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Multiple introduction of honeybee colonies increases cross-pollination, fruit-set and yield of 'Black Diamond' Japanese plum (*Prunus salicina* Lindl.)

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SUMMARY

Japanese plum (*Prunus salicina* Lindl.) belongs to the *Rosaceae* family, which carries the *S*-RNase-mediated gametophytic self-incompatibility system, which prevents self-fertilisation, and thus promotes out-crossing. The plum cultivar 'Black Diamond'® has become, one of the most important cultivars in Israel in the last decade, yet its yield is low in comparison with its known potential. Honeybees (*Apis mellifera*) are the most important pollinators for plums and several studies have demonstrated an apparent relationship between the number of honeybees and the size of the fruit crop. Therefore, in this study, we focussed on improving bee management in a 'Black Diamond' orchard. In four consecutive years of experiments, we examined the effects of increasing the density of bee colonies, and of making multiple introductions of colonies, on honeybee activity and on their effectiveness as pollinators of 'Black Diamond'. We showed that four separate introductions, each of 0.625 colonies ha^{-1}, every 2 – 3 d from 10% full bloom to 3 d after full bloom, with a total density of only 2.5 colonies ha^{-1}, resulted in the greatest level of fruit set.

Japanese plum (*Prunus salicina* Lindl.) belongs to family *Rosaceae*, which carries the *S*-RNase-mediated gametophytic self-incompatibility (GSI) system. This system prevents self-fertilisation and thus promotes cross-pollination. Pollen rejection occurs when the *S*-haplotype of the pollen matches one of the two *S*-haplotypes in the pistil. For example, a pistil carrying the S_a-haplotype inhibits the growth of S_a pollen, but not pollen with other haplotypes (McCubbin and Kao, 2000). Hence, cultivar pairs can be incompatible (both cultivars having identical S-haplotypes), semi-compatible (cultivars sharing one of the two S-haplotypes), or fully-compatible (the two cultivars carry different *S*-haplotypes). In commercial orchards, cultivar pairs are either fully- or semi-compatible. However, it was shown in some apples cultivars that under sub-optimal conditions for pollination and fertilisation, the potency of cross-fertilisation between semi-compatible cultivars was lower than that of fully-compatible cultivars (Goldway *et al*., 1999; Schneider *et al*., 2005).

Honeybees (*Apis mellifera*) are the most important pollinators for plums (Free, 1993; Calzoni and Speranza, 1996; 1998; Delaplane and Mayer, 2000). Several studies have demonstrated a positive relationship between honeybee foraging activity in an orchard and later fruit yield (Free, 1993). For example, Free (1962) found that plum trees near to honeybee colonies were visited by more bees, and had greater fruit set than distant trees. The recommended density of beehives for plum orchards ranges from 2.5 colonies ha^{-1} (McGregor, 1976; Crane and Walker, 1984; Kevan, 1988; Scott-Dupree *et al*., 1995) to 5.0 colonies ha^{-1} (Mayer *et al*., 1986; Standifer and McGregor, 1977). However, there are other factors that limit cross-pollination by bees, such as their tendency to restrict their mobility to one row, usually containing a single cultivar (Williams and Smith, 1967; Eisikowitch *et al*., 1999), and their preference for the flowers of other fruits or competing flora, which they find more attractive (Free, 1993).

Al-Tikrity *et al*. (1972) were the first to recommend the sequential introduction of bee colonies into crown vetch fields. By applying sequential introductions of hives into pear orchards in Washington State, the number of bees, and consequently fruit-set, were raised (Mayer *et al*., 1994). This approach was also examined in 'Red Delicious' apple and in 'Spadona' pear in Israel where half of the colonies were introduced at 10% full bloom (FB) and half at FB, resulting in an increase in the number of bees per tree, bee mobility between rows, and the percentage of "topworkers" (in apples). Consequently, fruit set and yield were increased (Stern *et al*., 2001; 2004).

The plum cultivar 'Black Diamond'®, which was bred by the Sun-World Corporation (Coachella, CA, USA) and also registered as Suplumeleven™, has become one of the most important plum cultivars in Israel over the last decade. 'Black Diamond'® usually blooms during the first half of March when it is cold, windy and wet (i.e., weather that is unfavourable for bee flight, pollination, pollen-tube growth and fertilisation). In addition, among those plum cultivars that bloom at the same time as 'Black Diamond'®, only semi- (and not fully-) compatible cultivars were found. One of these cultivars is 'Angeleno', which serves as the pollinator of 'Black

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Diamond'® in the orchard in which the experiments took place (Sapir *et al*., unpublished data). Semi-compatibility adds to the possible deficiency in pollination of 'Black Diamond'®.

The aim of the present study was to evaluate different beehive insertion regimes in a 'Black Diamond'® orchard, in order to enhance bee activity and, consequently, to increase fruit-set and yield.

TABLE I
Location of treatments in each experiment in 'Black Diamond'® Japanese plum

		Treatment		
Expt.	Year	North†	Centre	South
1	2003	DD[2]	Control[1]	SI[3]
2	2004	DD[2]	Control[1]	SI[3]
3	2005	SIDD[4]	SI[3] (Control)	MIDD[5]
4	2006	MIDD[5]	SI[3] (Control)	MI[6]

[1]Control = recommended introduction of 2.5 colonies ha^{-1} at 10% FB. [2]DD = double-density. [3]SI = sequential introduction (×2). [4]SIDD = sequential introduction (×2) with double-density. [5]MIDD = multiple introduction (×4) with double-density colonies. [6]MI = multiple introduction (×4).
†The orchard was divided into three parts.

MATERIALS AND METHODS

Orchard design

The experiments were conducted in a commercial Japanese plum orchard, located at Kfar-Blum, in the northern part of Israel (330° N; 35.5° E; 100 m a.s.l.). This is a semi-arid area with high temperatures (*ca*. 38°C max.) and low humidity (< 35% RH) during the Summer (May – October). Annual precipitation (November – April) is approx. 500 mm, with low temperatures (*ca*. 2°C min.) between December and February. The soil was 0.8 m deep and consisted of a well-draining, basaltic protogromosol (> 60% clay) with a pH of 7.7 and a $CaCO_3$ content of 9% (w/w). The main cultivar in the orchard was 'Black Diamond'®, with 'Angeleno' as pollinator. Both cultivars were grafted on 'Marianna' rootstock. Trees were planted in 1998, and were similar both in size and in crop load in all years before the experiment. Two rows of 'Black Diamond'® were planted at a spacing of 2.5 m × 4.5 m (900 trees ha^{-1}) with two adjacent rows of 'Angeleno'. Rows were oriented North-South. There was a good overlap between bloom in the two cultivars each year.

Experimental design: In all experiments, the orchard was divided into three parts - North, Centre and South (Table I). Each part received a different treatment, and each treatment was separated by a "buffer-zone" of at least 300 m in order to reduce overlapping foraging between bees from the different treatments. Table II describes, in detail, each treatment during the 4 years of experiments.

Experiments 1 and 2 - Sequential introduction (SI) vs. *double density (DD) in 2003 and 2004*: As a control, the standard recommendation in Israel was applied [i.e., all colonies were introduced at a density of 2.5 colonies ha^{-1} at the start of bloom (10% FB)]. The double-density treatment (DD) involved introduction of 5.0 colonies ha^{-1} at 10% FB. The sequential introduction (SI) treatment involved introduction of 1.25 colonies ha^{-1} at 10% FB, and a second introduction of 1.25 colonies ha^{-1} at FB, giving a total of 2.5 colonies ha^{-1}.

Experiment 3 - Sequential introduction of double density (SIDD) vs. *multiple introduction of double density (MIDD) in 2005*: Colonies were introduced sequentially in all treatments. The SI (= control) treatment involved the introduction of 1.25 colonies ha^{-1} at 10% FB, and an additional introduction of 1.25 colonies ha^{-1} at FB, making a total of 2.5 colonies ha^{-1}. The SIDD treatment involved introducing a double-density of 2.5 colonies ha^{-1} at 10% FB and a second introduction of 2.5 colonies ha^{-1} at FB, giving a total of 5.0 colonies ha^{-1}. The MIDD treatment involved the introduction of 1.25 colonies ha^{-1} on each of four occasions: 10% FB, 50% FB, FB, and FB + 3 d, giving a total of 5.0 colonies ha^{-1}.

Experiment 4 - Multiple introduction of double-density (MIDD) vs. *multiple introduction (MI) in 2006*: Colonies were introduced sequentially in all treatments. The SI (= control) treatment involved introducing 1.25 colonies ha^{-1} at 10% FB, and a second introduction of 1.25 colonies ha^{-1} at FB, making a total of 2.5 colonies ha^{-1}. The MIDD treatment involved the introduction of 1.25 colonies ha^{-1} on each of four occasions: 10% FB, 50% FB, FB and FB + 3 d, giving a total of 5.0 colonies ha^{-1}. The MI treatment involved the introduction of 0.625 colonies ha^{-1} on each of four occasions: 10% FB, 50% FB, FB, and FB + 3 d, giving a total of 2.5 colonies ha^{-1}.

Bee-foraging activity

Bee foraging activity on 'Black Diamond'® trees was assessed at a distance of approx. 50 m from the hives. The number of bees per tree was counted with a manual

TABLE II
Experimental treatments to evaluate the effects of high colony density and sequential or multiple colony introductions on honeybee foraging activity, fruit-set and fruit yield in 'Black Diamond'® Japanese plum

			No. of colonies ha^{-1} at each introduction				
Expt.	Year	Treatment	10% FB	50% FB	FB	FB+3	Total
1	2003	Control[1]	2.5	–	–	–	2.5
		DD[2]	5.0	–	–	–	5.0
		SI[3]	1.25	–	1.25	–	2.5
2	2004	As 2003					
3	2005	SI[3] (Control)	1.25	–	1.25	–	2.5
		SIDD[4]	2.5	–	2.5	–	5.0
		MIDD[5]	1.25	1.25	1.25	1.25	5.0
4	2006	SI[3] (Control)	1.25	–	1.25	–	2.5
		MIDD[5]	1.25	1.25	1.25	1.25	5.0
		MI[6]	0.625	0.625	0.625	0.625	2.5

[1]Control = recommended introduction of 2.5 colonies ha^{-1} at 10% FB. [2]DD = double-density. [3]SI = sequential introduction (×2). [4]SIDD = sequential introduction (×2) with double-density. [5]MIDD = multiple introduction (×4) with double-density colonies. [6]MI = multiple introduction (×4).

counter, while circling the tree at a distance of about 1 m for 60 s (Free and Spencer-Booth, 1963; Mayer *et al*., 1986). Activity was measured in the morning (08.00 - 10.00 h) on ten trees per treatment in each experiment. The number of days on which bee foraging activity was measured varied between years.

Fruit-set and yield

While at the 'balloon' stage, 2,000 flowers were labelled randomly in one row of 'Black Diamond'®, adjacent to an 'Angeleno' row, in each treatment in each experiment (100 flowers per branch × two branches per tree × ten trees). The percentage fruit-set was determined approx. 4 – 6 weeks after FB. In 2003, the fruit yield on each of ten trees was recorded at harvest.

Statistical analysis

Percentage fruit-set data were subjected to arcsin transformation before analysis, to provide a normal distribution. Data were analysed for statistical significance by the general linear model (GLM) procedure. Duncan's new multiple range test was applied to compare treatments when ANOVA showed significant differences among the means.

RESULTS

Experiment 1 (2003)

SI vs. *DD*: In this preliminary experiment, bee-foraging activity was determined on only one typical day at full bloom (FB) of 'Black Diamond'®. Nonetheless, considerable differences were observed between treatments (Table III). The highest number of bees per tree (8.1) was in the sequential introduction (SI) treatment, followed by the double-density (DD) treatment (6.7). Yet, the total number of hives ha^{-1} in SI was half that in the DD treatment (Table II). In the control treatment, the total number of hives was the same as in the SI treatment, and the number of bees per $tree^{-1}$ was the lowest (5.7). Doubling the hive density from 2.5 colonies ha^{-1} in the control, to 5.0 colonies ha^{-1} in the DD treatment, or introducing the hives sequentially (SI), to a total density equivalent to that in the control (2.5 colonies ha^{-1}), increased the initial fruit set by approx. 75%, from 8.5% to about 15%, and the total fruit yield by about 30%, from 64 to 84 kg $tree^{-1}$ (Table III). No difference in the yield of large fruit (> 55 mm) was observed, indicating there was no overcropping. There was a positive correlation between the number of bees $tree^{-1}$ min^{-1} at FB and initial fruit-set (R^2 = 0.46; P < 0.05; Figure 1A).

TABLE III

Effect of sequential introduction (SI) of honeybee colonies and doubling colony density (DD) on the number of bees $tree^{-1}$ min^{-1} at FB (15 March 2003), initial fruit set (8 April 2003) and yield of 'Black Diamond'® Japanese plum (Expt. 1. 2003)

Treatment				Yield (kg $tree^{-1}$)***	
Name	Total number of colonies ha^{-1}	No. of bees* $tree^{-1}$ min^{-1}	Fruit set** (%)	Total	Large fruit (> 55 mm)
Control[1]	2.5	5.7 ± 0.89	8.5 b[†]	64.2 b	48 a
DD[2]	5.0	6.7 ± 0.53	15.8 a	83.1 a	57 a
SI[3]	2.5	8.1 ± 0.85	14.1 a	84.3 a	52 a

[1]One introduction of 2.5 colonies ha^{-1} at 10% FB.
[2]One introduction of 5 colonies ha^{-1} at 10% FB.
[3]Two introductions of 1.25 colonies ha^{-1} at 10% FB and at FB.
*Numbers of bees $tree^{-1}$ min^{-1} are means ± SE for ten replicate trees per treatment.
**Fruit set values are means of 2,000 flowers per treatment (100 flowers × two branches × ten trees).
***Yield data are the means of ten trees per treatment.
[†]Results within a row followed by different lower-case letters differ significantly by Duncan's new multiple range test (P < 0.05).

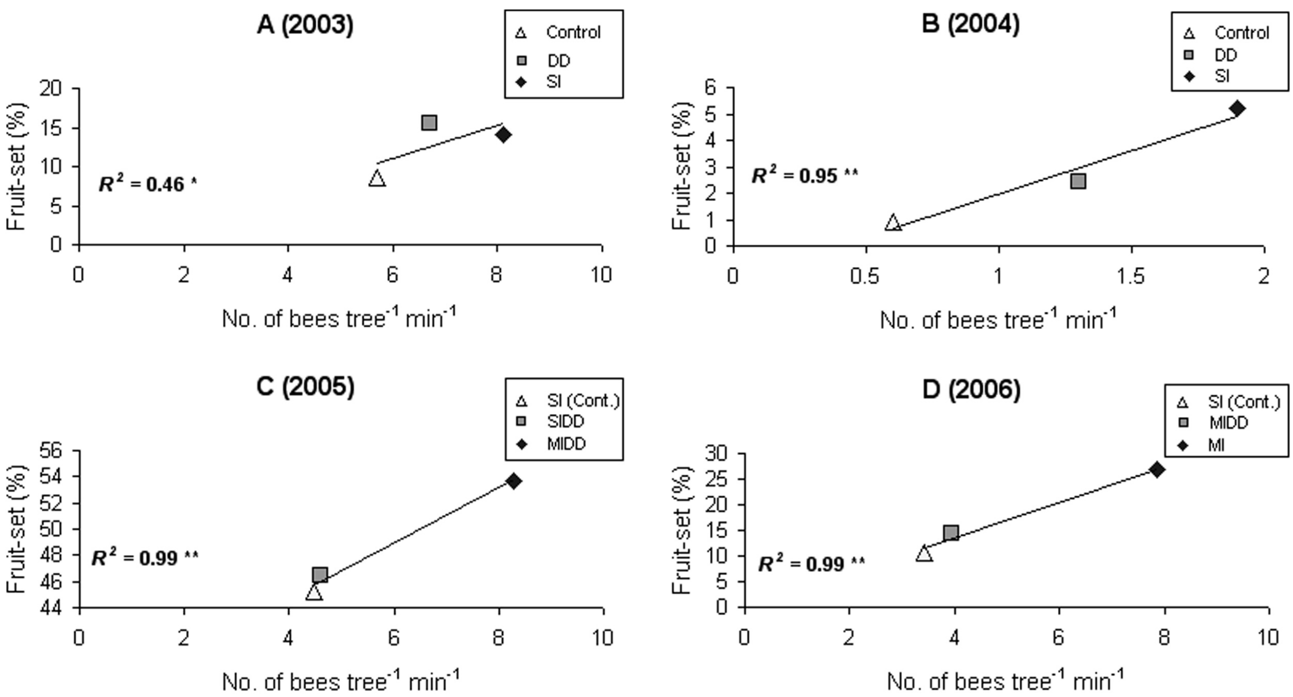

FIG. 1

Relationship between the average number of honeybees $tree^{-1}$ min^{-1} and the percentage of initial fruit-set, 6 weeks after FB in each year (Panel A, 2003; Panel B, 2004; Panel C, 2005; Panel D, 2006). In 2005, initial fruit-set was recorded 4 weeks after FB because of a heavy yield which demanded early and strong thinning. *, ** = significant at P < 0.05 and P < 0.01, respectively.

Experiment 2 (2004)

SI vs. *DD*: Encouraged by the positive results from the preliminary studies in 2003 (Experiment 1) we expanded the survey. Bee activity on trees was determined on three successive days during FB. Bee-foraging activity was low in 2004, with only 1 – 2 bees $tree^{-1}$ min^{-1} (Figure 2), compared to 6 – 8 bees $tree^{-1}$ min^{-1} in 2003 (Table III). No significant difference in bee number per tree was observed between the three treatments prior to the second introduction of the SI treatment (3 March). However, during the 2 d that followed the second introduction (4-5 March), there was a considerable increase in bee number per tree in the SI treatment, and a decrease in the DD and the control treatments. Despite the fact that the increase in bee number per tree in the SI treatment occurred during a single day, that day was during FB, a stage with a major impact on the level of fruit-set. Consequently, the percentage fruit-set in the SI treatment was much higher (5%) than that in the DD (2.5%) or control (0.8%; Figure 3) treatments. Again, there was a positive correlation between the average number of honeybees $tree^{-1}$ min^{-1} and the initial fruit set (R^2 = 0.95; $P < 0.05$; Figure 1B).

Experiment 3 (2005)

SIDD vs. *MIDD*: The results from 2003 and 2004 indicated that a high colony density, and even more so, sequential introduction, enhanced bee pollination activity. Therefore, in 2005, we tested whether further enhancement could be achieved using a double-colony density, combined with sequential introduction (SIDD), relative to sequential introduction alone (SI = control). In addition, we examined whether combining double-density with four introductions (MIDD) every 2 – 3 d would improve bee activity compared to SI or SIDD.

In 2005, 'Black Diamond'® and 'Angeleno' began to bloom at the end of February, approx. 2 weeks earlier than normal. Weather conditions changed during bloom. Until FB (2 March) it was warm with no rain (i.e., favourable conditions for bee foraging). The weather then turned cold and wet until the end of bloom, causing negligible bee activity. Thus, we could not test the effect of the second introduction at FB in the SI treatment, or

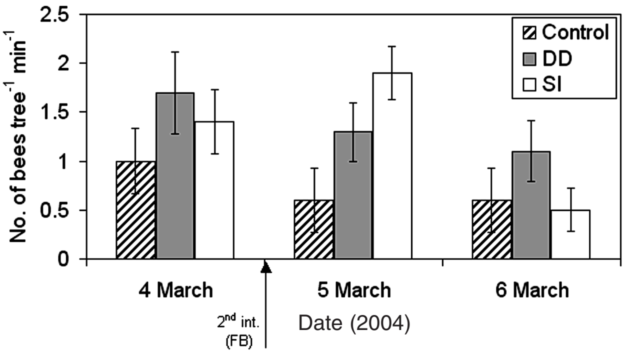

FIG. 2

The effect of sequential introduction (SI) of honeybee colonies, or of double colony density (DD) on the average number of bees $tree^{-1}$ min^{-1} during FB of 'Black Diamond'® plum (4 – 6 March; Expt. 2; 2004). The DD treatment was at 5.0 colonies ha^{-1} *vs*. 2.5 colonies ha^{-1} in the control. Both were introduced at 10% FB (1 March). The SI treatment was 1.25 colonies ha^{-1} at 10% FB (first introduction) and 1.25 colonies ha^{-1} at FB (second introduction, arrow). Data are the means of ten replicate trees per treatment ± SE.

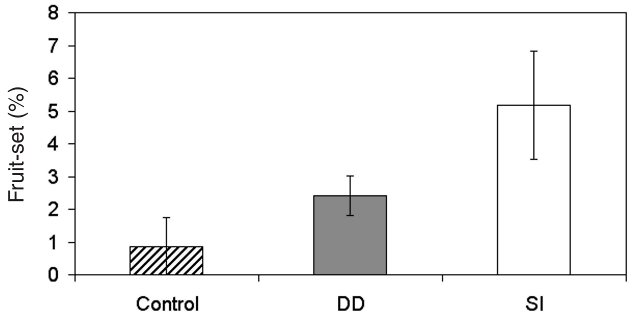

FIG. 3

Effect of sequential introduction (SI) of honeybee colonies, or of double colony density (DD) on initial percentage fruit set in 'Black Diamond'® plum (Expt. 2; 2004). The DD treatment was at 5.0 colonies ha^{-1} *vs*. 2.5 colonies ha^{-1} in the control and the SI treatment. Colony introduction was as described in Figure 2. Fruit-set data are the means of 1,000 flowers per treatment (100 flowers × one branch × ten trees) ± SE.

the effect of the third or fourth introductions in the MIDD treatment. In essence, we could only test the effect, until FB, of a constant density of 2.5 colonies ha^{-1} (SIDD), or of sequential introductions at 50% FB, to reach a total of 2.5 colonies ha^{-1} (MIDD) relative to 1.25 colonies ha^{-1} during the entire period (SI). The higher colony density in the SIDD treatment did not increase bee activity relative to the SI treatment. However, sequential introduction at 50% FB in the MIDD treatment greatly increased bee activity (Figure 4).

In the MIDD treatment, an increase of *ca*. 20% in the initial fruit-set ratio was observed (SI = 45 ± 2.8; SIDD = 46 ± 3.6; MIDD = 54 ± 3.9). It should be noted that differences between the treatments were observed despite the high fruit-set of 'Black Diamond'® in this year. Similarly, fruit-set in 'Angeleno' increased significantly (SI = 2.0%, SIDD = 6.0%, MIDD = 8.2%). Initial fruit set was determined 4 weeks after FB, as later data were biased due to heavy thinning. There was a positive correlation between the average number of honeybees

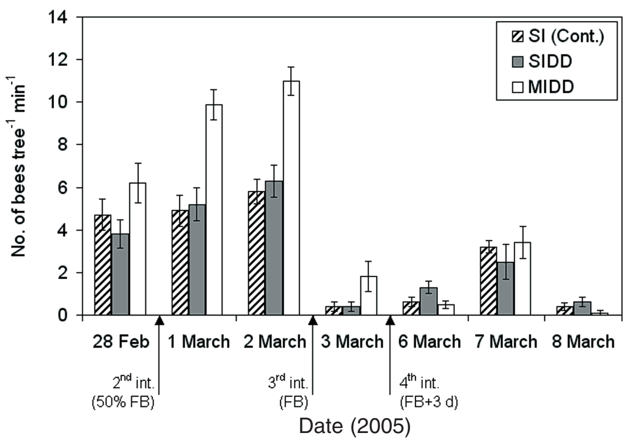

FIG. 4

The effect of sequential introductions of double density colonies (SIDD), or multiple introduction of high density colonies (MIDD), on the average number of bees $tree^{-1}$ min^{-1} during the flowering period of 'Black Diamond'® plum (Expt. 3; 2005). The SI (control) treatment involved the introduction of 1.25 colonies ha^{-1} at 10% FB (27 February) and 1.25 colonies ha^{-1} at FB (total = 2.5 colonies ha^{-1}). The SIDD treatment involved the introduction of 2.5 colonies ha^{-1} at 10% FB and 2.5 colonies ha^{-1} at FB (total = 5.0 colonies ha^{-1}). The MIDD treatment introduced 1.25 colonies ha^{-1} at each of four times: 10% FB, 50% FB, FB, and FB + 3 d (arrows; total = 5.0 colonies ha^{-1}).

tree^{-1} min^{-1} in the first half of the blooming period and initial fruit-set (R^2 = 0.99; $P < 0.01$; Figure 1C).

Experiment 4 (2006)

MI vs. *MIDD*: Results from the 2005 season confirmed the enhancement of bee activity following sequential introduction, and revealed an additional advantage of multiple introductions (i.e., spreading the effect over the period of bloom, thus reducing potential damage due to episodes of adverse weather). Subsequently, we determined the impact of a reduced colony density at each introduction, from a total of 5.0 colonies ha^{-1} (MIDD as in 2005) to 2.5 colonies ha^{-1} (0.625 colonies ha^{-1} at each introduction; MI). This treatment was also compared to SI with the same total of 2.5 colonies ha^{-1}. The results again exhibited, the significant advantage of MI compared to SI on bee pollination activity (Figure 5). Bee activity was unexpectedly low in the MIDD treatment from the beginning (1 – 2 March), relative to the SI treatment (with the same colony density), and to the MI treatment (with half the colony density), possibly due to using some weak colonies in that treatment. Nonetheless, introducing new colonies at 50% FB (second introduction) almost doubled the number of bees tree^{-1} min^{-1} in both the MI and MIDD treatments; whereas there was only a slight increase in bee activity without introducing new colonies during the same period (SI). Introducing new colonies at FB increased bee activity, especially in the MIDD treatment, and had only a minor effect on the MI (which was already high) and SI treatments over the following 2 d. Introducing new colonies towards the end of bloom no longer affected bee activity. Thus, during 2006, sequential introductions seemed to have their greatest effect earlier in the blooming period, a mild effect at FB, and no effect later in the blooming period.

Greater overall bee activity in the MI treatment translated into a higher percentage fruit-set. As in

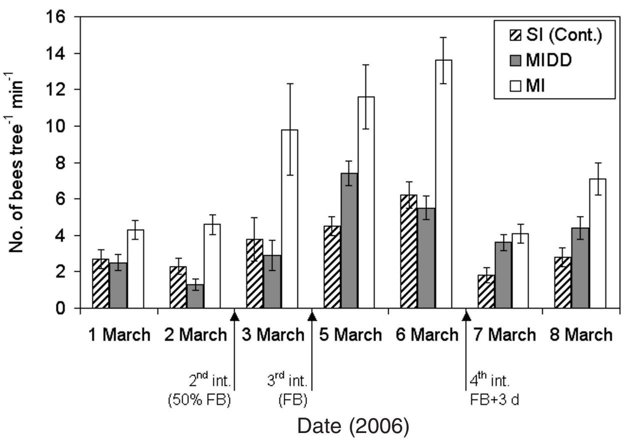

FIG. 5
The effect of multiple introductions of double-density colonies (MIDD), or multiple introductions (MI), on the average number of bees tree^{-1} min^{-1} during the flowering period of 'Black Diamond'® plum (Expt. 4; 2006). The SI control treatment consisted of 1.25 colonies ha^{-1} introduced at 10% FB (1 March) and 1.25 colonies ha^{-1} at FB (total = 2.5 colonies ha^{-1}). The MIDD treatment consisted of 1.25 colonies ha^{-1} at each of four introductions: 10% FB, 50% FB, FB and FB + 3 d (arrows; total = 5.0 colonies ha^{-1}). The MI treatment consisted of 0.625 colonies ha^{-1} at each of four introductions: 10% FB, 50% FB, FB, and FB + 3 d (arrows; total = 2.5 colonies ha^{-1}).

previous experiments, there was a positive correlation between the average number of honeybees tree^{-1} min^{-1} and initial fruit-set (R^2 = 0.99; $P < 0.01$; Figure 1D).

DISCUSSION

In Israel, 'Black Diamond'®, like other Japanese plum varieties, does not fulfill its full carrying capacity. Insufficient cross-pollination is one of the most important factors responsible for low productivity in Japanese plum (Sapir *et al*., 2004). Based on the work of others on plums (Free, 1993; Calzoni and Speranza, 1996; 1998; Delaplane and Mayer, 2000), and our work on pears and apples (Stern *et al*., 2001; 2004), we assumed that increasing bee activity would improve cross-pollination and, subsequently, yield.

The Extension Service of the Israeli Ministry of Agriculture recommends introducing 2.5 colonies ha^{-1} into the orchard at 10% FB. Here, it was shown that increasing the density of honeybee colonies to 5.0 colonies ha^{-1}, when all the colonies were introduced at 10% FB (DD), increased bee activity on the trees and improved fruit-set and yield (Table III; Figure 2; Figure 3). Moreover, introducing the colonies sequentially (half at 10% FB, and half at FB), with the recommended ratio of colonies (1.25 ha^{-1} at each introduction) to achieve a total of 2.5 colonies ha^{-1}, further increased the number of bees tree^{-1} (Table III; Figure 2) and improved fruit-set and yield to the same level, or more, than the DD treatment (Table III; Figure 3).

Our data also emphasised the importance of timing of high bee activity during the period of bloom. Bee activity in an orchard is affected by competing flora (Zohary, 1962). One-to-two days after the colonies were placed in the orchard, a high proportion of the bees foraged in the open fields rather than in the orchard (Stern *et al*., 2004). Therefore, high bee activity may last for only a few days; yet, if this activity was before or during FB, fruit-set and yield were significantly enhanced (Figure 2; Figure 4; Figure 5). Multiple introductions of colonies (MI), each at a low density of 0.625 colonies ha^{-1}, prevented the colonies from establishing constancy on competing flowers in the vicinity, as was suggested by Mayer *et al.* (1986) and Free (1993). A total of four insertions every 2 – 3 d led to a dramatic improvement in fruit-set (Figure 4; Figure 5). This was found both for 'Black Diamond'® and 'Angeleno', which pollinate each other.

The advantage of multiple *vs*. single sequential introductions, evident in our study, may result from several complementary factors. The level of bee activity on the target crop is the result of complex decision-making processes at the individual and colony level, and is affected by the condition of the colony, the weather, the target crop, and competing flora (Seeley, 1995). Furthermore, in crops with a relatively short period of bloom, such as plum, there is only a short and often unpredictable period of peak bloom, during which high bee activity would be most effective. A mismatch of 1 – 2 d in the timing of a sequential introduction of bee colonies (too early or too late) could render this ineffective.

Consequently, both bee activity and bloom cannot be predicted precisely, and the beneficial effect of any single introduction of bee colonies, at any particular time, cannot be guaranteed. Multiple introductions increase the chance that at least one will have the desired effect of increasing bee activity during the critical time. For example, in 2005, a "Winter climate" episode, which greatly reduced bee foraging activity, occurred from FB until the end of bloom. Thus, while colony introductions at FB had no effect, the introduction of colonies at 50% FB in the MIDD treatment was, in fact, a sequential introduction; the favourable treatment. As a result, bee activity levels increased and fruit set was high. In 2006, new colonies introduced at 50% FB (second introduction) greatly enhanced bee activity in one treatment (MI), but not in another (MIDD), showing that, even at a particular time, different colonies located in different parts of the orchard may behave differently. Thus, we suggest that multiple introductions should be the preferred treatment, especially where "Winter climate" episodes are common, such as during March in Israel.

During each year of study, initial fruit-set was positively correlated with bee foraging activity (Figure 1). Combining the results of all our experiments, we still found a positive correlation between the number of honeybees tree^{-1} min^{-1} and percentage fruit-set (Figure 6). Hence, although bee activity may differ between years (due to weather conditions, etc.) and is affected by honeybee colony management, particular levels of bee activity consistently corresponded to particular percentages of fruit-set. For this reason, we recommend that a level of 7 – 8 bees tree^{-1} min^{-1} should be reached, in order to ensure high fruit-set and yield in Japanese plum. This optimum level of bee activity is comparable to that for pear (6 – 7 bees tree^{-1} min^{-1}), but is less than for 'Red Delicious' apple (12 – 14 bees tree^{-1} min^{-1}), due to differences in pollination efficiency and floral attractiveness (Stern *et al*., 2001; 2004).

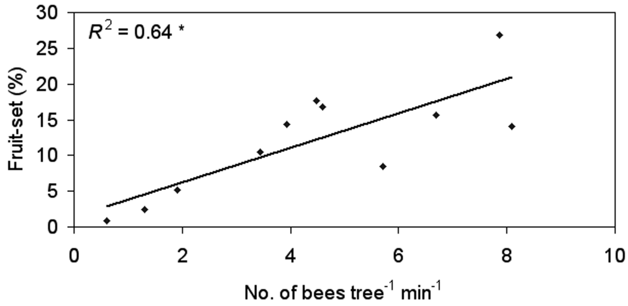

FIG. 6
The correlation between the average number of honeybees tree^{-1} min^{-1} and the percentage of initial fruit set 6 weeks after FB in all treatments (three treatments per year × 4 years). In 2005, one record (MI) was not included due to a high yield, which demanded heavy thinning. *, significant at $P < 0.01$.

In summary, as for 'Red Delicious' apples (Stern *et al*., 2001), but in contrast to 'Spadona' pear (Stern *et al*., 2004), an increase in bee colony density from 2.5 to 5.0 colonies ha^{-1} in 'Black Diamond'® Japanese plum improved bee activity on trees, cross-pollination, fruit-set and yield. In addition, introducing naïve bees to the orchard, by sequential introduction or, more so, by multiple introduction, further improved bee activity and levels of fruit-set. This effect was found even at a relatively low final density of 2.5 colonies ha^{-1}. Hence, multiple introductions of naïve bees every 2 – 3 d during bloom were more efficient compared to increasing the colony density. It is also much cheaper for the farmer.

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