Effects of natural pollinators on broad bean pollination and crop yield in plots with a Frisian clay area BEESPOKE seed mix based flower strip.

Ate S. Boerema, Marieke A. Strijkstra, Jeroen Sagel, Leonhard Teicke, Arjen M. Strijkstra

University of Applied Sciences Van Hall Larenstein, Leeuwarden, NL

Contact: arjen.strijkstra@hvhl.nl

Introduction: Broad beans (*Vicia faba L.)* is a crop that can be an important source of plant based protein for both human and farm animal consumption. Most varieties of broad beans are primarily self-pollinating (Free, 1993) but presence of pollinators can increase yields by 10-40%, depending on the variety (van Loo, 2022). The shape of the flower suggests that especially natural pollinators, such as (long tongued) bumblebees, are optimal for pollination. Stable and well maintained flower strips incorporated in the arable landscape can help to support these natural pollinators by providing nesting sites and food outside the flowering period of the crop. We assessed an easy to use method for finding pollination efficiency in a broad bean field with a flower strip designed for the Frisian clay area, by manipulating pollination with exclusion tents. We measured the effect of the flower strip by measuring pollinator behaviour at different distances to the flower strip.

Methods: Pollinator abundance and behaviour (observations), exclusion of pollinators from the crop (exclusion cages) and measures of crop yield were obtained in 6 plots planted with summer broad beans (varieties: Cartouche: n=5, and Banquise: n=1) in the BEESPOKE Frisian clay research area (see Figure 1). Plots had a developed 2nd year (n=2, 2021), 3rd year (n=2, 2022), or a developing 1st year (n=2, 2022) BEESPOKE seed-mix based flower strip (Strijkstra et al., 2023) adjacent to the crop.

Figure 1. *Example of a broad bean field next to a 3rd year BEESPOKE clay area flower strip. In the left side an exclusion tent can be seen.*

Exclusion tents (2m x 2m x 2m; mesh size 0.7 x 0.2 mm) were used to exclude pollinators and measure the influence of the absence of pollinators on crop yield. Cages were placed in the middle of the selected plots in May or June, prior to the start of flowering. Cages were removed after flowering ended.

Pollinator observations were done by slowly walking transects (+/- 20m^2 in 10 min) at several locations in the crop (at 1m and 10m from the flower strip, at the middle of the plot, and at 1m and 10m from the opposite side) when plants were flowering in June and July. The number of honeybees, solitary bees and the number and species of bumblebees on and near the plants were counted. Different types of visits (entry via the front of the flower, nectar collection via a hole in the back of the tube of the flower (robbing), and extra-floral nectar gland visits) were also recorded.

Crop yield was measured by collecting 10-20 plants from each transect and from the tent, at the stage that plants were ready for harvesting as cattle silage (5 locations) or dried in the field (1 location). Plants were cut just below the first node visible above the soil. Number of nodes, total plant mass, number of developed seeds (big beans), number of developed pods (big pods), and the number of seeds per pod (as an index for pollination success) were obtained.

Pollinator abundance: Bumblebees were the main pollinator species observed (77%), followed by honeybees (*Apis mellifera;* 20.8%) and other solitary bees (2.2%). Four different bumblebee species were observed pollinating broad bean flowers: 42.6% buff-tailed bumblebees (*Bombus terrestris complex), 4%* common carder bees (*Bombus pascuorum)*, 5.6% red-tailed bumblebees (*Bombus lapidarius)*, 1.5% garden bumblebees (*Bombus hortorum),* and 23.2% unidentified bumblebees. The last category likely contains a similar ratio of the other species observed. Additionally, pest-controlling species (primarily hoverflies, ladybugs and soldier beetles) were also observed throughout the crop at 12.6 individuals/10 min (SEM 1.9).

Interestingly, the number of bumblebees was relatively high closer at 1m from the flower strip at 16.8 individuals /10 min (SEM 3.18). This decreased to 10.35 individuals /10 min in the middle (SEM 2.27) and to 9.5 individuals /10min (SEM 1.55) at 1m from the border on the opposite side of the field.

Especially the numbers of buff-tailed and red-tailed bumblebees were higher closer to the flower strip, suggesting that the flower strip provides real habitat for these species. The number of honey bees was much lower at around 3.2 individuals /10 min (SEM 2.4) at all distances from the flower strip, which does not suggest a relationship of this species with the flower strip habitat (Data in Appendix 1, Figure 1).

Visiting behaviour: Especially the shorter tongued pollinator species (buff-tailed bumblebees and honeybees) were observed to collect nectar from a hole at the base of the flower tube during many of their visits. The buff-tailed bumblebee was indeed observed creating these holes in the back of the flower. Buff-tailed bumblebees still visited from the front of the flower in 39.8% of the cases, contributing to pollination. In several cases the same individual was observed to use both types of flower visits. Only honeybees and hoverflies were observed collecting nectar from the extra-floral glands. (Data in Appendix 1, table1).

Crop yield: Exclusion of pollinators decreased the big bean yield significantly with around 7.5 big beans per plant stem (-22%), from 41.6 big beans /plant (SEM 3.9) in the open situation, to 34.1 big beans /plant (SEM 3.9) in the exclusion tents. The total amount of big pods produced also decreased slightly with around 10%, from 13.6 big pods/plant (SEM 1.1) to 12.3 big pods/plant (SEM 1.3). Pollination success, as indicated by the amount of big beans /big pod, also decreased with around 10% in the exclusion tents, suggesting that the presence of pollinators increased both the amounts of pods and the amount of beans per pod. Total plant weight (1 stem) appeared quite variable, and plants in the tents produced on average 1.2 (SEM 0.2) more nodes. It was also observed that plants in the exclusion tents flowered longer, which suggests a continued need for animal pollination by the plants in the absence of pollinators. (Data in Appendix 1, table 2).

Conclusion: Crop yield (number of big beans) increased by 22% through the presence of pollinators. This is in line with previous reports on effects of animal pollinators. Flowers were primarily visited and pollinated by bumblebees, in particular by the buff-tailed bumblebees. The number of bumblebees observed 1 m into the broad beans next to the flower strip was around 30% higher than 10m into the crop and even 61% higher

than in the remainder of the field. This suggests that the BEESPOKE flower strip may provide useful habitat for these natural pollinators that are important for broad beans in the Frisian clay area.

References:

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Appendix 1: Overview of pollinator abundance in relation to the distance to the flower strip.

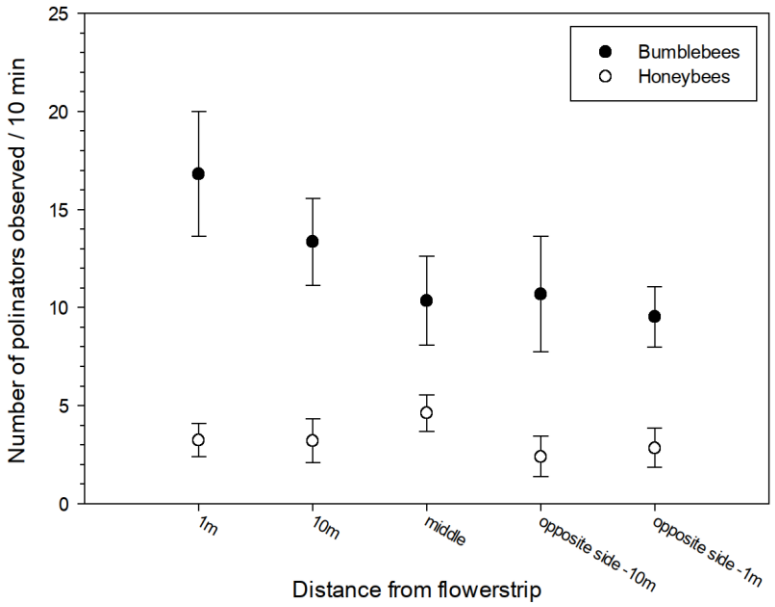

Figure 1: *The number of pollinators observed at different distances from the flower strip. All species of bumblebees combined (filled circles) and honeybees (open circles). There was a significant effect of distance from the flower strip in the amount of bumblebees of observed (One-Way-RM Anova; p<0.001). The number of bumblebees at 1 meter from the flower strip was significantly higher (post-hoc Holm-Sidak) than the number of bumblebees in the middle (p=0.007), at 10 m from the opposite edge (p=0.010), and at 1m from the opposite border (p=0.002). The number of honeybees did not differ with distance from the flower strip (One-Way-RM Anova; p=0.88).*

Table 1: *Types of visits per identified pollinator species, when observed on a plant. Pollinators were observed to visit extra-floral glands or holes at the back of the flower tube for nectar, or pollinate while entering the front of the flower.*

species	scientific name	backside of the flower (% visits)	entry front of the flower (% visits)	extra-floral nectar gland (% visits)
buff-tailed bumblebee	*Bombus terrestris complex*	60.2	39.8	0.0
common carder bee	*Bombus pascuorum*	11.0	89.0	0.0
red-tailed bumble bee	*Bombus lapidarius*	1.7	98.3	0.0
garden bumblebee	*Bumbus hortorum*	0.0	100.0	0.0
honeybee	*Apis mellifera*	77.3	17.5	5.2

Appendix 2: Crop yield parameters and the effect of excluding pollinators.

Table 2: *Crop yield comparison of different yield parameters in the situation with pollinators were excluded (tent) and the open situations at different distances to the flower strip (open). Significant differences (paired t-tests, Bonferroni corrected) were observed in the number of nodes per plant/stem (1.2 nodes more inside the exclusion tent, SEM 0.2; +5%,) and the number of big beans per plant/stem (7.5 big beans less inside the tent, SEM 1.3; -22%). The pollination index (big beans per big pod) showed a trend (p<0.10) towards more big beans per big pod outside the tent. A decrease was seen in 5 of 6 sites, except the Hoekstra site: this may have been caused by late placement of the exclusion tent, which slightly overlapped with early flowering.*

location	year	variety	number of nodes			total plant mass			number of big pods			number of big beans			pollination index: beans / pod		
			tent	open	tent - open	tent	open	tent - open	tent	open	tent - open	tent	open	tent - open	tent	open	tent - open
Hoekstra *	2021	banquise	26.7	26.3	*0.3*	152.6	176.8	*-24.2*	9.9	15.7	*-5.8*	29.3	42.9	*-13.6*	3.02	2.77	*0.25*
Van der Wal **	2021	cartouche	24.4	22.6	*1.8*				14.4	15.1	*-0.8*	37.9	45.0	*-7.0*	2.64	2.96	*-0.32*
Venema	2022	cartouche	25.8	24.8	*1.0*	141.0	201.3	*-60.3*	16.6	15.8	*0.8*	48.2	53.8	*-5.6*	2.88	3.41	*-0.53*
Fokkema	2022	cartouche	26.3	25.1	*1.2*	206.0	200.4	*5.6*	14.6	14.5	*0.1*	40.1	47.3	*-7.2*	2.74	3.26	*-0.52*
Van Kuiken 1 ***	2022	cartouche	23.7	22.2	*1.6*	136.5	111.8	*24.7*	9.6	10.1	*-0.5*	23.7	30.1	*-6.4*	2.52	3.00	*-0.48*
Van Kuiken 2 ***	2022	cartouche	23.3	22.0	*1.3*	130.8	119.0	*11.9*	9.0	10.4	*-1.4*	25.4	30.4	*-5.0*	2.88	2.94	*-0.06*
		average	**25.0**	**23.8**	***1.2***	**153.4**	**161.8**	*-8.5*	**12.3**	**13.6**	*-1.3*	**34.1**	**41.6**	***-7.5***	**2.78**	**3.06**	*-0.28*
		SEM	0.6	0.7	*0.2*	13.6	19.5	*15.2*	1.3	1.1	*0.9*	3.9	3.9	*1.3*	0.07	0.10	*0.13*
	t-test	***p-value***		***0.002***			*0.609*			*0.243*			***0.002***			*0.082*	

* Lower nodes flowering prior to exclusion tent placement.

** Plants collected and analysed just before harvest, no fresh weight available.

*** Planted later due to weather conditions.