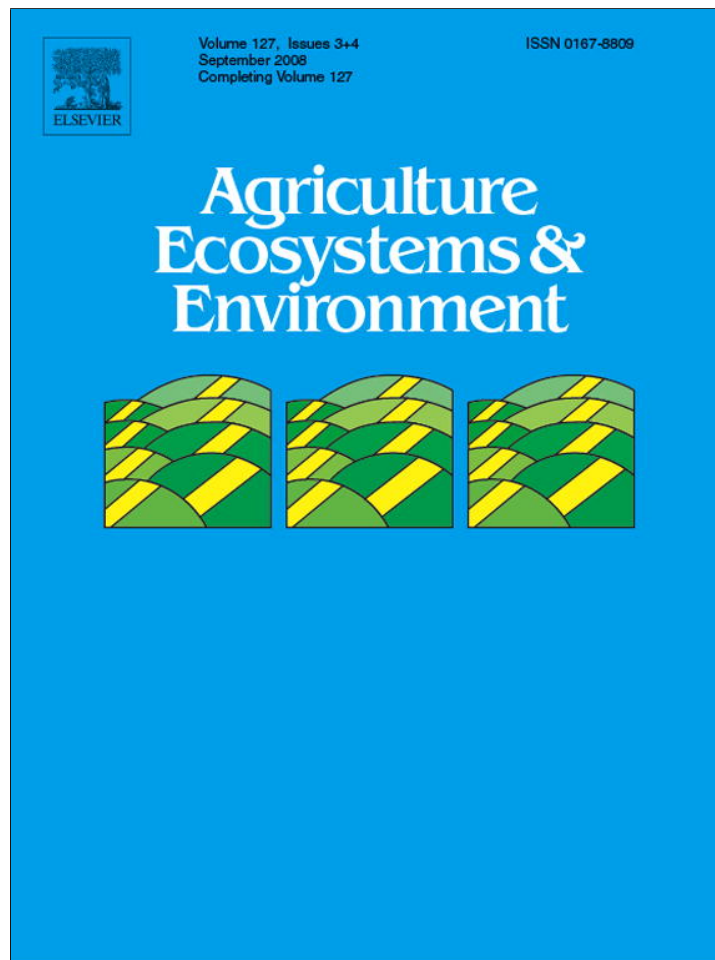


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ABSTRACT

The work of pollinators is crucial to the sustainability of plant communities in natural and agricultural ecosystems; however, pollinators are declining in much of the developed world due to a variety of parasites, diseases, and environmental stresses. These experiments are the first to examine directly the impact of honey bee, *Apis mellifera*, nest invaders on plant pollination and fitness. A cost to pollination could occur under two scenarios: (1) at the colony level where nest invaders compromise the health of the foraging cohort and reduce their efficacy as pollinators or (2) at the community level where invaders simply kill bee colonies and reduce the local pollinator population. Honey bee colonies were manipulated to achieve different levels of the parasitic mite *Varroa destructor* or nest-invading beetle *Aethina tumida* and tented under one of two model plants: canola (*Brassica napus*) or rabbiteye blueberry (*Vaccinium ashei*). On the basis of single-bee flower visits, fruit-set was reduced in blueberry with bees from varroa-parasitized colonies. However, on the basis of colonies, there were no differences in blueberry fruit-set, number of blueberry pollen tetrads deposited on the stigma, and pod-set in canola among colonies with different levels of nest invaders or no-invader controls. Thus, within the range of nest invader densities used in this study, individual inefficiencies were erased by compensatory multiple flower visits by this colonial pollinator. By failing to affirm the functionality of scenario (1) this study indirectly supports scenario (2): the major contribution of honey bee nest invaders toward a pollinator deficit is the simple eradication of colonies.

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1. Introduction

The role of pollination in terrestrial plant and animal communities is so fundamental that the state of a community's pollinators constitutes an indicator of its health and sustainability (Kevan, 1999). In the case of agro-ecosystems, pollination is recognized as a natural asset, and if natural pollinators are insufficient then managed pollinators are imported as deliberate inputs (Free, 1993; Delaplane and Mayer, 2000). However, there is evidence that pollinators, both wild and managed, are declining in much of the developed world (National Research Council, 2007). In the case of the western honey bee, *Apis mellifera* L., this problem is due to a variety of cosmopolitan pests, diseases, and environmental toxins taxing managed and feral populations (Cox-Foster et al., 2007). The honey bee is the pollinator of choice in much of its modern range, owing to its manageability and large forager populations (Winston, 1987; Hoopinger and Waller, 1992; Free,

1993; Delaplane and Mayer, 2000). The annual value of honey bee pollination in the USA has been estimated at over \$14 billion (Morse and Calderone, 2000). It is therefore a matter of public interest to sustain high densities of vigorous bee colonies.

Varroa mites (*Varroa destructor* Anderson and Trueman) and small hive beetles (*Aethina tumida* Murray) are virulent nest invaders of *A. mellifera*. Varroa decreases a colony's honey yield (De Jong et al., 1982) and number of pollen foragers (Janmaat et al., 2000). For individual bees, varroa reduces body weight, life span, sperm load in drones, size of mandibular glands, flight activity, and insecticide tolerance (Schmid-Hempel, 1998). Adult small hive beetles reduce colony bee populations, brood area, and flight activity (Ellis et al., 2003).

This study examines whether nest invaders limit the efficacy of honey bees as pollinators. A cost to pollination could occur under two scenarios: (1) at the colony level where nest invaders compromise the health of the foraging cohort and reduce their efficacy as pollinators or (2) at the community level where invaders simply kill colonies and reduce the local population of pollinators. It is arguable that a cost to pollination could occur at a level preceding (1), that is, at the level of individual pollinator. However in the case of the honey bee, the pathology of the host and behaviors of its specialist, obligate nest invaders *V. destructor* and

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*A. tumida*² are best understood in the context of eusocial colonial life. If invaders do affect the pollinating performance of individual honey bees, it must be understood in terms of colony-level parasitism, not individual. Just as colony is the unit of natural selection in social bees (Seeley, 1995), colony is the meaningful level for understanding parasites (Schmid-Hempel, 1994; Neumann and Moritz, 2000; Tarpy, 2003), and it is at this level the present experiment was conducted.

In this paper we treat scenario (1) above as a testable hypothesis. Varroa mites and small hive beetles served as the model nest invaders, and canola (*Brassica napus* L.) and rabbiteye blueberry (*Vaccinium ashei* Reade) as the model plants. Blueberry and canola both require insect pollination to some degree. For blueberry, we used rabbiteye varieties that are self-sterile and require cross-pollination (Delaplane and Mayer, 2000). Canola is 70% self-pollinated, but insects provide additive pollination benefit (Westcott and Nelson, 2001). Hence, the two crops differ in their degree of dependence on insect pollination, allowing one to make more general inferences on the effects of pollinator parasites on plant fitness.

2. Materials and methods

2.1. General

The study was conducted at the Horticulture Farm of the University of Georgia, Oconee County, GA, USA (33°50'N, 83°26'W) on a 16-year-old plantation of *Vaccinium ashei* and a cultivated field of *Brassica napus*. Blueberry anthesis at this location occurs from mid-March to mid-April and fruit ripens from mid-June to late-July. Canola anthesis occurred during April, and pod-set occurred in May.

In 2005 and 2006, honey bee colonies were manipulated to derive three classes of nest invader density: (1) varroa (VM), (2) small hive beetle (SHB), and (3) no-invader control (NIC). In February, we sampled full-sized Langstroth colonies for varroa using standard sticky screens. Using this information, we identified high varroa and low varroa colonies. A subset of colonies was made nearly varroa-free by treating colonies with coumaphos, botanical oils (Api-Life VAR™, Chemicals LAIF, Italy) or powdered sugar; dusting bees and mites with powdered sugar has been shown to dislodge a large fraction of the mite population (Fakhimzadeh, 2001). The resulting groups had the following 24 h mite drop counts (mean ± S.E.): 2005–VM (42.9 ± 6.7), NIC (3.1 ± 0.6); 2006–VM (56.7 ± 14.9), NIC (2.7 ± 0.4). In mid-March, four-frame nucleus colonies, each with a population of ca. 6400 honey bees (0.55 kg bees), a density found sufficient to pollinate blueberries under these conditions (Dedej and Delaplane, 2003), were established from the colonies in which the varroa populations were manipulated. Bees from each group were mixed in a large cage and subsequently distributed among nuclei, each with two empty frames, one frame of honey, one frame of capped brood, and a 2.5-cm queen pheromone strip (Bee Boost™, Phero Tech, Canada). In 2006, the no-invader control group was dusted with powdered sugar within the cage to further reduce mite loads. Varroa levels for each nucleus were confirmed using overnight sticky screens and alcohol samples. SHB colonies were established by introducing ca. 300 lab-reared beetles after colonies were placed under cages.

2.2. Canola pollination efficacy

In 2005, 21 1.8 m³ canola plots were established. The following treatments were randomly assigned to plots: VM or SHB (6 plots each), NIC, no-bee control (NBC), or open control (OC) (3 plots each). A plot of each treatment consisted of a cage (1.8 m³ frame covered with Lumite screen (Bioquip, USA)) housing the plants and one four-frame nucleus of honey bees.

Pollination efficacy measurements for canola included percent pod-set, number of seeds per pod, weight of seed per plant, and rate of honey bee flower visits. Ten plants within each plot were marked and used to determine plant fitness for the plot. Since the canola plant has continuous growth and bloom, we determined pod-set based on the total number of flowers per plant using the flowering phenology described by Eisikowitch (1981): each flower remains open approximately 3 days. The number of open flowers on each plant was counted every fourth day until flowering was complete. The daily values were summed to yield the total number of flowers per plant. The rate of honey bee flower visits was determined per plot by recording the number of bee visits per 2 min.

Plants were harvested when seeds in bottom-most pods were black. Upon harvest, the number of pods was counted. Percent pod-set per plant was calculated by dividing the total number of pods by the total number of flowers. Plants were allowed to dry indoors at room temperature for 2 weeks. After drying, all pods were removed, weighed (g), and threshed for seed. The total weight of seeds per plant (g) and the weight of 20 seeds per plant were used to determine the average number of seeds per pod.

2.3. Blueberry pollination efficacy

In 2005, 21 1.8 m² blueberry plots were established. The following treatments were randomly assigned to plots: VM or SHB (6 plots each), NIC, NBC, or OC (3 plots each). In 2006, 25 1.8 m² blueberry plots were established and the same treatments randomly assigned to 5 plots each. Each plot except for OC was enclosed by a cage (1.8 m³ frames covered with Lumite screen (Bioquip, USA)) with the plants and one four-frame nucleus of honey bees. Each plot contained two mature 'Climax' plants and three potted pollenizers ('Premier', 'Tifblue', and 'Brightwell'). Open plots were not provided with potted pollenizers because the orchard is already planted in alternating rows of 'Climax' and 'Premier' plants. Treatments remained on plots until bloom was complete at which time the cages were removed to minimize shade effects.

Pollination efficacy was measured in blueberry by determining percentage fruit-set, number of seeds per fruit, berry weight (g), and rate of honey bee flower visits (Dafni, 1992; Dedej and Delaplane, 2003). The number of unopened flowers per raceme was determined for 40 tagged racemes (in 2005, 80 on open plots to account for wind loss) on the 'Climax' plants in each plot before the introduction of bees. Remaining racemes were recovered upon fruit maturity and appropriate measurements made. Percentage fruit-set equaled the number of fully formed fruit per raceme divided by initial number of unopened flowers. The number of seeds per fruit was determined by crushing berries and counting all seeds. The rate of honey bee flower visits was determined by observations of the number of legitimate (at corolla aperture) bee visits to open flowers per 2 min.

In 2006, counts of pollen tetrads were conducted on randomly selected foragers and stigmas collected from each plot on an arbitrarily selected day. Honey bee foragers were collected directly from flowers and placed within a pre-weighed 20 ml vial. Forager weights (mg) were recorded and specimens stored frozen until

² The notion that *A. tumida* is an obligate associate of *A. mellifera* is functionally credible yet open to criticism. Beetles can reproduce on a diet of fruits, but longevity and reproductive success are dramatically higher on bee nest materials (Ellis et al., 2002).

pollen counts were completed. The method of counting pollen tetrads on foragers was modified from protocols described by Dafni (1992). Only the pollen on the body available for deposition onto stigmas was of interest; therefore, the hind legs of the bee were removed with scissors. The following were mixed with the forager in the 20 ml vial: 0.9 ml 70% ethanol, four drops of detergent, and three drops of 0.5% basic fuchsin. The suspension was shaken for 90 s with a vortex mixer (Thermolyne MaxiMixII, Type 37600 Mixer). One drop of suspension was placed on a hemacytometer and pollen tetrads counted with a compound microscope. Six samples were taken for each vial, and the suspension was shaken for 10 s between samples.

For direct counts of pollen tetrads on stigmas, five stigmas were randomly removed with forceps from open flowers within each plot. Stigmas were refrigerated until counts were made within 24 h of collection. The method of counting pollen tetrads on stigmas was modified from protocols described by Dafni (1992). Using a scalpel, the tip of the stigma (4 mm length) was removed and placed on a clean, glass slide. Three drops of Calberla's solution (described in Protocol 9, Dafni (1992)) were applied to the stigma and allowed to soak for 10 min. A cover slip was placed firmly on the stigma and direct counts of tetrads made with a compound microscope.

2.4. Pollinator efficacy based on single-bee flower visits

In the 2006 plots for VM, SHB, and NIC used to measure blueberry pollination efficacy, racemes with unopened blueberry flowers were covered with fine-mesh bags to prevent visitation to flowers and flowers allowed to open. On the day of observation, bags were removed and one bee allowed to visit the open flower cluster. Any unvisited flowers were removed, one visited stigma collected for pollen tetrad counts, the number of remaining flowers recorded, and the cluster rebagged and tagged for later recovery. Remaining racemes were recovered upon fruit maturity and percentage fruit-set, number of seeds per fruit, speed of ripening, and berry weight (g) were determined. Direct counts of pollen tetrads on stigmas were made within a few hours of collection using methods described above.

2.5. Statistical analysis

For canola, the effects of treatment on pod-set, the number of seeds per pod, and total seed weight per plant were tested with a completely randomized design analysis of variance using the MIXED procedure (SAS Institute, 2002–2003) and recognizing average number of varroa per plot as a random effect. Treatment effects on bee flight activity were determined with a repeated measure analysis of variance using the MIXED procedure and recognizing day and average number of varroa per plot as random variables. Cages were treated as replications. Least square means

were separated by Tukey's test and differences accepted at the $\alpha \leq 0.05$ level.

For blueberry, the effects of treatment on berry weight, fruit-set, and the number of seeds per fruit were tested with a randomized block design analysis of variance using the MIXED procedure (SAS Institute, 2002–2003) recognizing year and the interaction of year with treatment as random effects. Means were separated by Tukey's test and differences accepted at the $\alpha \leq 0.05$ level. The number of pollen tetrads per stigma and per forager were measured only 1 day on 1 year, so its analysis employed a one-way analysis of variance (Proc GLM) recognizing residual error as test term. Least square means were separated by Tukey's test and differences accepted at the $\alpha \leq 0.05$ level (SAS Institute, 2002–2003).

The effects of treatments on blueberry bee flight activity were tested with a repeated measure randomized block analysis of variance using the MIXED procedure and recognizing year, day, and all interactions as random effects (SAS Institute, 2002–2003). Least square means were separated by Tukey's test and differences accepted at the $\alpha \leq 0.05$ level (SAS Institute, 2002–2003). For one-visit comparison, treatment effects on fruit-set, berry weight, days to ripen, number of pollen tetrads per stigma, and number of seeds per berry were determined with a repeated measure analysis of variance using the MIXED procedure with day as random effect (SAS Institute, 2002–2003). Cages were treated as replications. Least square means were separated by Tukey's test and differences accepted at the $\alpha \leq 0.05$ level (SAS Institute, 2002–2003).

3. Results

3.1. Canola pollination efficacy

We followed 210 plants throughout the study and collected 11,002 pods for weight and seed measurements. Pod-set was affected by treatment ($F = 11.74$; d.f. = 4, 7; $P = 0.0035$). Pod-set for NIC, VM, SHB, and OC were significantly higher than NBC (Table 1). The number of seeds per pod and the total weight of seeds per plant were not affected by treatment ($F = 1.70$; d.f. = 4, 16; $P = 0.1984$) and ($F = 0.31$; d.f. = 4, 8; $P = 0.8660$), respectively (Table 1). The rate of honey bee flower visits was affected by treatment ($F = 5.43$; d.f. = 3, 11; $P = 0.0149$). Rate of visitation was significantly reduced in open plots compared to caged plots, and visitation was lower in SHB plots than varroa (Table 1).

3.2. Blueberry pollination efficacy

In 2005, 960 racemes were tagged with 5267 flowers that were observed until fruit maturation. We collected and weighed 1904 berries and counted 39,916 seeds. In 2006, 1000 racemes were tagged with 5656 flowers that were observed to maturation. We collected and weighed 2608 berries and counted 43,812 seeds.

Table 1
Results from spring 2005 canola pollination study^a

Treatment ^b	No. of bee visits/2 min	% pod-set	No. of seeds per pod	Seed wt per plant (g)
VM	68.6 ± 3.2 (66) a	55.8 ± 2.8 (6) a	11.7 ± 1.2 (6)	1.8 ± 0.5 (6)
SHB	49.7 ± 2.8 (66) b	59.1 ± 3.0 (6) a	13.7 ± 1.1 (6)	2.1 ± 0.3 (6)
NIC	66.5 ± 4.9 (33) ab	62.5 ± 5.2 (3) a	11.0 ± 0.5 (3)	1.8 ± 0.4 (3)
OC	16.8 ± 1.7 (33) c	50.6 ± 2.1 (3) a	15.8 ± 2.1 (3)	2.6 ± 0.6 (30)
NBC	NA	36.1 ± 0.8 (3) b	12.3 ± 1.0 (3)	2.0 ± 0.4 (3)

Column means with different letters are different at $\alpha \leq 0.05$; column means without letters are not significantly different. Least square means were separated by Tukey's test.

^a Values are mean ± S.E. (n).

^b Varroa mite (VM), small hive beetle (SHB), no-invader control (NIC), open control (OC), and no-bee control (NBC).

Table 2
Blueberry pollination results^a

Treatment ^b	No. of bee visits/2 min	% fruit-set	Average berry weight (g)	No. of seeds per berry
VM	38.5 ± 2.5 (96) a	54.5 ± 0.03 (11) a	0.70 ± 0.03 (11) a	68.0 ± 2.0 (11) a
SHB	34.6 ± 2.2 (97) a	57.9 ± 0.04 (11) a	0.72 ± 0.04 (11) a	67.5 ± 2.4 (11) a
NIC	38.1 ± 2.8 (76) a	43.9 ± 0.04 (8) ab	0.67 ± 0.04 (8) a	66.8 ± 1.4 (8) a
OC	2.6 ± 0.4 (76) b	35.7 ± 0.03 (8) b	0.62 ± 0.04 (8) ab	74.9 ± 1.2 (8) a
NBC	NA	17.6 ± 0.03 (8) c	0.55 ± 0.07 (8) b	41.2 ± 3.9 (8) b

Column means with different letters are different at $\alpha \leq 0.05$. Least square means were separated by Tukey's test.

^a Values are mean ± S.E. (n).

^b Varroa mite (VM), small hive beetle (SHB), no-invader control (NIC), open control (OC), and no-bee control (NBC).

Table 3
Pollen counts on blueberry stigmas and foragers^a

Treatment ^b	No. of pollen tetrads per stigma	No. of pollen tetrads per bee	Average bee weight (mg)
VM	61.2 ± 11.8 (5) a	1133.3 ± 374.2 (5)	83.1 ± 2.8 (5)
SHB	48.3 ± 6.4 (5) ab	1466.7 ± 764.5 (5)	85.8 ± 2.8 (5)
NIC	57.1 ± 7.9 (5) a	733.3 ± 487.6 (5)	91.8 ± 5.4 (5)
OC	74.8 ± 15.3 (5) a	1000.0 ± 380.1 (5)	87.1 ± 1.6 (5)
NBC	12.0 ± 3.6 (5) b	NA	NA

Column means with different letters are different at $\alpha \leq 0.05$; column means without letters are not significantly different. Least square means were separated by Tukey's test.

^a Values are mean ± S.E. (n).

^b Varroa mite (VM), small hive beetle (SHB), no-invader control (NIC), open control (OC), and no-bee control (NBC).

Table 4
Single-flower visit 2006 results for blueberry^a

Treatment ^b	% fruit-set	Average berry weight (g)	Days to ripen	No. of pollen tetrads per stigma	No. of seeds per berry
VM	2.2 ± 1.5 (15) b	0.95 ± 0 (2)	92.0 ± 0 (2)	43.3 ± 10.5 (15)	52.8 ± 9.8 (2)
SHB	44.8 ± 10.0 (13) a	0.63 ± 0.06 (10)	87.7 ± 2.3 (10)	88.9 ± 21.9 (14)	44.0 ± 2.8 (10)
NIC	33.2 ± 7.5 (14) a	0.7 ± 0.08 (10)	83.5 ± 3.4 (10)	66.9 ± 10.8 (14)	45.5 ± 3.9 (10)

Column means with different letters are different at $\alpha \leq 0.05$. Least square means were separated by Tukey's test.

^a Values are mean ± S.E. (n).

^b Varroa mite (VM), small hive beetle (SHB), and no-invader control (NIC).

Treatment had a significant effect on bee flight activity ($F = 36.1$; d.f. = 3, 20; $P < 0.0001$). The number of bee visits per 2 min was significantly less in open plots than in all other treatments (Table 2). Fruit-set was affected by treatment ($F = 17.11$; d.f. = 4, 4; $P = 0.0115$). Fruit-set values for VM, SHB, NIC, and OC were significantly greater than NBC (Table 2). Fruit-set for SHB and VM were significantly greater than OC (Table 2). Berry weight was significantly affected by treatment ($F = 3.56$; d.f. = 4, 40; $P = 0.0142$). Berry weight for VM, SHB, and NIC were significantly greater than NBC (Table 2). The number of seeds per berry was significantly affected by treatment ($F = 3.71$; d.f. = 4, 4; $P = 0.0346$). The number of seeds per berry for NBC was significantly less than all other treatments (Table 2).

The number of pollen tetrads counted on randomly collected stigmas was affected by treatment ($F = 5.71$; d.f. = 4, 20; $P = 0.0031$). The number of tetrads on stigmas collected from cages without bees was significantly lower than in VM, NIC, or OC cages (Table 3). The number of pollen tetrads per bee and bee weight were not affected by treatment ($F = 0.34$; d.f. = 3, 16; $P = 0.7995$) and ($F = 1.12$; d.f. = 3, 16; $P = 0.3694$), respectively.

3.3. Pollinator efficacy based on single-bee flower visits

Percent fruit-set was affected by treatment ($F = 10.24$; d.f. = 2, 39; $P = 0.0003$). Average fruit-set from one bee visit for varroa plots was significantly lower than other treatments (Table 4). There were no treatment effects for berry weight ($F = 1.96$; d.f. = 2, 17; $P = 0.1706$), days to ripening ($F = 1.0$; d.f. = 2, 19; $P = 0.3874$),

number of pollen tetrads per stigma ($F = 2.28$; d.f. = 2, 38; $P = 0.1161$), and number seeds per berry ($F = 0.53$; d.f. = 2, 19; $P = 0.5977$).

4. Discussion

4.1. Canola pollination efficacy

Experimental treatments did not affect the number of seeds per pod or seed weight per plant, and all values were numerically similar (Table 1). Moreover, pod-set was not different among colonies with different levels of nest invaders but was significantly reduced in cages without bees. This supports earlier research that shows an additive benefit of insect pollination in this crop which is otherwise responsive to wind (Fries and Stark, 1983; Westcott and Nelson, 2001). Pollination efficacy is not compromised at the bee colony level with the nest invader densities used in this study.

Bee flight activity was affected by treatment (Table 1). Varroa-infested colonies had the highest flight activity followed in decreasing order by control colonies, small hive beetle colonies, and open plots. Kralj and Fuchs (2006) found that varroa-infested bees stay outside the colony longer, which suggests a proportionally higher investment in foraging activity. Also, this study supports earlier findings by Ellis et al. (2003) that the presence of adult small hive beetles reduces flight activity in Western honey bees. Lower visitation rate in open plots is an artifact of our design: lower bee densities are expected outside the cages.

4.2. Blueberry pollination efficacy

As with canola, the blueberry experiment demonstrates that honey bee pollination is not compromised at the colony level with the nest invader densities used in this study. Actually, fruit-set in varroa and small hive beetle plots was significantly higher than open plots and numerically higher than no-invader controls (Table 2). Lower fruit-set in open plots is a result of lower bee density and flower visitation. Plots where honey bees were excluded had the lowest fruit-set, confirming the importance of bee pollination to *V. ashei*. In a parallel finding, the number of pollen tetrads per stigma was numerically reduced in plots without honey bees (Table 3). In terms of berry weight and the number of seeds per berry, values were lowest in cages without honey bees (Table 2). Large fruit and high seed content have been associated with cross-pollination of blueberry which would not occur without bees (Moore et al., 1972). This study affirms that honey bees are effective blueberry pollinators (Dedej and Delaplane, 2003; Sampson and Cane, 2000), but more importantly shows that fruit-set and fruit quality are not compromised by the presence of varroa and small hive beetles.

Counter-intuitively, fruit-set was numerically higher in small hive beetle and varroa plots than in the no-invader control (Table 2). Woyciechowski and Kozłowski (1998) found that honey bee workers infected with *Nosema apis* or workers with worn wings undertake foraging in poorer weather conditions more often than do healthy workers or workers with unworn wings. One may predict that workers compromised by varroa or small hive beetles will similarly sustain foraging efforts under sub-optimal conditions. This or similar compensatory actions may explain the sustained fruit-set performance in our VM and SHB plots.

4.3. Pollinator efficacy based on single-bee flower visits

When fruit-set was compared on the basis of single-bee flower visits, fruit-set was significantly lower for foragers from varroa colonies than those from control and small hive beetle colonies (Table 4). Fruit-set was numerically highest in small hive beetle-infested colonies. This trend is paralleled by the number of pollen tetrads per stigma after one visit, which was highest for the small hive beetle plot and lowest for the varroa plot (Table 4). This means that in terms of a single visit, foragers from colonies with varroa pressure deposited less pollen onto a stigma per visit which, in turn, resulted in lower fruit-set. Foragers from small hive beetle and control colonies were more efficient pollinators on a per bee basis.

The results from our other experiment which showed that varroa-parasitized colonies have comparatively high fruit-set (Table 2), demonstrates that overall colony effort is able to compensate for compromised performance at the individual bee level. This trend is also apparent for small hive beetle and control treatments; fruit-set is higher when investigated in terms of colony performance (Tables 2 and 4). This supports other work that has shown that honey bees are effective blueberry pollinators, but their success is dependent upon bee density and multiple flower visits (Dedej and Delaplane, 2003).

The number of seeds per berry, berry weight, and days to fruit maturity were not affected by treatments (Table 4). Berry weight was similar to that found in our previous experiment, but the number of seeds per berry was less for single-visit pollination. Again, since the number of seeds per berry is an indication of pollination quality, multiple visits are necessary to more effectively pollinate blueberry.

5. Conclusions

These experiments are the first to examine directly the impacts of honey bee pests and parasites on plant pollination and fitness. Within the range of conditions described herein, these studies show that honey bee nest invaders do not compromise the pollinating efficacy of the host bees. We believe that the range of experimental conditions was sufficient to compare the effects of nest invaders because bees from colonies with nest invaders showed a predictable downward trend in body mass (Table 3). Pod-set and fruit-set by pollinators from colonies with nest invaders were numerically equal to or higher than colonies without these pressures. This study also highlights the importance of colonial pollinators which can compensate for individual inefficiencies by fielding large forager populations and effecting multiple flower visits (Dedej and Delaplane, 2003).

Other studies have shown that parasitized or diseased bees engage in comparatively longer foraging trips (Kralj and Fuchs, 2006) under unfavorable conditions (Woyciechowski and Kozłowski, 1998). Both of these behaviors are risky to the forager but provide pollination benefits to the plant. Therefore, it seems that in the short-term plant fitness is not compromised by honey bee pests and parasites. But in the long-term if pest and parasite populations are left unchecked, bee colony populations collapse and die. This is the ultimate cause of the pollinator deficit and why it is imperative to continue research into controlling honey bee pests, parasites, and diseases.

Our study is also valuable because it helps elucidate the community level impacts of pollinator parasites. Is plant pollination compromised if (1) the pollinating efficacy of a colony's foraging cohort is compromised by parasites (i.e., parasites acting at the colony level), or (2) when parasites simply depopulate pollinator nests (parasites acting at the community level)? Although we did show reduced pollinating capacity in individual foragers from varroa-parasitized colonies, this deficiency was offset at the colony level because of the ability of this eusocial pollinator to effect multiple flower visits. By failing to affirm the functionality of scenario (1), this study indirectly supports scenario (2). This conclusion is strengthened by noting that experimental colonies with nest invaders were operating near known damage thresholds for varroa (Delaplane and Hood, 1999) and small hive beetles (J.D. Ellis, W.M. Hood, K.S. Delaplane, unpublished data). At invader densities significantly higher than these we can expect accelerating degrees of bee colony morbidity. Thus, we conclude that the major contribution of honey bee nest invaders toward a pollinator deficit is the simple eradication of colonies. These kinds of studies are necessary to understand the impacts of honey bee pests and parasites at the levels of agricultural landscapes and plant communities.

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References

- Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchinson, S.K., Simons, F.J., Egholm, M., Pettis, J.S., Lipkin, W.I., 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science express*, published online September 6, 2007; 10.1126/science.1146498.
- Dafni, A., 1992. *Pollination Ecology: A Practical Approach*. Oxford University Press, New York.

- Dedej, S., Delaplane, K.S., 2003. Honey bee (Hymenoptera: Apidae) pollination of rabbiteye blueberry *Vaccinium ashei* var. 'Climax' is pollinator density-dependent. *J. Econ. Entomol.* 96, 1215–1220.
- De Jong, D., Morse, R.A., Eickwort, G.C., 1982. Mite pests of honey bees. *Ann. Rev. Entomol.* 27, 229–252.
- Delaplane, K.S., Hood, W.M., 1999. Economic threshold for *Varroa jacobsoni* in the southeastern USA. *Apidologie* 30, 383–395.
- Delaplane, K.S., Mayer, D.F., 2000. *Crop Pollination by Bees*. CABI Publishing, Oxon, UK.
- Eisikowitch, D., 1981. Some aspects of pollination of oil-seed rape (*Brassica napus* L.). *J. Agric. Sci. Camb.* 96, 321–326.
- Ellis, J.D., Neumann, P., Hepburn, R., Elzen, P.J., 2002. Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets. *J. Econ. Entomol.* 95, 902–907.
- Ellis, J.D., Hepburn, R., Delaplane, K.S., Neumann, P., Elzen, P.J., 2003. The effects of adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae), on nests and flight activity of Cape and European honey bees (*Apis mellifera*). *Apidologie* 34, 399–408.
- Fakhimzadeh, K., 2001. Effectiveness of confectioner sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials. *Apidologie* 32, 139–148.
- Free, J.B., 1993. *Insect Pollination of Crops*, 2nd ed. Academic Press, London.
- Fries, I., Stark, J., 1983. Measuring the importance of honeybees in rape seed production. *J. Apic. Res.* 22, 272–276.
- Hoopingarner, R.H., Waller, G.D., 1992. Crop pollination. In: Graham, J.M. (Ed.), *The Hive and the Honey Bee*. Dadant and Sons, Hamilton, IL, pp. 1043–1082.
- Kralj, J., Fuchs, S., 2006. Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie* 37, 577–587.
- Janmaat, A.F., Wintson, M.L., Ydenberg, R.C., 2000. Condition-dependent response to changes in pollen stores by honey bee (*Apis mellifera*) colonies with different parasitic loads. *Behav. Ecol. Sociobiol.* 47, 171–179.
- Kevan, P.G., 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agric. Ecosyst. Environ.* 74, 373–393.
- Moore, J.N., Reynolds, B.D., Brown, G.R., 1972. Effects of seed number, size, and development on fruit size of cultivated blueberries. *HortScience* 7, 268–269.
- Morse, R.A., Calderone, N.W., 2000. The value of honey bees as pollinators of U.S. crops in 2000. *Bee Culture* 128, 15 pp. (special section).
- National Research Council, 2007. *Status of Pollinators in North America*. National Academies Press, Washington, DC.
- Neumann, P., Moritz, R.F.A., 2000. Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (*Apis mellifera* L.). *Insectes Soc.* 47, 271–279.
- Sampson, B.J., Cane, J.H., 2000. Pollination efficiencies of three bee (Hymenoptera: Apidae) species visiting rabbiteye blueberry. *J. Econ. Entomol.* 93, 1726–1731.
- SAS Institute, 2002–2003. *SAS/STAT User's Guide*, version 9.1. SAS Institute, Cary, NC.
- Schmid-Hempel, P., 1994. Infection and colony variability in social insects. *Phil. Trans. Roy. Soc. Lond. B* 346, 313–321.
- Schmid-Hempel, P., 1998. *Parasites in Social Insects*. Princeton University Press, Princeton.
- Seeley, T.D., 1995. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Harvard University Press, Cambridge, MA.
- Tarpy, D.R., 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc. Roy. Soc. Lond. B* 270, 99–103.
- Westcott, L., Nelson, D., 2001. Canola pollination: an update. *Bee World* 82, 115–129.
- Winston, M.L., 1987. *Biology of the Honey Bee*. Harvard University Press, Cambridge, MA.
- Woyciechowski, M., Kozłowski, J., 1998. Division of labor by division of risk according to worker life expectancy in the honey bee (*Apis mellifera* L.). *Apidologie* 29, 191–205.