Honey bee (*Apis mellifera*) distribution and behaviour on hybrid radish (*Raphanus sativus* L.) crops

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Abstract Commercial hybrid vegetable seed production involves movement of pollen between two distinct groups of plants (parent lines) – a pollen donor and seed production lines. The aim of this study was to observe the distribution and behaviour of honey bees (*Apis mellifera*) on hybrid radish (*Raphanus sativus* L.) crops in New Zealand. Honey bees were observed to have an even distribution across the parent lines, although individual bees largely maintained fidelity to the particular line on which they foraged. Of the bees observed on male-fertile flowers 72% did not switch to the other line. When plants from the different lines were manipulated so that they appeared to be a single plant, 69.5% of honey bees remained constant to the line visited. To maximise the effectiveness of honey bees as pollinators of hybrid radish, techniques should focus on better matching of lines or breeding of lines to reduce pollinator differentiation.

Keywords hybrid crops, radish, honey bee, *Apis mellifera*, behaviour.

INTRODUCTION

In New Zealand, radishes are grown for the production of hybrid seed, which produces plants that are advantageous for earliness, high yield, root quality and uniform development, resistance to insect pests and disease, and wider adaptability (Singh et al. 2001). Hybrid seed is obtained by growing together two different parental lines (a male-sterile line and a hermaphrodite male-fertile line) and relying mainly on insects to cross-pollinate the flowers (Free 1993). The standard field layout is three male-fertile rows for every eight male-sterile rows, with an empty row in between the lines. Only the male-sterile rows are harvested for seed. Generally, hybrid radish crops produce smaller

yields than open pollinated varieties (a single hermaphrodite line) (D. Harrison, South Pacific Seed, pers. comm.). Originally from Europe and Asia, radish (*Raphanus sativus* L.) is a fast-growing vegetable crop of the family Brassicaceae (Chandrashekhar 2006). Their flowers open in the morning and remain fresh until their second day. Stigma receptivity is reported to be limited to several hours a day (Kremer 1945). Both the male-fertile and sterile flowers are approximately 2.5 cm across, cross-shaped with four white, pink or purple petals. Every flower is capable of producing a pod containing six seeds (McGregor 1976). The flowers are visited by a range of Hymenoptera, Diptera, Coleoptera

and Lepidoptera, but honey bees are usually introduced for crop pollination (Chandrashekhara 2006; Sahli & Conner 2007). Honey bees have proven to be effective pollinators of open-pollinated varieties of radish (Kremer 1945; Priti et al. 2001). Pollination in hybrid crops requires pollen to be transferred between parental lines that are phenotypically and genotypically different. Honey bees forage on the male-sterile line for nectar and on the male-fertile line for nectar and pollen.

Research on hybrid radish in Japan showed that individual honey bees continually visited either parental line, with few movements between the lines (Kobayashi et al. 2010). Research on hybrid Brussels sprouts (*Brassica oleracea*) showed honey bees to be highly selective in their visits to parental lines, limiting the amount of movement between male-fertile and male-sterile lines (Free & Williams 1973, 1983; Faulkner 1974, 1976). This suggests that honey bees may not be effective pollinators of hybrid crops. Consequently, seed production in these crops may be sub-optimal.

Previous researchers (Kobayashi et al. 2010) have suggested that selective foraging in hybrid crops occurs because individual bees develop a preference for a particular line. This concept does not appear to have been tested with manipulative experiments. Another possibility is that the blocked planting regime of hybrid crops facilitates selective foraging behaviour. The optimal foraging theory predicts that honey bees will forage on the next closest flower, to maximise their net energy intake relative to time (McArthur & Pianka 1966). In hybrid crops the next closest flower will usually be one from the same line.

Preliminary observations of honey bees on hybrid radish in New Zealand indicated that bees have an uneven distribution across the parental lines (N.E.M. Page-Weir, Plant & Food Research, pers. comm.). In this study, the distribution and foraging behaviour of honey bees in hybrid radish seed crops was recorded, to establish whether issues similar to that observed overseas are evident in New Zealand. In addition, hybrid radish plants were manipulated to determine the

reason for selective foraging behaviour in hybrid radish crops. Such information could be used to improve the efficacy of hybrid pollination systems and thus increase production yields.

METHODS

Studies were conducted in hybrid radish crops located at Rakaia, Methven and Ashburton, Canterbury, in December 2007 and 2008, when fields were at peak flowering (flower density > 1000/m²). The crops were planted with three rows of male-fertile plants for every eight rows of male-sterile plants. Honey bee hives were present at all sites throughout the trial period.

Distribution

On 5 December 2007, the distribution of honey bees across the parental lines was recorded at the Rakaia (Site A) and Methven (Site B) sites. Counts of honey bees were made while walking six 10-m transects (three down male-fertile rows and three down male-sterile rows) at each site. A male-fertile and sterile transect were marked out in two opposing corners of the crop and in the centre of the crop (Figure 1). Honey bee counts were conducted on each of these transects during three time periods: 1000–1100 h, 1200–1300 h and 1400–1500 h. The number of honey bees foraging on radish flowers 1 m in from each transect was recorded. Rows were typically broader than 1 m width, thus each transect included plants from only one line. The width of the rows did vary between male-fertile and sterile-lines so to ensure consistency in observations between rows and fields, a tape measure was used to mark out 1 m to one side of the transect. An unpaired T-test was used to assess the differences in the numbers of bees observed on the two parental lines.

Crossing between male-fertile and male-sterile flowers

On 9 December 2008, 60 individual honey bees (38 nectar gatherers and 22 pollen gatherers) were observed whilst foraging on radish flowers at the Ashburton site (Site C). Honey bees with pollen in their corbiculae were recorded as pollen gatherers, and those without pollen recorded

Figure 1 Field design for counting honey bees along transect lines within hybrid radish fields.

as nectar gatherers. Bees foraging on the male-fertile line were chosen at random throughout the field and observed from a distance of 0.5 m to minimise disturbance. Each bee was observed for 20 consecutive visits. A sequential record was kept of the parental line of each flower visited and the length of time taken to complete 20 flower visits. Only bees that completed 20 consecutive visits were included in the analysis. The recording of any bee stopped when it left the vision of the observer or left the crop before 20 flower visits were completed and these data were not used.

Floral choice

On 18 December 2008 at site C, male-fertile and male-sterile flowers were manipulated to determine whether honey bees exhibit a

preference. A male-fertile plant from a male-fertile row and a male-sterile plant from the adjacent male-sterile row were intertwined between the two rows. Twist ties were used to secure the two plants together. The manipulated plant was observed for approximately 40 min, in order to record the foraging behaviour of visiting bees. For each bee that foraged on the manipulated plant, the parental line of the flowers visited was recorded. The parental line of the flower visited directly before and after the bee landed on the manipulated plant was also recorded. This was repeated using five different intertwined male-fertile and male-sterile plants. An unpaired T-test was used to assess the differences in the number of bees that foraged on a single parent line and those that crossed between both lines.

RESULTS

Distribution

The numbers of honey bees observed on the male-fertile and male-sterile lines did not differ significantly at either site, $P=0.5$ and $P=0.8$ for sites A and B respectively (Table 1).

Crossing between male-fertile and male-sterile flowers

The bees observed foraging on the male-fertile flowers at Site C occasionally crossed over to male-sterile flowers, with 72% of all the bees continuing to forage on the male-fertile flowers. This equates to a mean selfing (movement within the same line) to cross-movement (between lines) ratio of 5:2. Most (86%, 19/22) pollen gatherers observed and 60% (23/38) of the nectar gatherers observed stayed on the male-fertile flowers.

Table 1 The number of honey bees observed on male-fertile and male-sterile radish lines at different times during the day.

Time of survey (h)	Site A		Site B	
	Male-fertile line	Male-sterile line	Male-fertile line	Male-sterile line
1000–1100 h	44	40	4	7
1200–1300 h	31	32	5	4
1400–1500 h	37	31	12	16
Total	112	103	21	27

Floral choice

Despite the manipulations to place lines next to each other, the majority (69.5%, $n=73$, 95% CI=59.8–78.1) of all the bees stayed foraging on the line on which they were initially foraging. This proportion was similar for bees that were initially foraging on male-sterile flowers and those that were initially foraging on male-fertile flowers (Figure 2).

DISCUSSION

This study found the distribution of honey bees between fertile and sterile lines of hybrid radish crops to be similar, although the rate of crossing between male-fertile and sterile-lines by individual bees was lower than crossing within the lines. A mean selfing to cross-movement ratio of 5:2 was observed. In addition, when the flowers of male-sterile and fertile plants were intertwined, most of the bees observed visited flowers of the parental line that they had previously visited. This suggests that individual bees have, or develop, a preference that limits their movement across parental lines.

The findings in this study are consistent with the results of some of the previous research carried out on Brassicaceae (Faulkner 1974,

1976; Free & Williams 1983; Kobayashi et al. 2010). These authors all reported that honey bees selectively rather than randomly visited plants, and that they visited one parental line, with little movement between the lines. An extreme example of selective foraging is seen in Brussels sprouts where a mean selfing to cross-movement ratio of 30:1 was observed. In the instances when bees did move between the lines, few flowers on the second line were visited (Faulkner 1974). Faulkner (1974) also found the number of bees visiting each of the parental lines of Brussels sprouts was very similar, indicating that the bees did not have a preference for either line. Similar constancy has been reported for kale (*Brassica oleraceae*) (Free & Williams 1973). However, Selvakumar et al. (2003) reported the movement of honey bees between the hybrid parental lines of cauliflower to be random and non selective. They suggested that honey bees did not exhibit selective behaviour in their cauliflower hybrids because they were phenotypically similar.

Honey bees have also been observed to differentiate between hybrid lines of carrot (*Daucus carota* L.). Erickson et al. (1979) found that in hybrid carrot fields, not only did the bees differentiate between the parental lines, but they also exhibited constancy to particular lines, ignoring others in the process.

Faulkner (1974, 1976) related the honey bees' constancy to a particular parental line to the bees becoming conditioned to physical aspects of the plant, such as plant size, flower colour and fragrances. However, Free (1993) reported that many flower species frequently display variations in colour, but this does not appear to influence foraging behaviour unless conditioning to a colour variant is reinforced by an additional reward such as nectar or pollen. Kobayashi et al. (2010) suggested this is the reason for selective visitation to the parental lines in hybrid radish, where male-fertile and sterile lines differed in their available rewards for honey bees. Male-fertile flowers produce pollen, while male-sterile plants produce more flowers with a higher volume of nectar per flower than the male-fertile plants. Individual bees would have learned the difference

Figure 2 The percentage of bees that only visited male-sterile (MS-MS) or male-fertile (MF-MF) radish flowers compared with those that switched from male-sterile to male-fertile (MS-MF) and male-fertile to male-sterile (MF-MS). Error bars are 95% confidence intervals.

between these rewards after visiting flowers from both lines, then memorised olfactory and visual cues that differed between the two lines.

Based on the finding of this study and previous research, it is possible that selective foraging is a widespread problem for growers in some hybrid *Brassica* crops (Free & Williams 1973; Faulkner 1974, 1976; Kobayashi et al. 2010) and other hybrid crops (Erickson et al. 1979). Further research in a range of hybrid crops is needed to determine how widespread this selective foraging is. Although some crossing occurred by honey bees, suggesting that a degree of cross pollination takes place, improved efficiency of honey bee pollination may be achieved by increasing crossing rates. Gaining further understanding of which cues are being used by honey bees to differentiate between the lines may allow better matching of lines, or breeding of lines to reduce pollinator differentiation.

ACKNOWLEDGEMENTS

The authors would like to thank South Pacific Seeds Ltd. and Midlands Seeds Ltd. for organising sites for this trial. This study was funded by the Foundation for Arable Research, Contract no. 23468.

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