HOW FAR DO HONEY BEES FLY TO FIELDS OF *BRASSICA NAPUS* (OILSEED RAPE)?

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Introduction

Herbicide-tolerant genetically-modified (HTGM) oilseed rape (*Brassica napus* L.) is one of the first GM crops to be evaluated in farmscale trials in the UK (Firbank *et al*. 1999). Oilseed rape self pollinates, but also benefits from cross-pollination effected by wind and insects, primarily bees (Williams *et al.* 1986; 1987). Small quantities of pollen will be transported by wind or bees over hundreds of metres, perhaps kilometres (Thompson *et al*. 1999). Debate on pollen movement from GM oilseed rape crops has focussed on a) the extent of gene flow into conventional crops and wild plants (Crawley *et al.* 1993; Raybould & Gray 1993; Scheffler *et al.* 1993; Champolivier *et al.* 1999; Downey 1999; Simpson *et al.* 1999; Ingram 2000) and b) the chances of GM pollen entering honey bee colonies and being incorporated into honey and other hive products (Ramsey *et al*. 1999; Treu & Emberlin 2000).

Patterns of bee movement are fundamental to predicting the extent of bee-mediated pollen movement away from oilseed rape crops. Maximum distances flown by bees from colony to forage have been used to predict pollen flow, and consequently make inferences on gene flow away from a crop (Emberlin *et al.* 1999). However, this is not the most important parameter, since most cross-pollination is likely to occur when individual bees, carrying pollen, fly between fields of oilseed rape on a single foraging trip. Distances flown by bees on individual trips, constancy to crops, and viability of pollen on the bees' bodies are more important parameters in determining the extent of gene flow away from a field. In addition, there may be some in-hive pollen transfer between nest-mates leading to low levels of cross-pollination between fields (Vaissière *et al.* 1994; Ramsay *et al.* 1999), and this will depend on the colony-to-forage distance. The "colony-to-forage" distance is also important in determining the presence or absence of pollen in the honey and hive products.

We made use of a field scale trial of HTGM oilseed rape at Rothamsted to study the following:

1. Do honey bees show a preference for HTGM oilseed rape or conventional oilseed rape?
2. What distances do honey bees fly from their colonies to oilseed rape fields (i.e. mean colony-to-forage distance) in an arable landscape, typical of Eastern England?

Methods

Over four weeks, we examined the spatial and temporal distribution of mass-marked honey bees, from a transect of colonies, as they foraged on flowering winter-sown oilseed rape fields, on the arable farm of Rothamsted Estate (Hertfordshire, UK). One 5 ha field, called Black Horse (BH), contained a trial of HTGM oilseed rape, which formed part of a SAPPIO LINK programme entitled "Botanical and Rotational Implications of Genetically-modified Herbicide Tolerance" (BRIGHT). The trial was testing four cultivars of winter oilseed rape and was situated over 200 m from the nearest conventional oilseed rape field (Fig 1). It had sixteen plots (24 m x 120 m), arranged in four randomised blocks. Each block contained four plots, one of each cultivar of oilseed rape. The cultivars were genetically-modified glufosinate-tolerant rape (Liberty Link, Aventis), genetically-

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modified glyphosate-tolerant rape (Roundup-Ready, Monsanto), non-GM Imazamox-tolerant rape (Cyanamid/Pioneer) and a conventional oilseed rape cultivar called Apex.

Four fields of conventional (non-GM) winter-sown oilseed rape, at varying distances from the GM trial and from the transect of honey bee colonies, were also surveyed (Table 1).

Table 1 Details of surveyed oilseed rape fields

Field name (and abbrev.)	Approx. distance (m) from HTGM trial	Perpendicular distance (m) from hive transect
*Black Horse (BH)	0	12
White Horse (WH)	650	10
Great Knott (GK)	990	500-600
Appletree (AT)	1160	10
Long Hoos (LH)	1650	600

*HTGM trial site

Three similar-sized honey bee (*Apis mellifera* L.) colonies were placed at each of six positions (A – F) along a transect (total = 18), running East from the GM trial (Fig 1; Table 1). Each colony was fitted with a pollen trap to sample pollen loads from returning foragers, and a powder dye dispenser to automatically mark bees leaving the colony. Six fluorescent, non-toxic powder dyes (from Radiant Color NV, Belgium and Sterling Industrial Colours, London, UK) were used to mark the bees. One colour of powder dye was used for all three colonies at each position along the transect (Table 2) so that a bee's origin was apparent when it was observed foraging on a field.

Table 2 Details of colony sites along transect (see also Figure 1)

Colony site	Distance (m) from HTGM trial	Dye colour used
A	12	magenta
B	304	green
C	484	red
D	662	pale orange
E	892	dark orange
F	1302	yellow

The pollen traps collected pollen for 24 h from Monday to Tuesday morning, each week for four weeks. On each Tuesday morning, the pollen from each trap was emptied into a clean plastic bag and frozen until analysis. Using a microscope for identification, the percentage of oilseed rape pollen present in each sample was determined.

After each pollen trap had been emptied, approximately 8 g of powder dye were placed in the dye dispenser on the colony. Standard searches were made for marked bees, along fixed transects, on the GM trial (Black Horse) and the four conventional rape fields, on Tuesday and Wednesday of each week. The order in which fields were surveyed for marked bees was random, as was the order of plot surveys on Black Horse. Bee surveys were performed in warm, sunny conditions, between 10.00h–16.30h, when bee activity was at its peak. On Black Horse, observers walked, at a steady pace, down the central tractor-wheelings on each 120 m long plot, examining a one-metre-wide strip to one side. The numbers of honey bees marked with each powder dye colour were scored. On each of the four conventional oilseed rape fields, eight transects, each of 50 m in length, were spread out across the field, and walked as described above in search of marked bees.

To estimate forager density, all foraging bees (marked and unmarked honey bees, bumble bees and solitary bees) were counted on each transect, whilst searching for marked bees, every Tuesday. The presence/absence of oilseed rape pollen in the bees' corbiculae was also recorded.

Disposable overalls and gloves were worn to minimise observer-mediated pollen transfer between fields and between colonies.

Results

The four weeks over which the experiment was conducted (26 April – 23 May 1999) were generally warm and bright with variable wind conditions (0-10 mph).

Pollen trap samples

Oilseed rape pollen accounted for between 0.5% and 50.6% of the pollen collected by a colony (average per trap = 12±2% oilseed rape pollen). Even colonies positioned next to an oilseed rape field, with no obscuring vegetation between colony and crop (position A, D and F), had low percentages of oilseed rape in the pollen traps. Other pollen present was identified to be most probably from *Crataegus monogyna* Jacq. (hawthorn) with a small percentage from *Vicia faba* L. (field bean).

Forager density

There were between zero and 5.3 foraging bees per 20 m^2. Most were honey bees (marked and unmarked), and there were a few bumble bees (*Bombus lapidarius, B.pascuorum, B.terrestris* or *B.lucorum* and *B.pratorum*) and some unidentified solitary bees. Forty of the 573 honey bees counted during these density counts (7%) had oilseed rape pollen in their corbiculae. Forager densities were highest on Black Horse (1.2 ± 0.2 bees/20 m^2), and on White Horse (1.7 ± 0.2 bees/20 m^2), and low on the other fields, particularly Great Knott (0.6 ± 0.1 bees/20 m^2) and Long Hoos (0.4 ± 0.1 bees/20 m^2). Bee density increased in weeks 3 and 4, particularly on Black Horse. This reflected the flowering stages of the crops. Great Knott and Long Hoos were in full flower during week 1 of the experiment and had almost finished flowering by week 4. Apple Tree and White Horse were in full flower during week 2. Black Horse was in full flower during weeks 3 and 4, when the other fields had almost completed flowering.

On Black Horse, the total number of bees per 120 m^2 transect varied from 0 to 32. A two-way anova (with randomized blocking) was performed on the transformed transect counts (log (x+1)) to compare bee density between different cultivars of oilseed rape and between weeks. The total number of bees per transect varied significantly between weeks ($F_{3,45}$ = 58.6, $P<0.001$) but not between crop cultivars ($F_{3,45}$ = 1.65, P=0.191) and there was no interaction between week and cultivar ($F_{9,45}$ = 1.25, P=0.290). Considering only honey bees, density varied significantly between weeks ($F_{3,45}$ = 70.42, $P<0.001$) and between crop cultivars ($F_{3,45}$ = 4.10, P=0.012), with the highest density on the GM Liberty Link cultivar, and the lowest forager density on the GM Roundup-Ready cultivar (Fig 2). There was no interaction between week and cultivar ($F_{9,45}$ = 1.42, P=0.209).

Marked bee surveys

At least 90% of the bees exiting the colony were dusted with dye powder, which remained visible on the dorsal thorax, in wing joints and between abdominal segments, even when the bee groomed. Marked bees from site D (pale orange) and E (dark orange) became indistinguishable, so they were recorded together as "orange". There were large differences in the numbers of bees of each colour observed (max = 374 magenta; min = 19 yellow), because of the different sampling effort expended on different fields, and because of differences in colony activity.

A total of 736 marked bees were observed over the four weeks. They constituted 67% of the foraging population on the oilseed rape fields. The majority of marked bees (558) were seen on Black Horse, where the sampling effort was greatest. Sixteen x 120 m transects were walked each day on Black Horse, compared to eight x 50 m transects per day on the other fields. 157 marked bees were seen on White Horse, but only 19 were seen on Apple Tree despite similar sampling efforts. No marked bees were seen on Long Hoos and only two bees, one green and one yellow, were seen on Great Knott. These two fields were therefore excluded from analysis.

When considering how far bees fly to a field of oilseed rape, one can consider either the distribution at each field and where they came from, or one can consider each colony site and which field the bees were going to. We shall do both.

Marked bee distributions varied over the four weeks , and the majority of bees came from the colony sites nearest to the field (Fig 3). 89% of marked bees observed on Black Horse were magenta or green, from sites A and B, but there was an influx of red bees, from site C, during week 4 (Fig 3a). On White Horse, 95% of marked bees were from sites C, D and E. Magenta and green bees, from sites A and B, were only seen on the field during week 2. On Apple Tree, only yellow bees (from site F) were seen in weeks 1-3, but six orange bees were seen in week 4.

Focussing on the colony sites, the mean density of each colour of marked bees per 100 m^2 of transect was calculated for each field, and plotted against distance of the field from the colonies. The number of marked bees per 100 m^2 observed foraging on crops declined sharply with distance from their colonies (Fig 4). Overall, 89.5% of the observed marked bees were foraging on the field closest to their colony, and 9.8% were foraging on the next closest field. Only 0.7% were observed further afield. The average observed colony-to-forage distance was 127 m. The maximum observed foraging distance was for a green marked bee, seen 955 m from her colony, on Great Knott. The maximum potential observable distance of 1770 m (the distance between colony site A and Long Hoos).

Discussion

Placing honey bee colonies along a transect, and mass-marking the foragers as they left their colonies, proved to be a very effective technique for determining the distribution of bees on oilseed rape fields in the surrounding landscape. However, as with most mark-recapture studies, the area surveyed was limited, restricting the observable range of movement. Collecting pollen from traps, in combination with mark - re-observation, gave a clearer picture of colony foraging patterns. The high percentage of non-oilseed rape pollen in the traps suggests that many marked bees did not go to the oilseed rape and were, unobserved, foraging on uncropped vegetation. The high densities of honey bees on the oilseed rape were primarily collecting nectar. This may affect the amount of cross-pollination occurring, and will limit the quantity of pollen entering a honey bee colony, and subsequently the honey.

Although the density of honey bees differed significantly between cultivars of oilseed rape on Black Horse, the differences did not provide evidence that bees avoided GM cultivars. Picard-Nizou *et al.* (1995) examined bee behaviour on insect-resistant GM oilseed rape, and found no difference in visitation patterns. The lower density of bees on the Roundup-Ready cultivar in our experiment may be linked to this cultivar suffering low flower production due to sulphur deficiency, because of plot positions on the field.

Forager density and marked bee distribution were influenced by the change in relative attractiveness of the different fields over time. For example, in week 4, when most fields were finishing flowering and Black Horse was still in full flower, forager density on this field increased, as did the proportion of marked bees coming from more distant colonies. As the number of oilseed rape flowers declined over time, the bees were travelling further from their colonies to forage.

The oilseed rape fields attracted honey bees from colonies several hundred metres away, but the vast majority of marked bees (90%) foraged on the field closest to their colony. The foraging site that a bee chooses on leaving a colony will depend both on distance to be flown (Cresswell *et al.* 2000), competitive forage in the vicinity and probably on visibility of the foraging site. Only two marked bees were observed foraging on Great Knott and Long Hoos, which were distant from the colony transect. Bees from most of the colonies would have had to fly over a large beech wood to reach these fields. Apple Tree had suprisingly few marked bees, perhaps because it was adjacent to a large, mixed woodland, and garden, containing several flowering tree and herb species.

Although the bees had a choice of oilseed rape fields within foraging range, at different distances from their colonies, they were all observed within 1km of their colonies. Maximum observable distance was 1.7 km. These results do not provide information on pollen movement between fields, but they do have implications for beekeepers with colonies positioned in the vicinity of GM crops. If there are several oilseed rape crops in the area, our results suggest that it is very unlikely that bees will travel beyond the nearest one or two fields to forage. However, a tiny proportion of bees may travel long distances to feed, even if they have comparable forage nearer to home. No pale orange or yellow bees were seen foraging on Black Horse, so we predict that there is only likely to be a discernible quantity of GM material in the pollen samples from colonies at sites A-D (situated at a maximum of 662 m from the GM trial). This prediction will be tested by analysing the DNA of the pollen collected in the traps.

If a beekeeper has colonies nearer to conventional oilseed rape crops, than to a GM crop, then the quantity of GM pollen returned to the colony is likely to be minimal, although it may increase as flowering finishes and bees spread further afield.

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References

Champolivier, J., Gasquez, J., Messean, A. & Richard-Molard, M. (1999) Management of transgenic crops within the cropping system. *Gene flow and agriculture:relevance for transgenic crops*. *BCPC Symposium proceedings No. 72.* (ed. P.J.W. Lutman). pp. 233-240. BCPC, London.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D. & Buxton, J. (1993) Ecology of transgenic oilseed rape in natural habitats. *Nature,* 363, 620-623.

Cresswell, J.E., Osborne, J.L. & Goulson, D. (2000) An economic model of the limits to foraging range in central place foragers with numerical solutions for bumble bees. *Ecological Entomology,* 25, 249-255.

Downey, R.K. (1999) Gene flow and rape - the Canadian experience. *Gene flow and agriculture:relevance for transgenic crops*. *BCPC Symposium proceedings No. 72.* (ed. P.J.W. Lutman) pp. 109-116. BCPC, London.

Emberlin, J., Adams-Groom, B. & Tidmarsh, J. (1999) *A report on the dispersal of maize pollen.* Soil Association Report.

Firbank, L., Dewar, A., Hill, M., May, M., Perry, J., Rothery, P., Squire, G. & Woiwod, I.H. (1999) Farm-scale evaluation of GM crops explained. *Nature,* 399, 727-728.

Ingram, J. (2000) The separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. *Plant Varieties and Seeds,* 13, 181-199.

Picard-Nizou, A.L., Pham-Delegue, M.H., Kerguelen, V., Doualt, P., Marilleau, R., Olsen, L., Grison, R., Toppan, A. & Masson, C. (1995) Foraging behaviour of honey bees (*Apis mellifera* L.) on transgenic oilseed rape (*Brassica napus* L. *var. oleifera*). *Trangenic Research,* 4, 270-276.

Ramsay, G., Thompson, C.E., Neilson, S. & Mackay, G.R. (1999) Honey bees as vectors of GM oilseed rape pollen. *Gene flow and agriculture:relevance for transgenic crops*. *BCPC Symposium proceedings No. 72.* (ed. P.J.W. Lutman). pp. 209-214. BCPC, London.

Raybould, A.F. & Gray, A.J. (1993) Genetically modified crops and hybridization with wild relatives: a UK perspective. *Journal of Applied Ecology,* 30, 199-219.

Scheffler, J.A., Parkinson, R. & Dale, P.J. (1993) Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). *Transgenic research,* 2, 356-364.

Simpson, E., Norris, C., Law, J., Thomas, J. & Sweet, J. (1999) Gene flow in genetically modifed herbicide tolerant oilseed rape (*Brassica napus*) in the UK. *Gene flow and agriculture:relevance for transgenic crops*. *BCPC Symposium proceedings No. 72.* (ed. P.J.W. Lutman). pp. 75-81. BCPC, London.

Thompson, C.E., Squire, G., Mackay, G.R., Bradshaw, J.E., Crawford, J. & Ramsay, G. (1999) Regional patterns of gene flow and its consequence for GM oilseed rape. *Gene flow and agriculture:relevance for transgenic crops*. *BCPC Symposium proceedings No. 72.* (ed. P.J.W. Lutman). pp. 95-100. BCPC, London.

Treu, R. & Emberlin, J. (2000) *Pollen dispersal in the crops maize (Zea mays), oil seed rape (Brassica napus ssp. oleifera), potatoes (Solanum tuberosum), sugar beet (Beta vulgaris ssp. vulgaris) and wheat (Triticum aestivum)* Soil Association Report

Vaissière, B.E., Torre Grossa, J., Rodet, G. & Malabœuf, F. (1994) Sociality and pollen flow: direct evidence for effective in-hive pollen transfer. *Les Insectes Sociaux, 12ème Congress de l'Union Internationale pour l''Etude des Insectes Sociaux UIEIS,* (eds A. Lenoir, G. Arnold & M. Lepage), pp. 290. Université Paris Nord, Paris, Sorbonne

Williams, I.H., Martin, A.P. & White, R.P. (1986) The pollination requirements of oil-seed rape (*Brassica napus* L.). *Journal of Agricultural Science, Cambridge,* 106, 27-30.

Williams, I.H., Martin, A.P. & White, R.P. (1987) The effect of insect pollination on plant development and seed production in winter oilseed rape (*Brassica napus* L.). *Journal of Agricultural Science, Cambridge.* 109, 135-139.

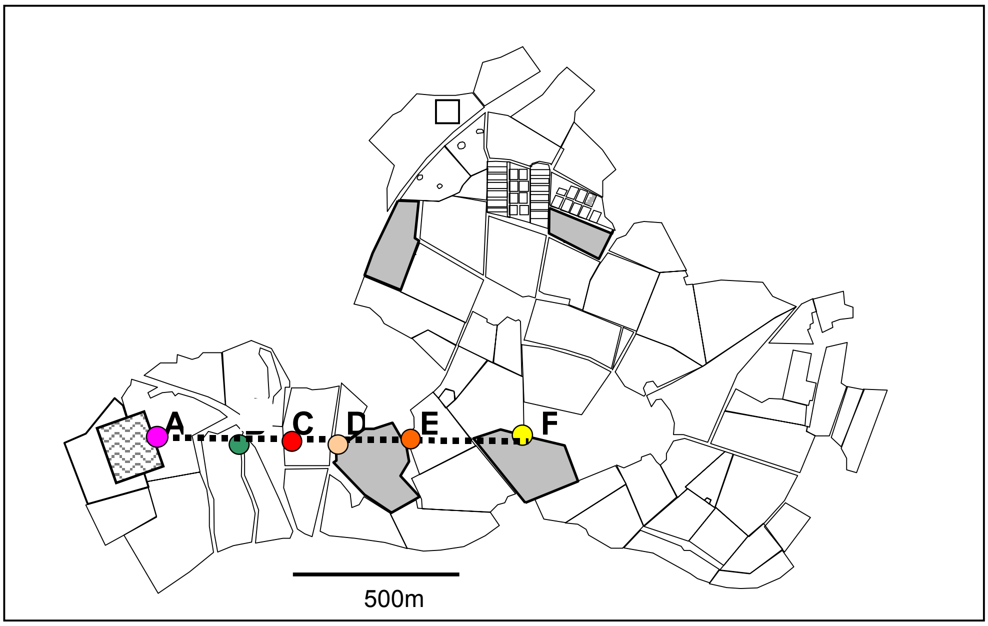

Figure 1 Outline of fields on Rothamsted estate. Hatched block (BH) = Black Horse, field of HTGM trial site. Grey blocks with initials (WH, AT, GK and LH) = surveyed fields of conventional oilseed rape (Table 1). Dotted line = transect of 18 honeybee colonies at sites A-F. Three colonies were placed at each coloured dot, and colour indicates powder dye added to colony (Table 2).

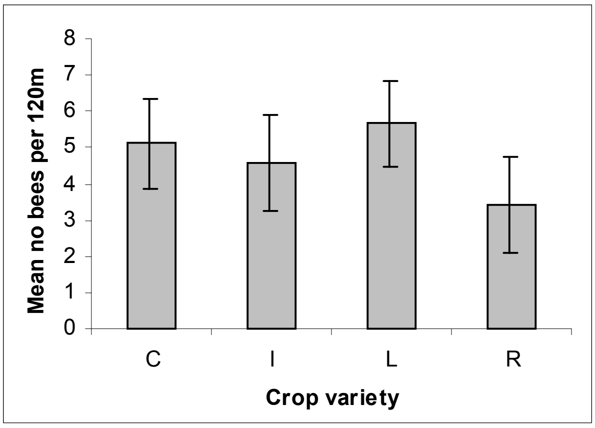

Figure 2 Back-transformed mean number of honey bees (± s.e.m) on crop cultivars on Black Horse, combining weeks (n=16 for each column i.e. 4 weeks x 4 plots). C = conventional variety (Apex); I = non-GM Imazamox-tolerant; L = GM Liberty Link, R = GM Roundup-Ready.

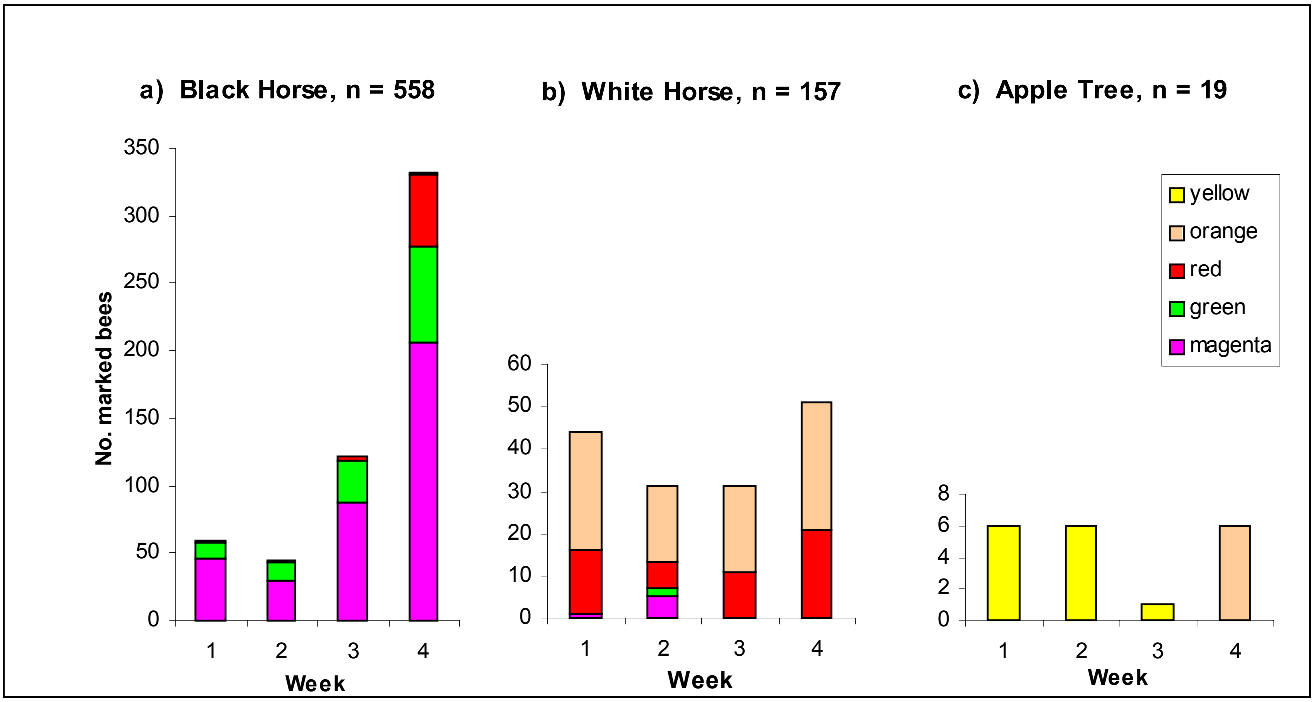

Figure 3 Numbers of marked bees of each colour seen each week on each field a) Black Horse b) White Horse c) Apple Tree (refer to Fig 1 for relative positions of fields and colonies). n = total number of marked bees observed on that field.

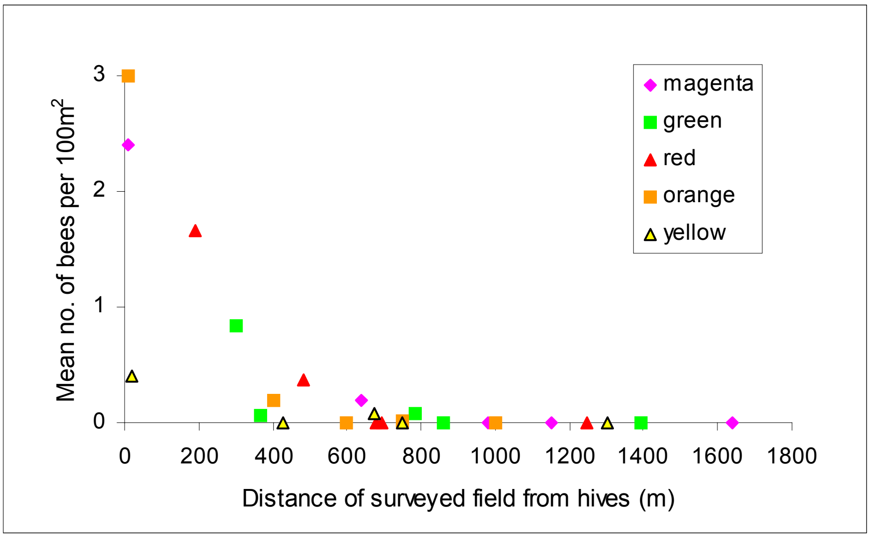

Figure 4 Mean number of marked bees per 100m^2 observed at different distances from their colonies. Different colours represent bees from different colony sites (Table 2).

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