3,589 ± 433 (63)a	3,053 ± 399 (58)a	3,928 ± 518 (45)a
Colony varroa mite pop[a,b]	1,307 ± 263 (72)ab	1,920 ± 393 (72)a	1,195 ± 207 (72)b	1,194 ± 236 (72)b	600 ± 197 (72)b
Mites per 100 bees[a,b]	13.7 ± 5.2 (72)a	23.4 ± 6.9 (72)a	12.6 ± 2.8 (72)a	10.3 ± 1.8 (72)a	6.9 ± 2.5 (72)a
Colony wt (kg)[a,b]	39.5 ± 2.2 (22)a	37.8 ± 1.9 (21)ab	35.4 ± 1.8 (20)bc	34.3 ± 1.1 (19)bc	31.1 ± 1.5 (13)c
% bees positive for *A. woodi*[a,b]	1.8 ± 0.6 (22)a	1.1 ± 0.5 (21)a	1.7 ± 0.6 (21)a	1.1 ± 0.6 (19)a	1.9 ± 1.0 (13)a

[a] Values are mean ± SE (*n*). *Please note table is oriented to read left to right.*
[b] Row values with the same letter are not different (*t* test on LSmeans; $\alpha \leq 0.05$).

significantly true of colony weight (Table 2). One-way effects similar to these have been shown previously (Sammataro et al. 2000; Delaplane and Hood 1997, 1999; Ellis et al. 2003a,b).

What stands out in the present data is the finding that average colony varroa numbers decreased as apiary *A. tumida* levels increased. Because *A. tumida* levels were established within apiary, these results represent the average varroa numbers across colonies receiving all varroa miticide treatments, so it is reliably an *A. tumida* effect. Moreover, the results are not an artifact of decreasing adult bees or brood that serve as mite hosts, as adult bee populations, mites per 100 bees, and square centimeters of total brood showed no tendency to decrease as *A. tumida* levels increased (Table 2). We reject our null hypothesis 1 and conclude that colony varroa numbers do change in response to changing apiary *A. tumida* levels. However, we find the direction of this change—fewer varroa with more *A. tumida*—unintuitive because literature examples lead us to expect a positive relationship such that increases in one stressor handicap the resistance mechanisms of bees generally and facilitate higher levels of another stressor (Tentcheva et al. 2004, Yang and Cox-Foster 2007). This may not always apply at the macroscopic scale where organismal defensive reactions toward one pest may give collateral benefit toward another. For example, *A. tumida* are known to stimulate hygienic behavior in honey bees (Ellis and Delaplane 2008) by which bees recognize diseases or invaders inside brood cells, open the cell, and remove its contents. This behavior is also effective against varroa (Spivak 1996). Similarly, *A. tumida* can instigate vigorous aggressive responses by worker bees (Elzen et al. 2001), one outcome of which may be physical dislodgement and injury to varroa mites.

One result in our study consistent with a positive relationship between stressors (*sensu* Yang and Cox-Foster 2007) is the finding that colony levels of *A. woodi* increased as colony levels of varroa increased (Table 1). Thymol has toxic properties against *A. woodi* (Calderone et al. 1997); however, thymol was used only year 2, and the mixed model failed to show either a year effect or interaction of year with varroa treatment. Therefore, the change in *A. woodi* is not explained wholly by the use of thymol and may suggest some dynamic with varroa. This is consistent with the results of Downey et al. (2000) who showed that bees parasitized by varroa as immatures are more likely to be parasitized subsequently by *A. woodi* as adults.

Another feature of the present data is an absence of interactions between the categorical main effects varroa and *A. tumida* on measures of bee morbidity. This is apparent in the absence of interactions in our mixed model between the two main effects, varroa and *A. tumida*. Therefore, we failed to reject null hypothesis 2 and conclude that varroa and *A. tumida* do not interact such that bee damage from one changes in response to changing levels of the other.

Because the present design repeats our earlier methods that established an August treatment threshold for varroa before *A. tumida* were found in our apiaries (Delaplane and Hood 1997, 1999; Hood 2004), the present data provide an opportunity to see whether the varroa threshold has changed now that *A. tumida* are generally established in the region. Repeating our earlier analyses, we examined colony metrics for December data only and found that colonies in which varroa treatment had been delayed until August performed as well as colonies which had been treated continuously with miticide and performed better than colonies in which miticide treatment had been delayed until October. This repeats our previous finding that varroa densities encountered in August were below an irrecoverable level. In the present experiment, that August varroa density was 20 ± 12 mites per 100 bees, not lower, as one would expect if *A. tumida* had proven an additional hardship on bees; but it was actually higher than the 13 mites per 100 bees level shown previously (Delaplane and Hood 1999). Thus, we conclude that the varroa threshold has not changed since the arrival of *A. tumida* in the southeastern United States. This is consistent with our present results showing no interaction between varroa and *A. tumida* in the short term represented by our experiment.

In summary, we found that colony varroa levels decreased as apiary-wide *A. tumida* levels increased; this suggests that at least at the macroscopic scale organismal defense reactions against one nest invader may provide collateral benefit toward another. In contrast, colony levels of *A. woodi* increased as colony levels of *V. destructor* increased. Concerning measures of bee morbidity, varroa and *A. tumida* did not interact such that damage by one was affected by changing levels of the other. A treatment threshold established

for varroa before the arrival of *A. tumida* has not changed during the years since *A. tumida* has become established in the region. Work like this is important to the long-term project of understanding the interactions and dynamics of the multiple stressors contributing to honey bee decline.

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