Increasing seed yield in *Papaver somniferum* L. with the use of honey bees (*Apis mellifera* L.)

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Abstract
Honeybees (*Apis mellifera* L.) are known to play a role in pollination of poppy (*Papaver somniferum* L.) and could be used in seed crops to increase yield. Experiments were conducted on commercial poppy crops in northern Tasmania to establish the relationship between a gradient of honeybees and seed yield of poppy. Three beehives were grouped in the centre of three commercial poppy fields and an additional three fields were chosen that had no hives as controls. Plots were established in a spiral design every 5 m from 10 m to 140 m radius from the centre, thus having plots ranging over distance from a higher density to a lower density of bees pollinating the flowers at treatment sites. There were significantly more seeds per capsule for all capsule size classes, at the sites with beehives compared to the control. In addition for the large capsules there was a significant radial effect at the bee sites, with more seeds per capsule closer to the hive than further away. There was no radial effect for the control. Insect pollinations were also monitored using pan traps at set distances from the centre of the crop at each site. There were significantly more native pollinators captured at the edge of the crop than at any other distance from the centre of the field. Honeybees have been shown to increase yield in commercial poppy crops, and can be used to increase yield in crops were the yield of genetic superior seed is important.

Introduction
Poppy (*Papaver somniferum* L.) is grown for the production of alkaloids for the pharmaceutical industry and seeds for the culinary trade. Interest lies in increasing seed yield in seed crops to increase the amount of genetically superior seed available for the following season. The species is largely self-pollinated but can out-cross in the presence of insects (Patra et al. 1992; Miller et al. 2005). Miller et al. (2005) found that approximately 30% of self-pollination was facilitated by insects. European honeybee (*Apis mellifera* L.) and flies were thought to be the major insects pollinating poppy crops from their field observations. Therefore honeybees could potentially be used to increase fertilisation and hence seed production. The objective of this research was to determine the effect of pollinating bees on final seed yield. Insect activity at the sites was also investigated.

Methods
Experiments were conducted in commercial poppy fields in the 2010-11 season. Six field sites; three controls and three sites with beehives, were established in northern Tasmania (41.6°S, 147.3°E). Field sites were chosen based on estimated flowering date, with sites beginning flowering within 1-2 days of one another, each site was mean of 10 km from one another and the closest two sites were 2 km apart. Herbicides, fungicides and irrigation were applied in line with commercial practices.

We chose a design that would reflect the presumed movements of bees and would detect influences due to extraneous factors, such as pollinators moving into the crop from the crop edge or wind. In each field, forty-five 2 x 1 m plots were established radially at various distances from the location of the beehives. The exact positions of the plots followed an Archimedes spiral design to ensure a greater sample effort closer to the hives but approximately equal effort in all cardinal directions. The aim was to direct sampling effort to where nonlinearity in the outcome was expected to be maximal. The plots were positioned on 12 radii, making each radial arm at a bearing of 30 degrees, Figures 1 and 2 show the design. Plots were positioned every 5 m with a minimum distance of 10 m ranging to a maximum of 140 m from the centre of the crop. The radial distance, r_p , was derived by; $r_p = 10 + 0.1 \times b_p$, where b_p was the bearing from the centre. Three beehives were placed in the centre of the crop at treatment sites at the beginning of flowering, 9th December 2010, and removed when the majority of the sites had completed flowering, 10th January 2011. The poppy capsules from each plot were harvested at maturity and each capsule was separated by diameter into one of three classes: large (> 25 mm), medium (15-25 mm) and small (< 15 mm). Capsules in each class were counted and seeds extracted and average seed weight per capsule in each class calculated for each plot. The relative importance of honeybee pollination on seed yield was assessed by calculating the average seed weight per poppy capsule. A spatial model was fitted of the form; $y_{s,p} = \alpha t_s + \beta r_p + \gamma t_s * r_p$, where the outcome, $y_{s,p}$, for plot, p, at site, s; a treatment effect t_s referred to the site; a radius r_p for each plot; and an interaction of the two. The α, β and γ were coefficients to be estimated. The treatment effect consisted of the presence (B) or absence (C) of a beehive at the site. The radius effect was a measure of the change in the outcome with increasing distance from the beehives to the individual plot. After exploratory analysis a spatial spherical correlation structure was adopted. A random effect for the site was also used. The model was fitted using Proc MIXED in SAS version 9.2.

Insect activity

Pollinating insect activity was estimated at all sites using coloured pan traps, which is a common passive sampling method for bees. To account for different colour preferences of pollinator species, pan traps were painted UV-bright yellow, blue and white. The traps were 11 cm in diameter and 6 cm in height, each filled with approximately 200 ml of water, to which a few drops of unscented dishwashing detergent were added to break surface tension. At each site, 21 pan traps were established in seven clusters (each containing the three colours separated by a distance of 5 m) and placed at approximately the same height as the surrounding flowers. Trap clusters were placed in one transect from the centre of the circle at 50 m intervals with the first cluster as close as practical to the centre extending to 250 m radius. An additional cluster was placed at the edge of the crop. Traps were placed in the field on 13th December, towards the peak of flowering and when there was reasonable weather. Trap contents were retrieved after 24 h, strained, rinsed and stored in a specimen jar containing 70 % ethanol and identification was performed.

The effect of treatment (beehive or control), distance from the centre of the circle and pan trap colour was evaluated separately for honeybees, native bees, small flies, total number of insects and total number of potential pollinators caught. Data were analysed using GenStat 11.0 using a split-split plot ANOVA with each site as a separate replicate. The number of individuals caught in pan traps was transformed (square-root with constant of 0.5 added) prior to analysis and comparisons between treatment means were made using the least significant difference (LSD). Total number of insects and total number of potential pollinators caught was not transformed.

Results and Discussion

Seasonal conditions and seed yield

There was a high frequency of cool, rainy, windy weather experienced during the flowering period (Anon. 2010; 2011) that would not have favoured seed set (Tetenyi 1995) or insect pollination of the flowers (Winston 1987). There was a significant radial effect at the bee sites, but not the control sites for large capsules (Figure 1), where there was more seed per capsule close to the hive than further away, indicating a higher concentration of bees at the centre of the site could have increased pollination and hence yield. Beyond 100 m distance from hives the mean dropped by 0.39 g seed per capsule, which was small compared to the overall mean, however small treatment effects were detectable due to the power of the design. Medium capsules showed a similar radial trend to that of large capsules with a radial effect close-to-significant (P = 0.08; Figure 2). There was an inverse radial trend for medium capsules at control sites (P = 0.09; Figure 2), such that there was less seed per capsule towards the centre of the site than further out. Native pollinators moving in to the trial sites from the crop edge, resulting in a higher abundance of pollinators in the outer reaches of the crop, and decreasing in abundance towards the centre, is a likely explanation for the inverse relationship at control sites. Experiments were located among diverse agroecosystems with pasturelands, crops and open woodland generally in close proximity to the poppy crop. More insects were trapped closer to the crop edge (Figure 3), which was expected in the diverse landscapes where the experiments were located.

There was an overall treatment effect for all capsule size classes where bee sites had significantly greater weight of seed per capsule for large, medium and small capsules. On average there was 0.30, 0.075 and 0.055 g more seed weight per capsule at bee sites than control sites for large, medium and small capsules respectively. As there was always a treatment effect resulting from the presence of a beehive the influence of the bees from the hive possibly included the whole experimental area and beyond, not just poppies in proximity to the hive. *A. mellifera* is known to typically forage up to 2 - 3 km from its hive, with foraging over much greater distances also possible (Beekman and Ratnieks 2000), whereas smaller native bees are more limited in foraging distance, perhaps over a few hundred metres (Greenleaf et al. 2007). More hives should generally result in higher flower visitation and hence greater pollination. However, it should be noted that in this largely self-pollinated crop (Tetenyi 1995), the contribution of insect activity to overall pollination is small relative to other crops. Strong radial effects are unlikely to ever be seen though the presence of beehives has increased seed per capsule in this experiment.

Insect activity

The pan traps were successful in capturing a number of insects during the 24 h the traps were in the field (Table 1). Honeybees (*Apis mellifera*) were caught in low numbers at all sites (Table 1) and there were no significant effects of treatment (beehive or control sites), distance from the centre or colour of traps. This may have been due to the cold weather; the average temperature at Launceston Airport at the time of the insect monitoring was only 11.4°C on 13th December and 14°C on 14th December. These temperatures are well below the optimum foraging temperature for honeybees of 22 - 25°C, and only marginally above the minimum temperature required for active foraging, being around 13°C (Winston 1987). However, other potential pollinators were caught in large numbers, particularly native bees and small flies (Table 1). UV-bright pan traps have been known to under-sample *A. mellifera*, but despite this, they are still recommended as the most suitable method for pollinator monitoring schemes (Westphal et al. 2008). There was a significant effect of distance from the site for potential pollinators (Figure 3), with more pollinators caught at the crop edge than any other cluster, the same effect occurred for the total number of insects caught (data not shown). Trap colour influenced capture success of native bees and small flies, where white traps caught fewer native bees than the other coloured traps and yellow traps caught more small flies than other coloured traps (Figure 4). Poppies are predominately self pollinated but the presence of honeybees have been shown here to contribute to pollination and yield. Though the increase in yield is small overall, in crops where genetic superior seed is important honeybee hives could be used to bolster yield of the crop.

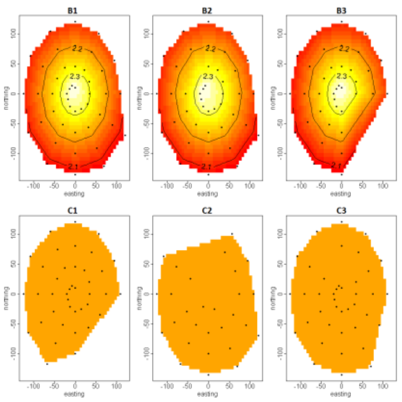

Figure 1. Fitted values for seed per large (>25 mm) capsules at each site. There was a significant radial effect at the bee sites (B1-3), but no significant radial effect for the controls (C1-3).

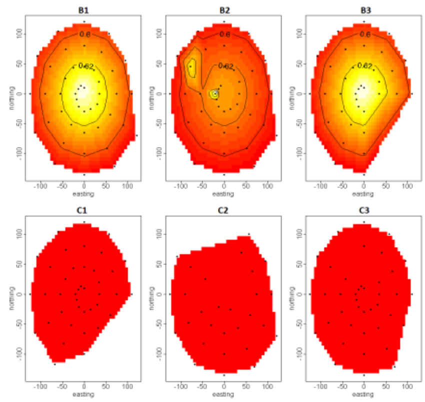

Figure 2. Fitted values for seed per medium (15-25mm) capsules at each site. There was a radial trend effect at the bee sites (*P*=0.08; B1-3) and no significant radial effect for the controls (C1-3).

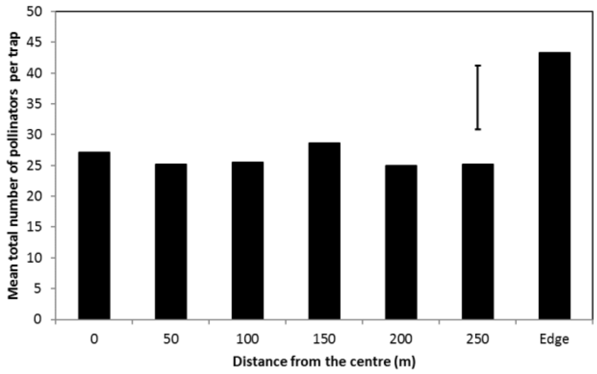

Figure 3. The mean total number of potential pollinators (flies, bumble, honey and native bees) caught per pan trap according to distance from the centre of the crop, edge is where traps were placed at the crop edge. Bar indicates LSD$_{0.05}$.

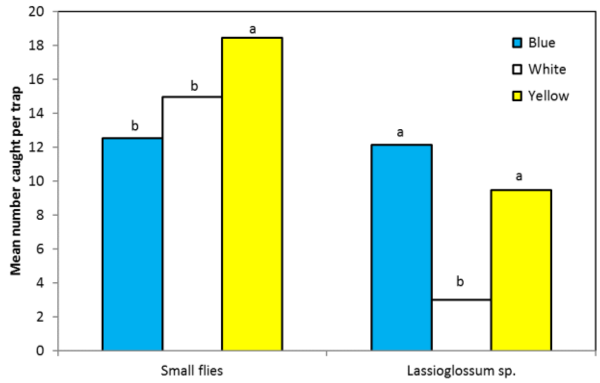

Figure 4. Influence of trap colour on capture success of small flies and native bees (*Lassioglossum* sp.). Within each group, a different letter above each bar indicates that the number of insects caught differs significantly between colours.

Table 1. Total number of different insects caught at each trial site.

	Bee sites			Control sites		
	B1	B2	B3	C1	C2	C3
Potential pollinating insects						
Bombus terrestris	2	1	0	2	10	0
Apis mellifera	3	3	10	3	10	2
Lassioglossum sp.	41	301	221	83	345	54
Homalictus sp.	0	14	0	0	33	0
Megachilidae	0	0	1	0	0	0
Small flies (predominantly *Drosophila* sp., *Fannia* sp., and Sciaridae)	295	128	267	336	454	336
Med-large flies (predominantly Calliphoridae and Tachinidae)	31	3	49	30	49	17
Other insects						
Small beetles (predominantly Staphylinidae)	10	4	10	27	6	10
Med-large beetles (predominantly *Phyllotocus* sp., Coccinellidae and Cantharidae)	1	4	0	4	5	1
Small wasps	33	13	20	8	33	14
Med-large wasps	0	0	0	0	3	0
Tenthredinidae	3	0	0	0	2	0
Thrips	5	24	92	5	9	12
Micromus sp.	9	0	0	3	0	2
Hemiptera	1	3	1	3	4	7
Moths	1	0	0	2	2	0

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References

Anon. (2010). Monthly weather review, Tasmania, December 2010. Available at (http://www.bom.gov.au/climate/mwr/tas/mwr-tas-201012.pdf).

Anon. (2011). Monthly weather review, Tasmania, January 2011. Available at (http://www.bom.gov.au/climate/mwr/tas/mwr-tas-201201.pdf).

Beekman M and Ratnieks FLW (2000). Long-range foraging by the honey-bee, Apis mellifera L. Functional Ecology 14, 490-496.

Greenleaf SS, Williams NM, Winfree R and Kremen C (2007). Bee foraging ranges and their relationship to body size. Oecologia 153, 589-596.

Miller JAC, Henning L, Heazlewood VL, Larkin PJ, Chitty J, Allen R, Brown PH, Gerlach WL and Fist AJ (2005). Pollination biology of oilseed poppy, Papaver somniferum L. Australian Journal of Agricultural Research 56, 483-490.

Patra NK, Ram RS, Chauhan SP and Singh AK (1992). Quantitative Studies on the Mating System of Opium Poppy (Papaver-Somniferum L). Theoretical and Applied Genetics 84, 299-302.

Tetenyi P (1995). Biodiversity of *Papaver somniferum* L. (Opium poppy). International Symposium on Medicinal and Aromatic Plants 191-201.

Westphal C, Bommarco R, Carre G, Lamborn E, Morison N, Petanidou T, Potts SG, Roberts SPM, Szentgyorgyi H, Tscheulin T, Vaissiere BE, Woyciechowski M, Biesmeijer JC, Kunin WE, Settele J and Steffan-Dewenter I (2008). Measuring bee diversity in different european habitats and biogeographical regions. Ecological Monographs 78, 653-671.

Winston ML (1987). The biology of the honey bee. Harvard University Press, Cambridge, MA.