

Resource Effects on Solitary Bee Reproduction in a Managed Crop Pollination System

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ABSTRACT Population density may affect solitary bee maternal resource allocation. The number of *Megachile rotundata* (F.), alfalfa leafcutting bee, females released for seed production of *Medicago sativa* L., alfalfa, may limit flower availability for nest provisioning. In turn, pollinator abundance also may affect crop yield. The *M. sativa* pollination system presents an opportunity to test for effects of density dependence and maternal manipulation on *M. rotundata* reproduction. A multiyear study was performed on *M. sativa* fields upon which *M. rotundata* densities were altered to induce low, medium, and high density situations. Numbers of adult bees and open flowers were recorded weekly; bee reproduction variables were collected once. Fields varied in plant performance for each site and year, and the intended bee densities were not realized. Therefore, the variable density index (DI) was derived to describe the number of female bees per area of flowers over the study period. As DI increased, percentages of pollinated flowers, established females, and healthy brood significantly increased, and the number of pollinated flowers per female and of dead or diseased brood significantly decreased. Sex ratio was significantly more female biased as DI increased. Overwintered offspring weights were similar regardless of DI, but significantly differed by year for both sexes, and for males also by field and year \times field interaction. Overall, resource limitation was not found in this field study. Other density-dependent factors may have induced a bee dispersal response soon after bees were released in the fields that circumvented the need for, or impact of, maternal manipulation.

KEY WORDS density dependence, maternal investment, *Megachile rotundata*, Megachilidae, *Medicago sativa*

Female bees are central place foragers, leaving from and returning to a common nest site where they provision nest cells with nectar and pollen on which larvae develop (Zurbuchen et al. 2010). Optimal foraging opportunities and reproduction might be expected if resources are unlimited and in close proximity to the nest, such as during peak bloom of a monoculture crop. Suboptimal foraging situations and negative impacts on reproduction could occur, however, if competition for resources escalates when the density of bees is relatively high and the availability of local resources is depleted or found at increasing distances from the nest site (Strickler and Freitas 1999; Bosch and Kemp 2005; Peterson and Roitberg 2006a,b; Zurbuchen et al. 2010). Therefore, the abundance and location of floral resources may impact the number of bee offspring produced, offspring size and sex ratio, and pollination efficiency. Fisher's, Optimal Allocation, and Conditional Allocation theories, as well as the multifaceted parental investment model, predict different outcomes for bee reproduction depending on changes that occur over the season, resource abundance and distance, age and size of bees, bee density at nesting sites, nest availability, egg limitation, and environmental conditions

(Fisher 1958, Frank and Swingland 1988, Rosenheim et al. 1996, Kim 1999, Strohm and Linsenmair 2000, Kim and Thorp 2001, Bosch 2008, Neff 2008). When resources become limited, the number of offspring produced is expected to decrease, because the resources are absent or at distances that increase the time and energetic costs of foraging. High foraging costs may result in maternal investment that favors the production of male over female offspring, because the larger females require more effort for resource provisioning. Furthermore, offspring size may decrease when resources are more difficult to provide in a timely manner. Extended time away from nests also increases the opportunity for invasion by parasites, predators, and usurpers.

For bee-pollinated flowers, an optimal bee service means that enough bees are present and capable of proper handling of flowers to assure that viable pollen is delivered to stigmas of all potentially successful ovules. Bee visitation can be affected by a flower's phenology, shape, size and complexity, olfactory and visual cues, numbers and arrangements in space, as well as by the geometry of the plant population, the development of flowers over time, and the longevity of awards offered (Handel 1983, Waddington 1983). The foraging behavior of bees and their likelihood of appropriate pollination (e.g., xenogamy or geitonogamy) also can be

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influenced by the aforementioned characteristics and mechanisms.

Alfalfa, *Medicago sativa* L. (Fabaceae), most commonly is pollinated using managed populations of *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), the alfalfa leafcutting bee, for commercial seed production in North America (Pitts-Singer and Cane 2011). The interaction between the crop pollinator and the plant is dynamic in the *M. sativa* cropping system. *M. sativa* has an indeterminate growth pattern and produces flowers over its long growing season. Although *M. sativa* is self-compatible, xenogamy (cross-pollination) is required to produce profitable amounts of quality seed (Rincker et al. 1988). However, once flowers are pollinated, plant resources are directed to the development of seeds rather than to additional flowers. If pollinated, flowers quickly wilt (in about 4 h); if not pollinated, flowers remain open for 5–7 d (Carlson 1928, Free 1993, Strickler 1999). In such a system, inundation of a blooming field with pollinating bees causes rapid depletion of floral resources (Strickler and Freitas 1999), but might assure a profitable seed yield, if many flowers are pollinated early in the season. Consequently, early depletion of floral resources may discourage female bees from nesting at local sites and inhibit their ability to reach their reproductive potential. Furthermore, the movement of bees between racemes within and between plants may be affected by flower abundance, which, in turn, may change the level of successful pollination (Strickler 1999, Strickler and Vinson 2000).

M. rotundata females are excellent pollinators of *M. sativa*, which requires “tripping” the flower to optimize pollination (Tysdal 1940). Tripping occurs when a bee lands on the keel of the flower causing a release of the pressure that holds the staminal column inside the keel; the staminal column abruptly springs forth from the keel and then remains upright and exposed against the banner petal of the flower. One bee visit that trips the flower is sufficient to create a pollination event and to expose the floral resources to make them available for the bees. *M. rotundata* females are not discouraged by the tripping event, but nectar-foraging *Apis mellifera* L. (Hymenoptera: Apidae), honey bees, learn to avoid this plant reaction by accessing the corolla from the side of the flower. Thus, in most cases, *M. rotundata* females are much more efficient *M. sativa* pollinators than are *A. mellifera* females and have been used as commercial pollinators in North America since the 1960s (Pitts-Singer and Cane 2011).

Although the current management system using *M. rotundata* results in profitable U.S. *M. sativa* seed yields (e.g., $\sim 1100 \text{ kg ha}^{-1}$), populations of this nonnative bee usually are not sustainable in U.S. commercial operations. To fulfill their pollinator supply needs, many U.S. *M. sativa* seed producers rely on *M. rotundata* production in Canada, where the bees tend to thrive in healthy populations, bee managers are very diligent in their efforts to maintain healthy bee stocks, summer adult emergence is not prevalent, and seed production and contract prices are less than in the United States (Pitts-Singer and Cane 2011). *M. rotundata* is solitary, but nests gregariously in artificial

nesting cavities, which are holes in bee boards provided for bees in or near *M. sativa* seed fields. Bee boards are made of polystyrene (e.g., L by W = 1.2 by 0.5 m) or wood (various sizes), each with $\geq 3,000$ evenly spaced holes (Richards 1984; Pitts-Singer and Cane 2011). Using *M. sativa* as a primary resource, female bees line nest cavities with leaf pieces and provision cells with pollen and nectar. Most populations of *M. rotundata* reared in the United States are not replaced through reproduction at commercial sites. Immature mortality is caused by pollen ball syndrome (no egg, or dead egg or first instar on uneaten provision; Pitts-Singer 2004), chalkbrood disease (a fungal disease of larvae), natural enemy attack, and unknown reasons (James and Pitts-Singer 2013). Bee populations are further reduced by adult bee migration from commercial sites (Pitts-Singer 2013a) and loss due to pesticides used to safeguard the crop from arthropod pests (Riedl et al. 2006, Hodgson et al. 2011). Loss is also due to so-called second generation bees, which are adults that emerge in the summer in the western United States and die before the winter; in more southerly U.S. regions, there may be three or four summer-emerging generations. These second-generation bees may not be able to replace themselves with overwintering progeny (i.e., diapausing progeny) before the nesting season ends because local resources are depleted late in the season or because necessary insecticide sprays kill these late-season nesters.

The potential tradeoff between optimal bee reproduction and high *M. sativa* seed yields must be considered in any economic assessment of the seed production system (Strickler 1996). Inundating fields with bees (up to 150,000 bees per ha) is a U.S. practice that is believed to assure profitable seed yield. However, previous studies of *M. rotundata* density in field cages revealed that high densities have significant negative effects on reproduction (Peterson and Roitberg 2006b, Pitts-Singer and Bosch 2010). The number of bee nests and cells per nesting female (Peterson and Roitberg 2006b, Pitts-Singer and Bosch 2010) and the size of female offspring (Peterson and Roitberg 2006b) were reduced when access to floral resources diminished. Nonetheless, studies of bees in cages cannot satisfactorily explain what occurs in open *M. sativa* fields where bees are free to emigrate or to use other resources beyond the field boundaries. Some studies have investigated *M. rotundata* density and *M. sativa* floral resources in commercial seed fields, but stopped short of intentional manipulation of bee or flower densities. Such studies showed that floral resources decreased to low levels in commercial fields within 4 wk after bees were introduced, floral resources closest to nesting sites declined more rapidly than more distant field locations, timing of bee release can affect the availability of floral resources, bees drifted between field domiciles in which bee nesting boards are housed, and releasing relatively small bee populations on fields might extend the duration of standing flower crop as bee forage and could improve bee reproduction (Goerzen et al. 1995, Strickler and Freitas 1999, Bosch and Kemp 2005).

The interactions between the pollinator and plant in the agricultural system just described could lead to a depletion of floral resources for the bees such that their reproductive potential would be limited. Unlike the mobile hives of *A. mellifera*, cavity-nesting *M. rotundata* rarely are moved between fields. The domiciles that contain artificial bee nesting sites are usually in permanent locations throughout the nesting season. Once local floral resources are depleted, these central place foragers must search beyond the target crop field for nest provisioning materials or for new nesting sites. Under the hypothesis that the availability of resources for reproduction leads to adjustments in progeny number, size, and sex ratio, the predicted response to limited *M. rotundata* larval food provisions would be a decrease in offspring number and size, and a biased sex ratio in favor of males.

To investigate the effects of bee densities and flower availability on bee reproduction and pollination in open fields, data were obtained over four years from three, small, isolated *M. sativa* seed fields in which varying densities of bees were released (same fields as in Pitts-Singer 2013a). A density index (DI; defined later) was calculated to provide a relative measure of *M. sativa* flowers available for the female bees for each field in each year. Bee cells were removed from the commercial nesting boards after the cessation of nesting, and cell contents were examined in order to determine if the destiny of each cell and the number, weight, and sex ratio of the surviving offspring differed according to the DI. The effects of the DI on the flowers tripped in the field (that represented bee pollination efficiency) were also examined. Results were compared to those from a study where *M. rotundata* were caged and could not abandon the site so that the number of nesting bees as well as *M. sativa* floral resources could be known. DIs were first described and calculated for this caged bee study (Pitts-Singer and Bosch 2010).

Materials and Methods

Three *M. sativa* seed fields (~0.6, 2.0, and 2.4 ha) were established in 2007 alongside hay fields on the property of the Utah State University Agricultural Experiment Station in Cache County, UT. These same fields were used for a 2007–2010 evaluation of retention of commercially released populations of *M. rotundata* (Pitts-Singer 2013a). Typical field management included application of a pre-bloom herbicide (pendimethalin, ammonium salt of imazamox, or clethodim products) and an insecticide (a carbofuran product) plus a mid-season insecticide (bifenthrin or endosulfan product); all fields were treated equally. Fields were separated by at least 22 km, and sites were never previously used for alfalfa seed production. Solid plywood bee domiciles (~1.2 m deep by 2.4 m wide by 1.8 m high) were erected at the edges of fields so that their openings faced the alfalfa and were oriented in a south-east direction as is common practice (Richards 1984, Pitts-Singer 2013a). Sufficient commercial, polystyrene Megablock bee boards (Beaver Plastics, Ltd., Alberta, Canada), each having 3,540 cavities, were housed in

the domiciles to accommodate at least two cavities per female bee; the exact number of boards was contingent on the number of intended female bees released. Hobo dataloggers (Onset Computer Corp., Bourne, MA) were mounted in the top of the inside of at least one domicile per field to record temperature and humidity throughout the bee nesting season in years 2007, 2008, and 2009.

Bee management methods are the same as in Pitts-Singer (2013a). Bee incubation was timed so that two bee releases, one week apart, occurred each year. Bees were incubated in a heated trailer at ~29°C, and incubation began at 3 and 4 wk prior to expected 10% *M. sativa* bloom. Prescribed high (111,197 bees/ha in 2007; 123,553 bees/ha in 2008–2010), medium (74,132 bees/ha), and low (37,066 bees/ha) densities of bees were released evenly in each domicile, and females nested using available resources.

Data obtained from Pitts-Singer (2013a) for use in this study were the estimated flowers ha⁻² and number of female bees present each week. To estimate flowers in the fields, permanent quadrats (0.09 m²) were established (9.9 quadrats/ha). Counted in those quadrats were the numbers of: 1) racemes, which included ones with buds, open flowers, and closed flowers, and 2) open flowers from only a subset of racemes ($n = \leq 40$); flowers were classified as tripped or untripped (= with nectar and pollen available as a bee resource). Despite working with a local consultant for alfalfa seed production, often the production of *M. sativa* seed the study fields was poor compared to industry standards due to pest problems, field management, and plot quality. Low seed yields also are known to occur in some seed production fields on commercial farms in the region due to pest and weather-related problems (Frank 2003; E. Anderson (farmer) and A. Evans (field consultant), personal communication). Therefore, seed yield could not be used to evaluate successful pollination, and the percentage of open flowers that were tripped was considered the measure of pollination efficacy in this study.

Estimation of nesting female bees was obtained from counts of bees in boards at night. With the aid of an otoscope (WelchAllyn, Skaneateles Falls, NY), bee board holes were inspected along a diagonal transect across the face of each board, alternating between the top-left to bottom-right and bottom-left to top-right. The estimated number of established females for the entire board was calculated from the number of holes examined and the proportion of those total holes per board containing female bees (Pitts-Singer 2013a). Counting along the diagonal helped to circumvent the fact that bee nesting preference and reproductive success varies according to locations and areas of the boards (Pitts-Singer 2013b). Other unmanaged and managed bees, including honey bees, were seldom or never seen on alfalfa flowers during data collection and, therefore, the only effective pollinators in the study fields were *M. rotundata*.

For this investigation, the reproductive success of the nesting bees was determined by diagnosing a subset of the cells produced. To obtain cells after nesting

activity had ceased, all bee boards were removed from the fields and stored at 16°C until early October, and then left at ambient conditions until cells were removed from boards. In winter (usually December), cells were punched out of the boards using a cooperators' specialized cell removal equipment (Richard Braul, Rosemary, Alberta Canada), keeping separate the harvest from each domicile and later recording total weight of cells from all boards pooled by domicile. For each domicile, a sample of cells (ranges = 2,331–2,736 in 2007, and 446–685 in 2008–2010) was taken for x-radiograph analysis (Pitts-Singer 2013b), from which was recorded the number of 1) live diapausing prepupae, 2) cells with pollen balls, 3) chalkbrood-infected cells, 4) cells with parasites, 5) cells with dead second generation pupae and adults, and 6) cells with dead larvae (instars 2–5) that died from unknown reasons. The total number of cells and the results for each cell category were pooled for each field to obtain the overall percentage of each cell category by field. Also, from the percentage of live prepupae and the weight of cells retrieved from the boards, we calculated the percentage of return on the released *M. rotundata* population (= % bee return on actual bee stock used in the field; see Pitts-Singer 2013a) and the live prepupae per kg. Up to 100 live prepupae per domicile were kept in cold storage (4–5°C) until they were incubated (29°C) for emergence the following summer. Once emerged, adult sex and weight of overwintered progeny were recorded.

Although certain *M. rotundata* densities were intended for pollinating the crop, the actual number of bees that emerged and the number that remained at the domiciles did not match the assigned treatment densities (Pitts-Singer 2013a; Table 1). Furthermore, the number of flowers available as resources for the bees was highly variable between fields, sampling dates, and years (Pitts-Singer 2013a). Therefore, a DI was generated so that a single variable could represent the interaction between the bees and flowers over the study period, just as was done for a previous study with bees in cages over *M. sativa* plants (Pitts-Singer and Bosch 2010). For each field in each year, the area under the curve was calculated for the estimated number of bees for each week of the nesting period, and the areas were summed. Similarly, the area under the curve of the estimated open flowers was calculated. The DI was computed as: Area of estimated bees / Area of estimated open flowers. Because the flowers counts were

1×10^3 , the DI values reported in the results are actually $DI \times 10^{-3}$ (as in Pitts-Singer and Bosch 2010).

Fields had originally been selected to serve as replicates of different treatments each year, with no field having the same treatment as another field in a given year (Table 1). However, it became clear during data analysis that the results from each field portrayed a performance quality due to field attributes that were not detected or described by the data collected. Based on the assumption that the bees and flowers in the fields performed in the same general manner, but with variation based on the level of field quality, mixed effects (random intercept) regression models were fitted to characterize the association between each dependent variable and the log-transformed DI. For each dependent variable model, the mixed model fit a best linear unbiased predictor specific to each of the three fields, allowing for incorporation of information from all sites simultaneously while also permitting site-specific inference between the relationship of DI and the dependent variable (PROC GLIMMIX, SAS 9.3 2002–2010; Littell et al. 1996). For visualization, the marginal linear predictor (population averaged predictor) for each analysis was plotted to show the overall relationships between the back-transformed model predictions and the actual variable data points. For the number of tripped flowers per highest obtained count of female bees (on one date per field and year), the percentage of bees released that were replaced with progeny, total live progeny produced by weight, sex ratio of overwintered progeny, a log transformation was performed to ensure a normal distribution of these dependent variables. For the percentage of females that established nests at domiciles, the percentage of tripped flowers, the percentage of cells that contained each category of cell contents as determined from x-ray analysis (live prepupae, pollen balls, chalkbrood cells, parasitized cells, dead larvae, or dead pupae and adults of second-generation bees), a beta distribution and a logit link function were used for the analysis of these dependent variables. For the two values in the percentage of females that established nests that exceeded 100% (i.e., 103 and 104%), the proportions were constrained between 0 and 1 for use of beta distribution.

The weights of overwintered male and female adults were tested for the effects of year, field, and their interactions using ANOVA (PROC GLM, SAS 9.3 2002–2010).

Results

The temperatures of the fields varied between the three years, and those differences are described (but the data not shown) in Pitts-Singer (2013a). In brief, the mean temperatures and relative humidity (RH) patterns were similar across fields and years. The Richmond site was more humid (1–10% higher mean RH on a given day) than the other fields, especially in the early part of the study periods. Seasonal mean high temperatures were 1–2°C warmer in 2007 than in the other years. The mean temperatures at the Wellsville site were warmer than at the other sites on most days

Table 1. Assigned (but unachieved) treatment assignment (Trt) and density indices (DI; see text) for each alfalfa seed field and year that describes the density of *M. rotundata* females that were present and the open flowers that were available to them during several weeks of the growing season

Year	Cache Junction		Richmond		Wellsville	
	Trt	DI	Trt	DI	Trt	DI
2007	Low	0.56	High	2.12	Medium	0.46
2008	Medium	0.70	Low	0.61	High	0.63
2009	Low	0.21	High	0.66	Medium	0.06
2010	High	0.82	Medium	2.72	Low	0.12

in 2007 and 2009, and in 2008 mean temperatures were warmer at the Richmond site than the others.

DIs are reported for each field and year of the study (Table 1). The DIs were very high at the Richmond site in 2007 and 2010. Wellsville had the lowest of the DI values for 2007, 2009 and 2010. In 2008, DIs were similar across fields. In no year did the DIs represent all of the intended relative densities of bees (high, medium, or low numbers of bees per ha) released on the field as treatments (Table 1). In 2009 at the Cache Junction site and in 2010 at the Wellsville site, the DIs were relatively low, which was the assigned treatment. The high DI at the Richmond site in 2007, but not 2010, reflects its high treatment assignment (Table 1).

The mixed logistic regression models did not show significant effects of the DI on the percentage of released females that established at the domiciles ($F = 0.65$, $df = 1,8$, $P = 0.444$). However, the highest percentages of female establishment were not obtained at the lower DI values (i.e., fewer bees per available flowers) as was expected.

Except for the Richmond site in 2007, the percentage of open flowers that were tripped was $< 30\%$ each year (Fig. 1A) and was nearly equal across sites in 2008. The relationship between the DIs and the percentage of open flowers that were tripped was positive and significant ($F = 19.11$, $df = 1,8$, $P = 0.002$; Fig. 1A). The exceptional Richmond site had the highest percentage of tripped flowers in each year and was much higher than the other sites in 2007 and 2009. Although the percentage of tripped flowers increased with increasing DIs, the number of tripped flowers per female bee (as estimated for the highest night count of females) was negatively related to the DI ($F = 16.64$, $df = 1,8$, $P = 0.004$). That is, the individual female bee efficiency decreased (Fig. 1B), with the lowest number of tripped flowers per female occurring at the Richmond site, except in 2008 (Fig. 1B).

The percentage of cells produced at each site that were live prepupae was highest in the first year of study for all fields, and was positively and significantly affected by the DI ($F = 20.96$, $df = 1,8$, $P = 0.002$; Fig. 2A). Contrary to prediction, the sites with the lowest DIs also had some of the lowest percentages of live prepupae. The relationship between the DI and weight of live brood cells was also positive and significant ($F = 26.21$, $df = 1,8$, $P = 0.0009$; Fig. 2B). Although the percentages of cells with live prepupae reveals information about the viability of bees at the study sites (Fig. 2A), the calculation of the live brood per kg of cells revealed differences between sites in the weight of those prepupae (Fig. 2B), with more lighter prepupae needed to create the same mass as fewer heavier ones. For instance in 2007, it appears that the percentage of live prepupae were nearly equal at the Cache Junction and Richmond sites (Fig. 2A), but the number of live prepupae per kg in that year was higher for the Richmond site (Fig. 2B). This means that those Richmond prepupae weighed less than the Cache Junction prepupae. Also, although the percentage of prepupae at the Richmond site in 2009 was lower than in 2007 and 2010, the number per kg in 2007 was higher than

expected because those prepupae weighed less in that year than in the other years. Indeed the weight of overwintered females was found to be less in 2009 than in the other years (Fig. 7B).

The percentage of released bees returned as viable progeny also was significantly and positively affected by the DI ($F = 6.22$, $df = 1,8$, $P = 0.037$) and was highest at the Richmond site and lowest at the Wellsville site (Fig. 3). This result means that at the higher DIs, the number of cells produced and the quality of those cells did not diminish as the number of bees per flower increased.

There was a negative and significant relationship of the DI with the percentage of cells that contained pollen balls ($F = 45.34$, $df = 1,8$, $P = 0.0001$; Fig. 4A), chalkbrood larvae ($F = 8.10$, $df = 1,8$, $P = 0.022$; Fig. 4B), other dead larvae ($F = 18.01$, $df = 1,8$, $P = 0.003$; Fig. 5A), or dead pupae or adults of second-generation bees ($F = 7.61$, $df = 1,8$, $P = 0.025$; Fig. 5b). The percentage of parasitized cells had no significant relationship with DI ($F = 1.48$, $df = 1,8$, $P = 0.259$). Cells from the Wellsville site had the highest proportion of pollen balls, dead larvae, and second-generation pupae and adults in most years compared to the other fields, reflecting the generally poor performance of the bees in that field.

The sex ratio of overwintered brood was positively and significantly related to DI ($F = 8.22$, $df = 1,8$, $P = 0.021$; Fig. 6). The proportion of males was highest at the Wellsville and Cache Junction sites in 2009, and at the Richmond site in 2008 and 2009. Mean adult weight of overwintered adult bees did not appear affected by DI (Fig. 7A). Male weights were affected by field, year, and by their interactions (field: $F = 3.59$, $df = 2$, 1255, $P = 0.027$; year: $F = 9.32$, $df = 2$, 1255, $P < 0.001$; field \times year: $F = 3.87$, $df = 4$, 1255, $P = 0.004$). The interaction of field \times year revealed that the Cache Junction site in 2010 had significantly heavier overwintered adult males than males from the other sites in all years, except for males from the Wellsville site in 2008. Males from the Wellsville site in 2008 were significantly heavier than males from other sites in 2009 only. Female weights only were affected by year (field: $F = 2.67$, $df = 2$, 1713, $P = 0.070$; year: $F = 28.44$, $df = 2$, 1713, $P < 0.001$; field \times year: $F = 2.19$, $df = 4$, 1713, $P = 0.067$; Fig. 7B), with bees in 2009 weighing less than bees in other years. Female adults obtained from sites with two of the lowest DIs in 2009 (DI = 0.06 and 0.21) weighed less than all other adults.

Discussion

In this managed crop pollination system, the examination of ecological theories under open field conditions did not reveal impacts of resource limitation or bee-plant interactions on overall *M. rotundata* offspring production and *M. sativa* pollination. Nesting sites with the highest numbers of female bees per open flowers (i.e., highest DIs) had the highest yields of viable progeny. Additionally, under the conditions of comparatively high DIs, allocation of resources by mother

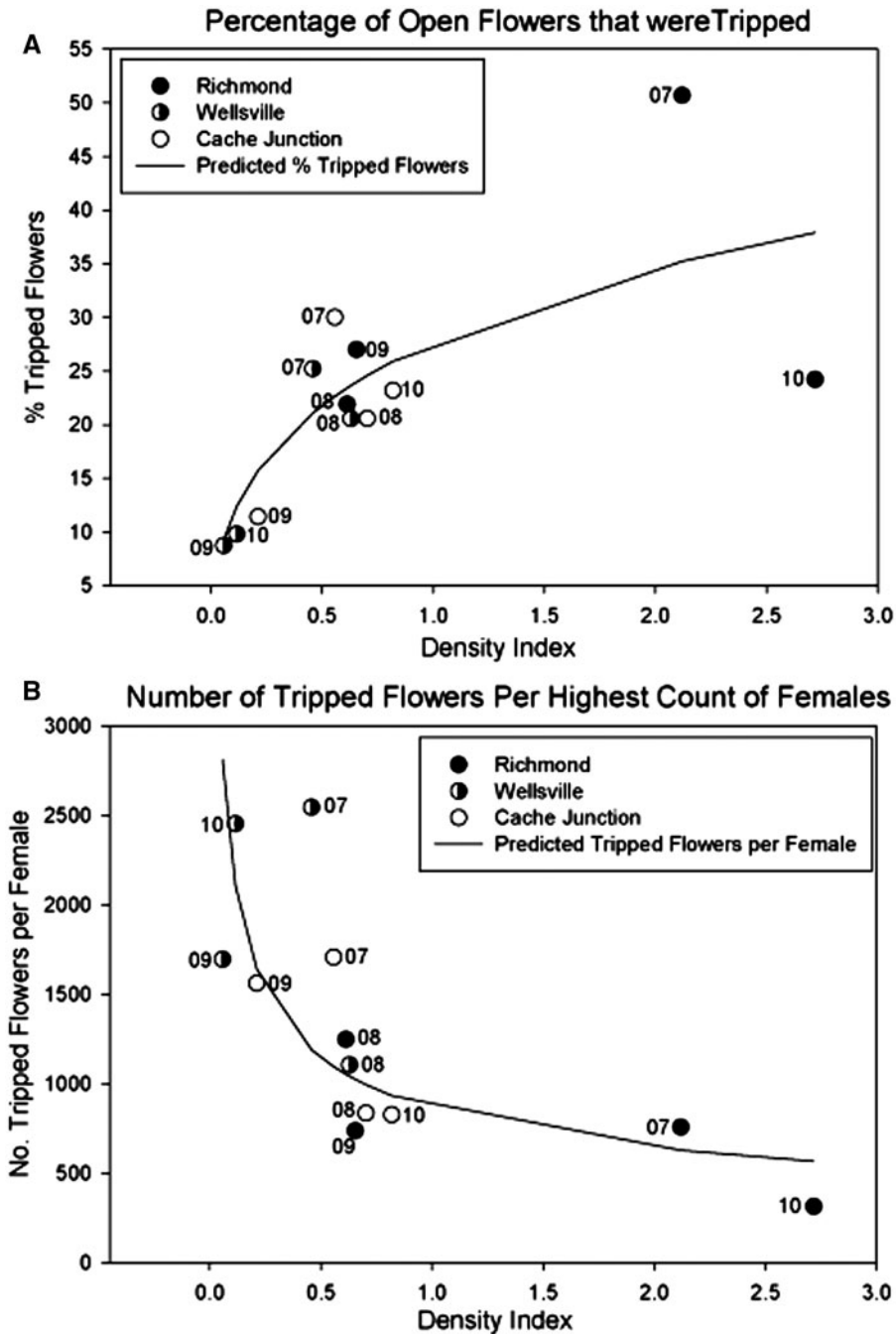


Fig. 1. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, (A) the percentage of the estimated number of flowers that were tripped and (B) the number of flowers tripped per the highest count of *M. rotundata* female bees (only one date per field and year) according to the DI. Numbers by symbols represent study years.

bees was not adjusted to increase the production of less-costly males or to produce smaller progeny in general. The bee return on the number of released bees was highest when DIs were high, exceeding 400% at the Richmond site in one year. That no maternal manipulation of resources was detectable each year is

likely explained by the fact that resources were never truly limited due to the number of foraging bees throughout the field season, because fewer than expected female bees remained at the local nesting sites (Pitts-Singer 2013a). Contrary to prediction, negative reproductive outcomes, such as incidence of pollen

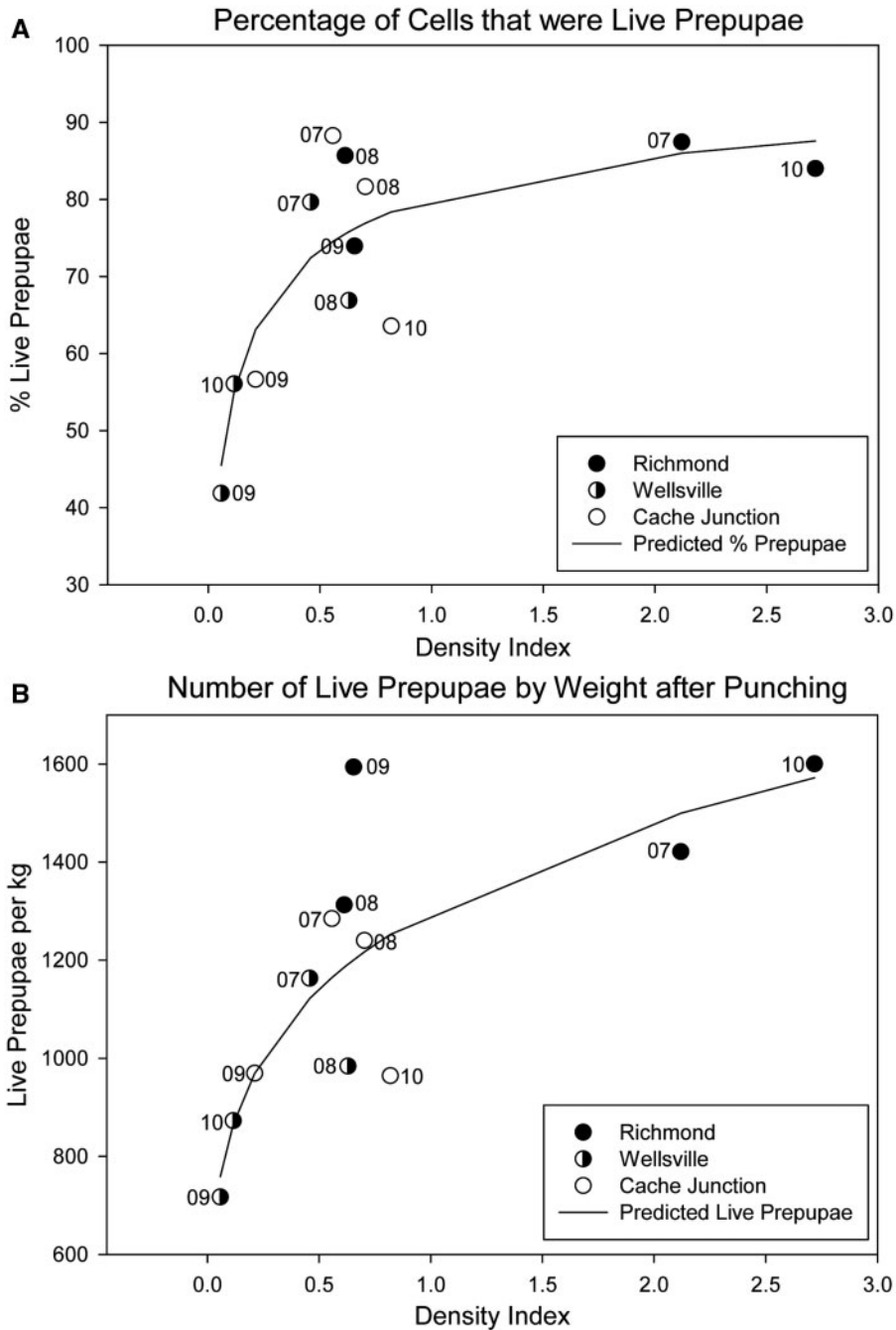


Fig. 2. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, (A) the percentage of all cells that contained live, diapausing prepupae and (B) the live prepupae per kg according to the DI. Numbers by symbols represent study years.

balls, chalkbrood disease, and dead larvae, significantly decreased as DIs increased and likely are attributable to factors unrelated to the density of bees that remained in the fields throughout each season. Although the DI is very useful for leveling differences between bee and plant responses at the three study sites, it only

captures the dynamic relationship between a plant and its pollinator over a field season. It does not capture other time-sensitive influences from the field such as temperature, humidity, photoperiod, and resource availability from the landscape beyond the field margins. These other environmental factors may help to

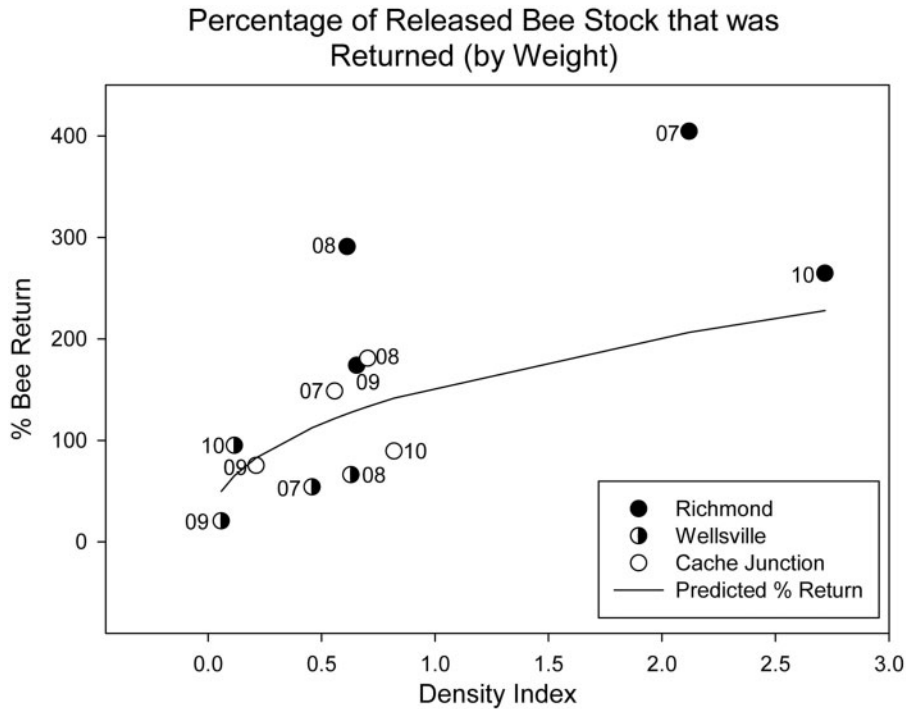


Fig. 3. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, the percentage of the stock (by weight) of those bees that were released in each field that was returned as new brood according to the DI. Numbers by symbols represent study years.

explain how and why bees did not reach the high densities that were intended as field treatments.

In a study in which *M. rotundata* were caged over blooming *M. sativa*, DI was first used as a measure of bee and flower density on account of the unevenness of floral resources between cages and the resulting variable number of female bees that survived to found nests at provided sites within those cages over time, regardless of an intended female density treatment assigned to each cage (Pitts-Singer and Bosch 2010). As in this current study, there was reduced establishment of nesting bees for the assigned density treatments of the cage study. It is interesting that the range of DIs in the field study (0.06–2.72) fell within the broader range of those in the cage study (0.03–3.95); the range of density treatments in the cage study was also broader. However, not all of the relationships between the DIs and most variables in this open field study support the findings of the cage study. Although the percentage of overwintering (diapausing) prepupae generated by the caged bees was not significantly related to the DIs, the increase in DIs was significantly related to a decrease in the percentage of bees that survived to adulthood (overwintering plus summer-emerging bees) and to the increase in the percentage of pollen balls. Thus, there was an overall loss of viable progeny in the cage study as DIs increased. In the field study, the percentage of cells containing live diapausing prepupae was significantly greater at the higher DIs, and the percentages of cells containing pollen balls, chalkbrood cadavers, and

other dead bees were significantly lower at the higher DIs. Records of the adults that emerged in the summer in the field study are not available for comparison to the cage study.

The quantity of bee cells produced in the two studies also was determined, but different methods were used. The number of nests and cells was used to assess reproductive output in the cage study, but in the current study, the production was expressed as the percentage of released bees returned to the population as live prepupae per kg of bee cells and the percentage of return on the released bee stock. In the cage study, as DIs increased, nest and cell production decreased (Pitts-Singer and Bosch 2010), with the lowest reproduction of *M. rotundata* occurring at the two highest DIs (above 3.0). In the field study, both the percentage of bee return and live bees per kg were highest at the highest DIs. Negative impacts attributed to the higher DIs in the cage study could not be avoided by the trapped female bees that had no choice but to remain in unfavorable, high bee density conditions with limited resources; they could not leave to find better nesting sites or other resources. Moreover, all DIs in the field (where bees could travel beyond the field margins) were lower (< 3.0) than the highest DIs in the cage study. The downturn in bee production in the cage study with DIs > 3.0 suggests that a DI of 3.0 may be a threshold at which bee reproductive success is inhibited.

A common outcome of the two studies was that the sex ratio of the overwintered progeny (males:females)

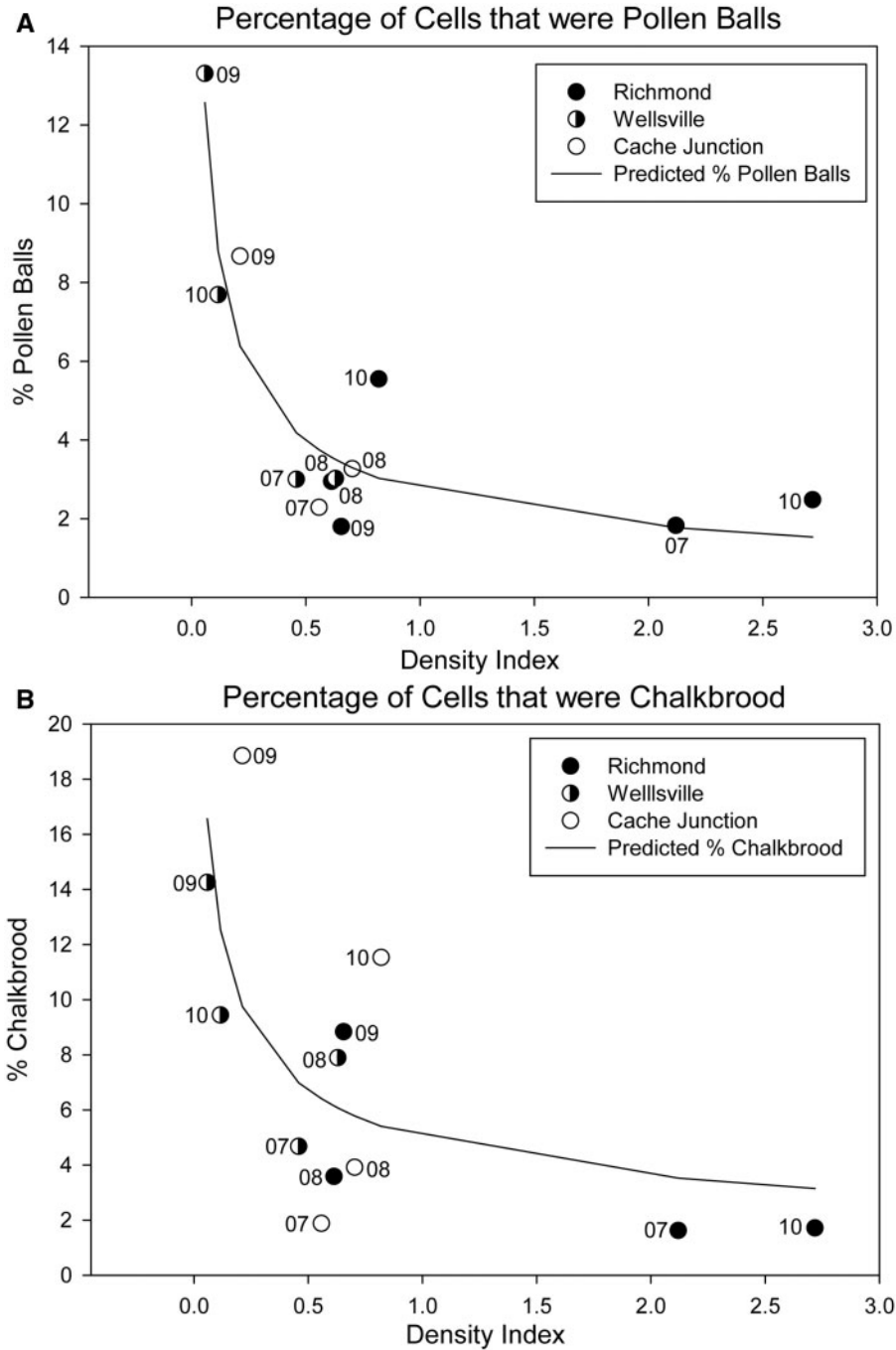


Fig. 4. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, (A) the percentage of all cells that were pollen balls and (B) the percentage of all cells that were chalkbrood according to the DI. Numbers by symbols represent study years.

were low and had similar ranges (in cages: 0.30:1–1.65:1; in fields: 0.34:1–1.69:1; Pitts-Singer and Bosch 2010). In the field study, there was a significantly negative relationship found between sex ratio and DI, but no significant correlation in the cage study. That bees in the field produced proportionately more females

when DIs were high, contradicts the hypothesis that increased competition for resources due to high bee abundance would bias offspring production toward the less costly sex, the males. Females determine the sex of their offspring by controlling the release of stored sperm (Gerber and Klostermeyer 1970), with fertilized

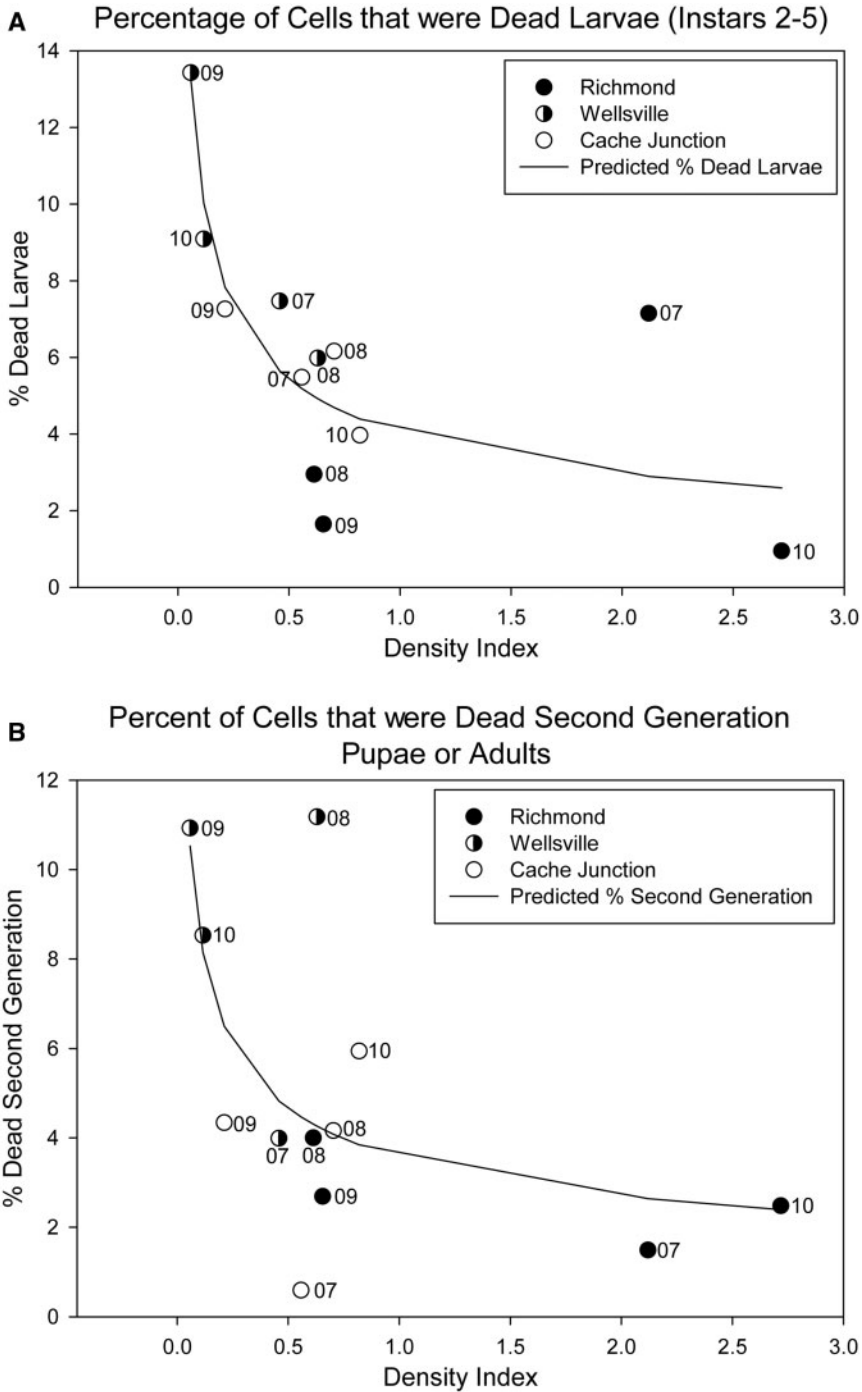


Fig. 5. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, (A) the percentage of all cells that were dead larvae (instars 2–5) and (B) the percentage of all cells that were dead pupae and adults of second-generation bees according to the DI. Numbers by symbols represent study years.

(diploid) eggs becoming females. In our study, it appears that floral resources were relatively high in 2008 and 2009 when the most male-biased of overwintered brood sex ratios were recorded, so it is not clear why

nesting females laid proportionately more male-destined eggs in these particular years. However, the sex ratio of brood that reached adulthood and emerged in the summer is unknown and may differ from this

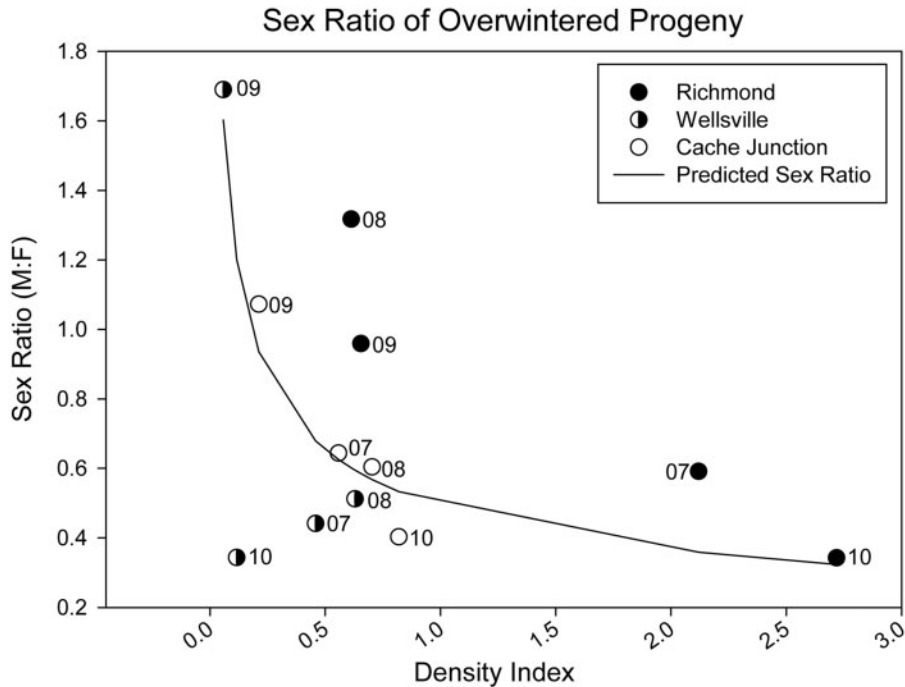


Fig. 6. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, the sex ratio (M:F) of overwintered progeny according to the DI. Numbers by symbols represent study years.

overwintered brood. The only assessment of second-generation bees in the field study was noting the percentage of cells that contained dead pupae or adults once the cells were removed from the bee boards, and this variable was negatively affected by DI. The number of second-generation bees that emerged during the nesting season and how this variable affected the results of this study were not assessed.

Another similar outcome between the two studies was the pollination performance of *M. rotundata*. Because seed yield was low in both fields, the tripping of open flowers was used as a measure of pollination success because it represents the potential of the crop to have produced seeds, if plant performance, seed predator abundance, and weather conditions had been more favorable prior to harvest. For both the cage and field studies, the percentage of open flowers that were tripped increased with increasing DIs, while the number of tripped flowers per female decreased. Thus, individual female efficacy was reduced as more bees per flowers occurred. The percentage of flowers tripped was up to 97% in some of the cages in 2004, when flower resources were more sparse than in the 2003 cages (% tripped range = 6–27%). This field study was more similar to the 2003 cage trial, with the percentage of tripped flowers in the fields ranging from only 9–51% of the open flowers. Again, it is apparent that floral resources were not limited for the bees that remained in the open fields over time.

Other previously analyzed data reported from the same research fields (Pitts-Singer 2013a) provide valuable and necessary information for further

interpretation of the current results. First, just as in the cage study, there was annual variation in flower abundance. Although *M. sativa* fields were irrigated and managed with pesticides as recommended, weed and insect problems as well as environmental conditions differed between fields and years such that bloom abundance and duration was not constant. Although floral resources seemed to be available for bees throughout the season in the three fields each year, only 46–79% of any intended stocking density was reached, and only at one of the fields (Richmond) in two of the four study years was there 100% establishment of females that actually entered the field (Pitts-Singer 2013a). It is unknown why so many bees left fields that had abundant nesting cavities and available floral resources. The Richmond site had much more successful nesting than the other sites, but information collected cannot explain why this site differed in this respect. Nonetheless, having knowledge of the actual number of females that survived to fly out into the field, and then chose to nest in the commercial bee boards, allows for an understanding that fields are not truly inundated with bees in a manner that diminishes bee reproductive success due to lack of resources for larval provisioning. Thus, even though the DIs increased in the fields, no high bee density situations were created that were detrimental to reproductive output. On the other hand, despite any measure of bee density and resource availability, for the fields that had less than 100% bee return, the true bee reproductive potential was far less than would be predicted in a completely optimal nesting situation in which a single

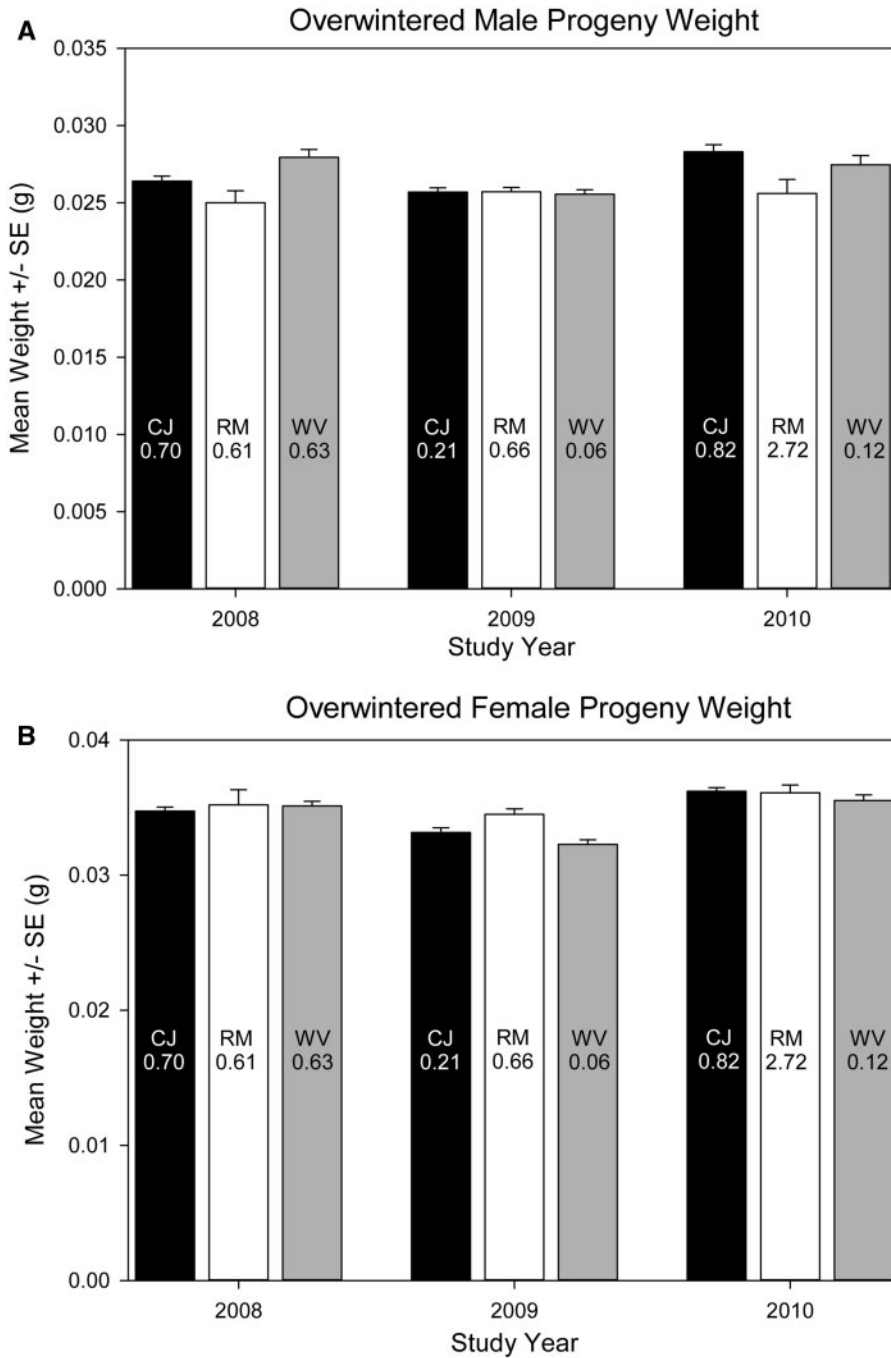


Fig. 7. From data collected in Utah alfalfa seed fields in 2007–2010 studies on *M. rotundata*, the mean (\pm SE) weight (g) of overwintered (A) male and (B) female adult bees according to year. The DI is shown on each bar; W is Wellsville; C is Cache Junction; R is Richmond.

M. rotundata female could produce up to 40 eggs in her lifetime (Gerber and Klostermeyer 1970).

Several studies have reported the influence of floral resources on progeny weight and sex allocation (e.g., Kim 1999; Kim and Thorp 2001; Peterson and Roitberg 2006a,b; Zurbuchen et al. 2010). In a cage study

with *M. apicalis*, Kim (1999) found an effect of resource level on maternal investment that increased female production and increased the amount of provision for those female offspring when resources were abundant. Peterson and Roitberg (2006a) showed that increased *M. rotundata* flight distance to *M. sativa*

resources caused a reduction in the production of daughters, and also a lower investment in individual offspring. However, in a caged study where floral resources, rather than distances, were varied, Peterson and Roitberg (2006b) found that reduced resources resulted in less investment per daughter and lower offspring production in general, but that sex ratio was unaffected. Perhaps the lack of influence on sex ratio by floral abundance in Peterson and Roitberg (2006b) and in this current field study means that the bee per flower ratio was below the critical threshold that would cause a change in resource allocation to bias production of male over female offspring.

Over the four study years, there was some significant variation in adult progeny weight due to year and site, but DI did not explain any differences in the weight of overwintering *M. rotundata* progeny; thus, no effect of maternal manipulation of progeny size in response to resource availability was observed. *Osmia bicornis* L. (Megachilidae) progeny weight is known to be affected by provision mass size and by temperature, with higher temperatures leading to smaller body size (Radmacher and Strohm 2010). In this study, however, the overwintered progeny from 2009 weighed less than progeny from 2008 and 2010 despite the fact that the estimated number of open flowers in the fields were relatively high in this year and temperatures were not extreme (Pitts-Singer 2013a). Focus in future studies on the effects of environmental parameters on *M. rotundata* maternal foraging activities and larval development throughout the nesting season may yield more fruitful interpretation of variation in progeny body size.

Exploration of this bee–plant system in both controlled and natural conditions offers a more acute understanding and valid interpretation of results than either type of study alone. Perhaps *M. rotundata* pollinator populations have a natural propensity to regulate population density in the field that avoids overcompetition for offspring production under managed conditions, such as for commercial *M. sativa* seed production. Although reproductive potential is often unmet, the causes of U.S. commercial population losses do not seem to be due to overcrowded situations during nesting, but seem more likely to be due to the immediate dispersal of bees from the commercial site, from failure of the progeny produced in commercial bee boards to survive for known and unknown reasons (James and Pitts-Singer 2013; Pitts-Singer 2013a,b), and from the emergence of second-generation bees and their limited ability to replace themselves at the end of the nesting season. Very few studies have shown whether bees released at field shelters remain at those sites over the nesting season (Bosch and Kemp 2005, Pitts-Singer 2013a), and one study has suggested that bees drift between domiciles and fields (Bosch and Kemp 2005). Dispersal in another solitary managed bee, *Osmia lignaria* Cresson (Megachilidae), used as an orchard pollinator also can be very high and unrelated to availability of crop bloom (Torchio 1982, Artz et al. 2013). How unmanaged, natural *M. rotundata* aggregations would react under similar conditions may never be realized because the availability of cavities in

such high numbers in nature would be highly unlikely to occur.

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