Winter field beans: Assessing autogamy in 7 varieties.

Report
Cluster agro-environment
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ondernemen in west-vlaanderen

inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

1. Content

2. Preface

During the last couple of decades, many reports on pollinator declines -both with respect to numbers and diversity- have been published. Less pollinators results in lower yields in many crops, but pollinators also play an important role in ecological resilience, food webs and population dynamics of both the plants that they pollinate, as well as the organisms that are dependent on these plants.
The loss of pollinators from the landscape thus has effects both on agricultural and ecological aspects.

The Interreg NSR project "BEESPOKE" aims to support pollinators in the agricultural landscape and in doing so improving the pollination of crops as well as the overall biodiversity of pollinators. To achieve this, bespoke flower mixtures tailored to crops and the environment (landscape characteristics like soil type, …) are being developed, and alternative management practices of crops that have a potential to support pollinator diversity are being tested (e.g. mixtures for productive grasslands, phased mowing of alfalfa).

For growers it is important to be able to register pollination deficits and pollinator presence in their fields. Therefore, the project aims to develop and trial easy protocols to investigate these parameters for pollinator dependent crops like strawberry, cherry, apple, pear and field beans.

Inagro, as one of the project partners, is focussing on alfalfa management as a potential pollinator supporting crop, and field beans as a pollination dependent crop.

This report describes the findings on differences in the capacity to self-pollinate in several winter field bean varieties, which is of relevance when investigating the pollination deficit on fields in the wider landscape where different field bean varieties may have been sown.

3. Materials and methods

The survey was performed within a larger field of field beans (variety winter field bean: Tundra), in a heavy soil polder. 7 Varieties of winter field beans were studied: Augusta, Axel, Diva, Hivena, Irena, Nebraska, and Tundra.

Exclusion cages (1m x 1m x 1,8m; mesh size 0,95 x 1,35mm) were used to exclude pollinators and were raised at more than 45m from the field borders.

Figure 2: Exclusion cages in the field at the start of the both pilots, 18th of May 2021

Figure 1: Plant size at the time where the marked plants were harvested for the observations, 22nd of June 2021.

Cages were mounted about 20 days after the first flowers opened on the plants, on the 18th of May 2021. On each plant, the lowest flower cluster with all flowers still closed was marked with woollen threads at the same time (See the marker in the blue ellipse on figure 3).
Manual pollination took place 16 days after marking these flower clusters, on open flower clusters above this marker, ensuring that no bee-pollination could interfere with the treatments in the exclusion cages .

Whole plants were harvested in the first half of July. Following parameters were registered: count original number of flowers per flower cluster (scars can clearly be seen where flowers used to be. See figure 4), count the number of green and black (=rotten or aborted) bean pods per cluster, count the number of beans per pod.

Figure 3: On each plant, the lowest flower cluster with all flowers still closed was marked with a woolen thread.

1 Exclusion cage was installed in each variety, 6 plants were marked for each treatment and 3 Treatments were applied to each variety:

1. "Exclusion"
    - (= Flowers excluded from pollinators, inside the cage),
2. "Open"
    - (= Flowers open to pollinators, outside the cage), and
3. "Open + trigger"
    - (= Flowers open to pollinators, outside the cage + manually trigger pollinated (= forced self-pollination) to account for the potential shortage of pollinators to provide the pollination. Trigger pollination was found to be as effective as manual cross pollination in previous studies, and confirmed in a study in parallel with this one.)

Figure 4: Scars where flowers used to be can easily be counted upon harvest (when harvesting the plants green). In this example, the 3 arrows indicate 3 scars at the top, but the scars can be anywhere on the flowering stem.

Pollinator visits were registered by walking 5m long transects parallel to the field border, for 5 minutes. 3 Transects were located at 5m from the field border, 3 at 20m and 3 at 45m. Transects were walked on May 14th and the 8th of June 2021. Transects were located outside the plots with different field bean varieties, in the larger field (monoculture variety "Tundra").

4. Results and discussion

Almost all observations were performed within the peak bean production period of the varieties (see figure 5), except in 'Irena' which already was ending bean production, and 'Hivena' which was still starting up.

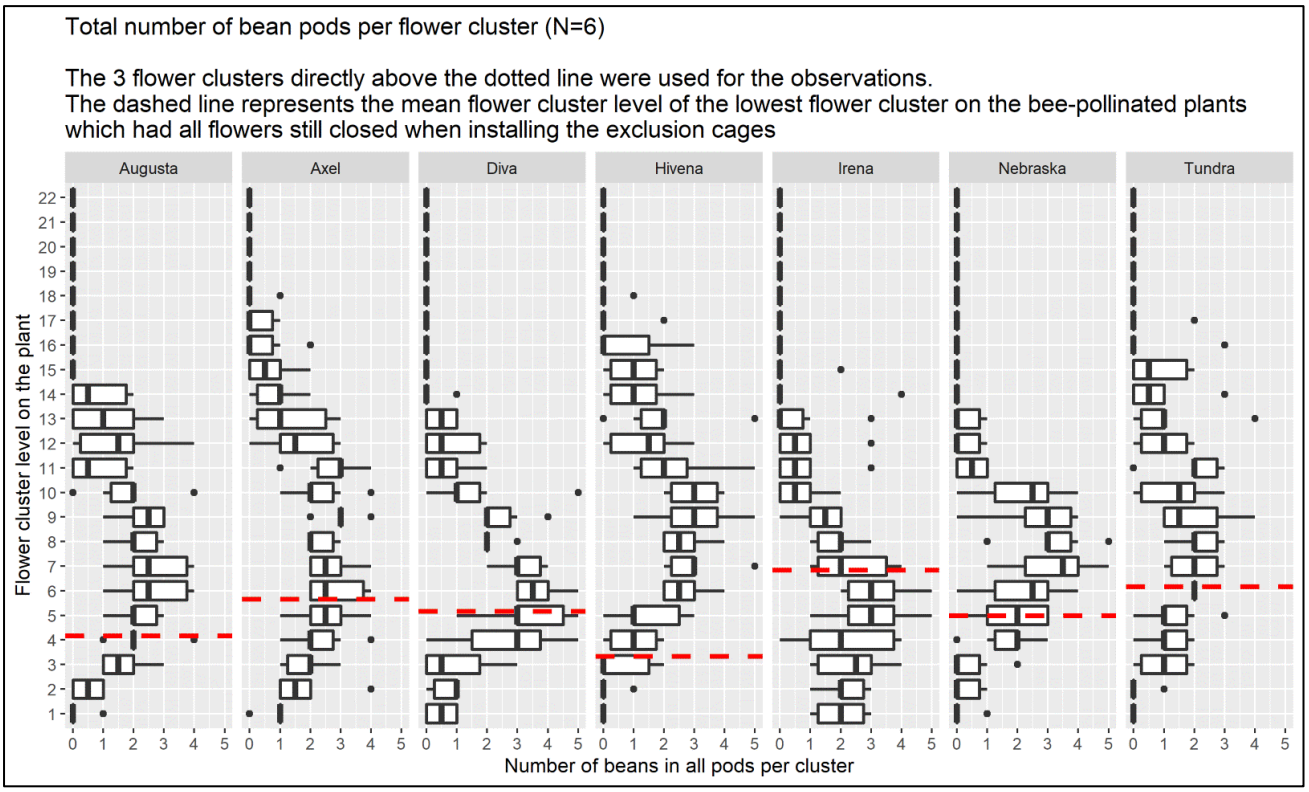

Figure 5: Timing of the observations (the 3 cluster above the red dashed line) with respect to the peak bean production on the plants. Observations in all the varieties started on the same day (=red dashed line).

However, the observations used in the analysis below in this report are performed only on 3 flower clusters per plant. Thus, the results are only based on a limited timeframe, and only on the level of individual flower clusters and not on yields from whole plants.

Figure 6 shows the number of bees that were found in the field on June the 8th (almost no pollinators were observed in the field on the 14th of May), extrapolated from the 7,5m² transects. The figure differentiates the observed flower visitation behaviour of each bee. "Robbing nectar" means that the bees were extracting nectar through a hole that was made at the base of the flower tube (only *Bombus terrestris/lucorum* was observed making these holes.), "Regular flower visit" means that the bee was accessing the flower from the front of the flower, "Passing by" means that the bee was not visiting a flower but just passing by, and "Extra floral nectar" means that the bee was tipping it's tongue on the nectar glands on the bracts of the flower clusters.

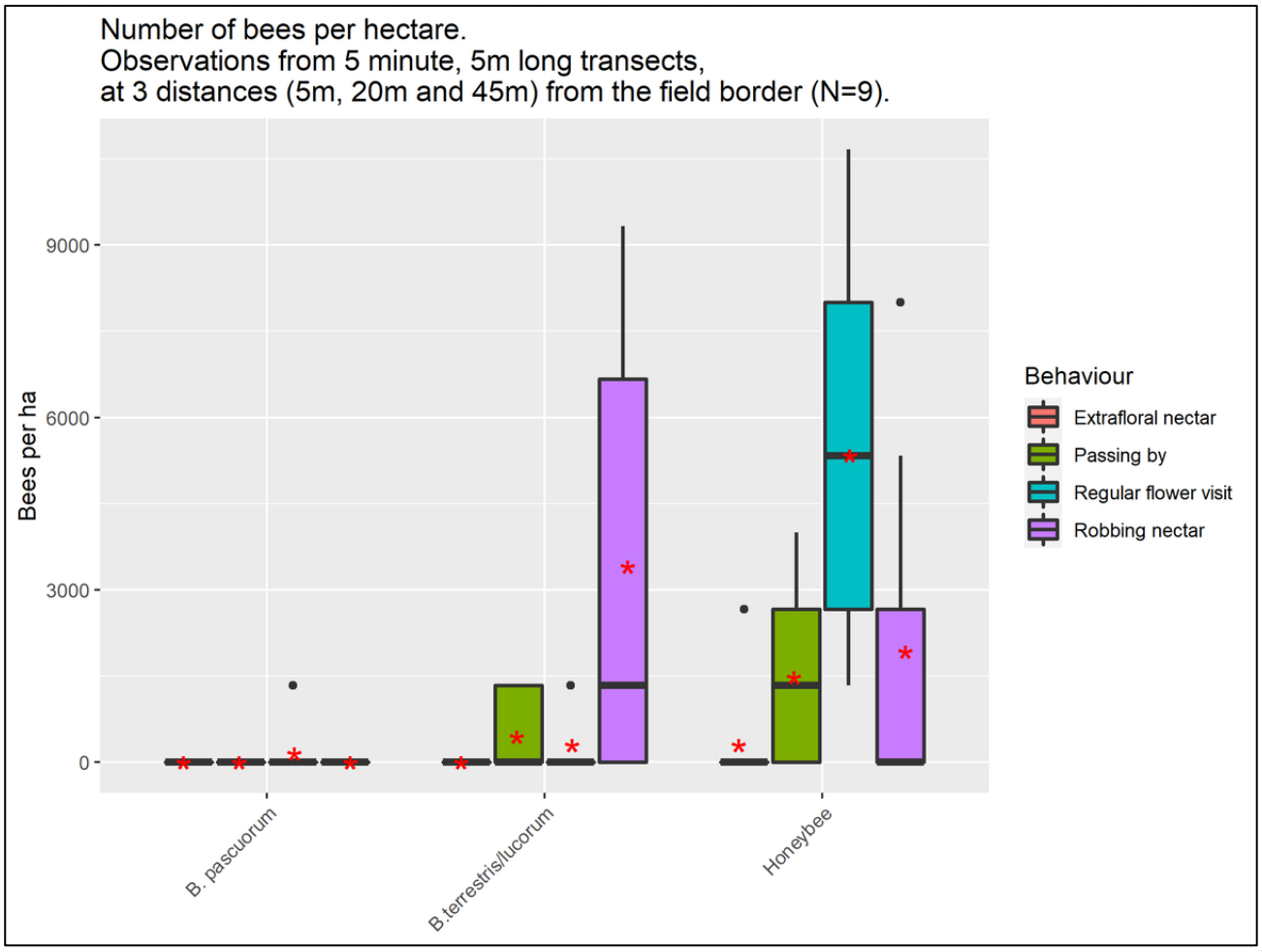

Figure 6: Number of bees, and their observed behaviour with respect to flower visitation on June the 8th 2021.

Figure 7 shows the extrapolated number of bees on different distances to the field border. The exclusion experiment on the field bean varieties was situated at least 45m away from the field borders.

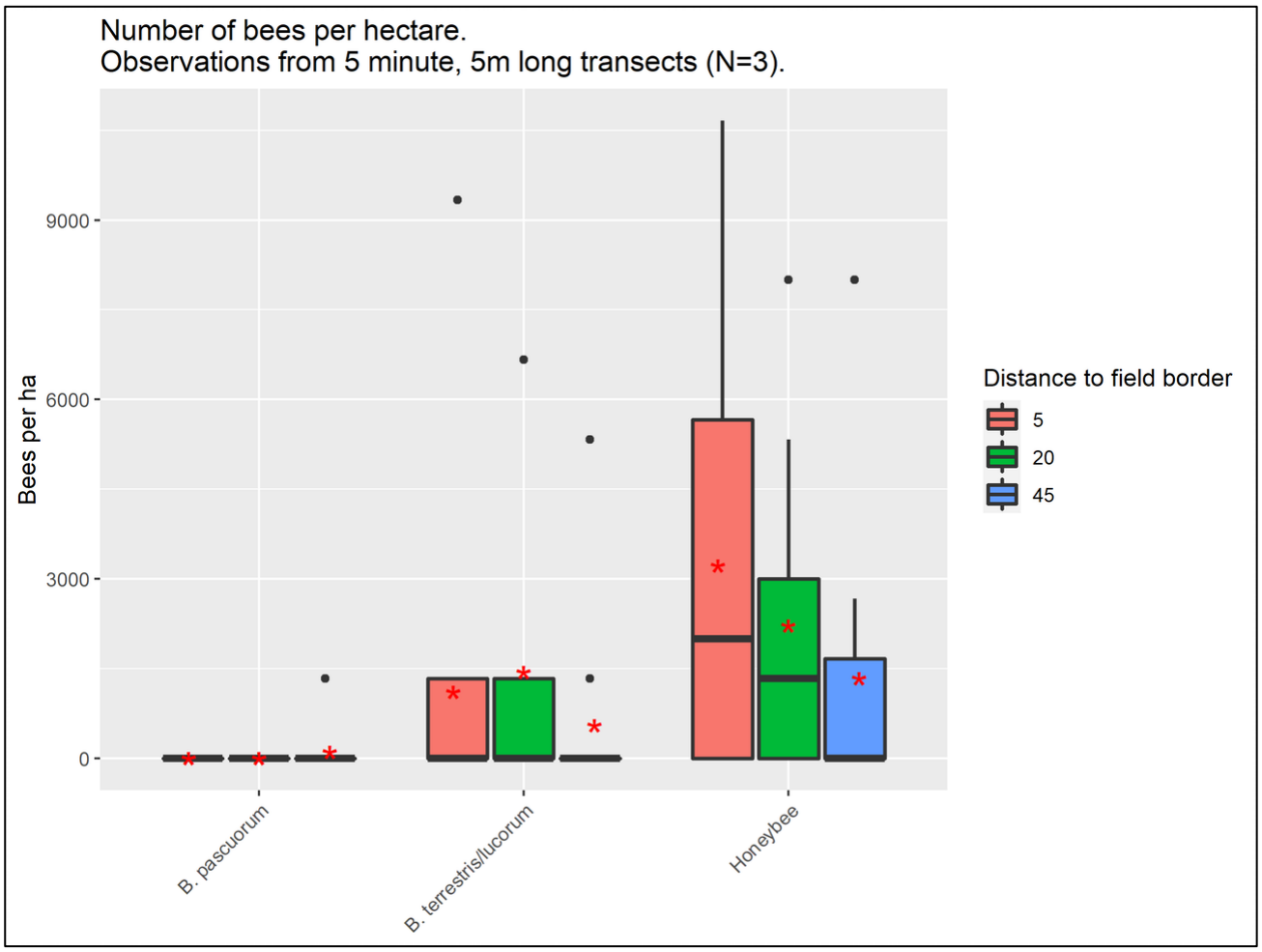

Figure 7: Number of bees at different distances to the field border (5m, 20m and 45m).

Figure 8 and table 1 show the variability of the number of flowers that are produced per cluster, for each field bean variety. The higher the number of flowers, the higher the number of potential bean pods (theoretically).

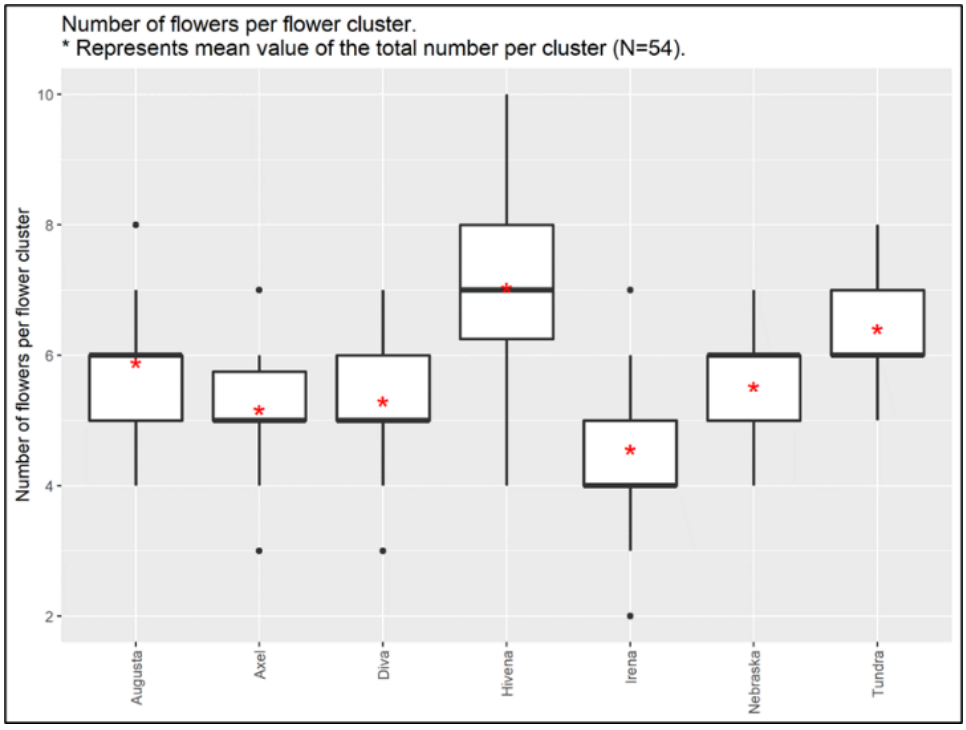

Figure 8: Average number of flowers per flower cluster, per variety.

Table 1: Significance table comparing all the varieties (Number of flowers per cluster)

dunn.test(), p.adj=bonferroni				
Variety 1	**Variety 2**	**Z**	**P.adjusted**	**Sign. Level**
Augusta	Axel	-3,082751354	2,15E-02	*
Augusta	Diva	-2,775092876	5,79E-02	ns
Augusta	Hivena	1,338414921	1,00E+00	ns
Augusta	Irena	-1,243637342	1,00E+00	ns
Augusta	Nebraska	-2,533523997	1,19E-01	ns
Augusta	Tundra	0,68891369	1,00E+00	ns
Axel	Diva	0,31215008	1,00E+00	ns
Axel	Hivena	4,485712262	7,63E-05	***
Axel	Irena	1,865963812	6,51E-01	ns
Axel	Nebraska	0,557245698	1,00E+00	ns
Axel	Tundra	3,826728759	1,36E-03	**
Diva	Hivena	4,173562182	3,15E-04	***
Diva	Irena	1,553813732	1,00E+00	ns
Diva	Nebraska	0,245095618	1,00E+00	ns
Diva	Tundra	3,514578679	4,62E-03	**
Hivena	Irena	-2,61974845	9,24E-02	ns
Hivena	Nebraska	-3,928466563	8,98E-04	**
Hivena	Tundra	-0,658983502	1,00E+00	ns
Irena	Nebraska	-1,308718113	1,00E+00	ns
Irena	Tundra	1,960764947	5,24E-01	ns
Nebraska	Tundra	3,269483061	1,13E-02	*

Figure 9 and table 2 show the average number of formed beans (seeds) per flower cluster, for each treatment in the survey. Data on the percentage of pollinated flowers per cluster show a very similar distribution, with an extra significant difference between the "exclusion" treatment and the "open" treatment in variety Augusta.

Clear differences in self-pollination capabilities between the varieties can be observed from figure 9. However, the lowest performing variety in the "exclusion" treatment (Nebraska) outperforms the highest yielding varieties (Axel, Diva, Irena) from the "exclusion" treatment when they are compared under normal field conditions ("open" treatment).

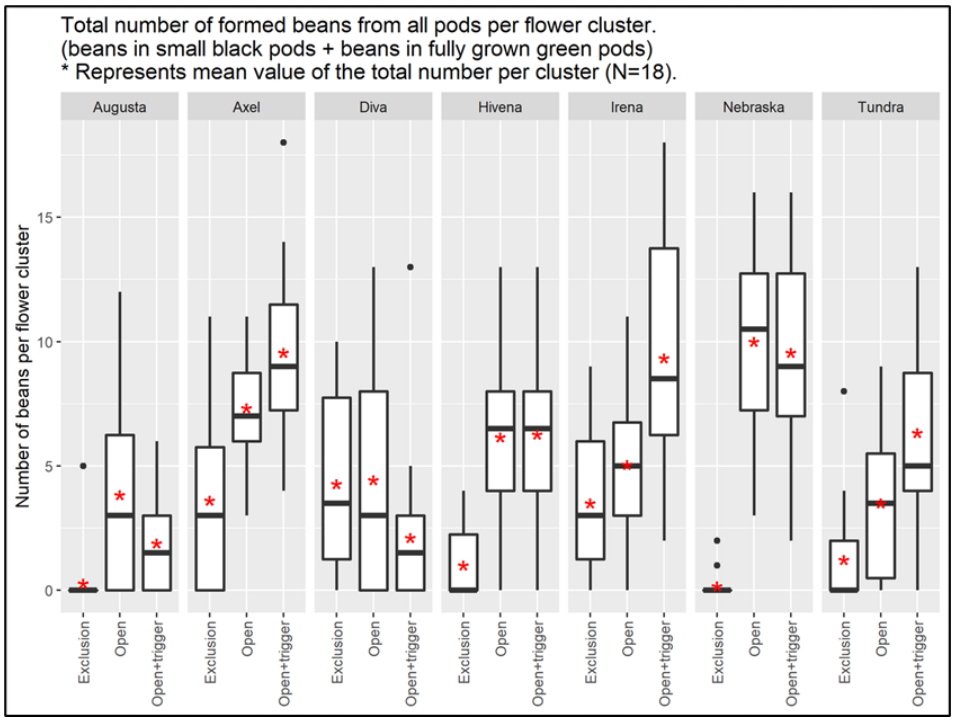

Figure 9: Variation and average of the total number of beans per flower cluster for each treatment in each variety.

Note that in some cases the "open" treatment results in higher yields than the "open+trigger" treatment, which theoretically should reach optimal pollination.

Table 2: Significance table comparing the differences from the treatments within each variety (Number of beans per flower cluster), and among the varieties between "Exclusion" and "Open".

dunn.test, p.adj= bonferroni					
Variety	Pol. type 1	Pol. type 2	Z	P.adjusted	sign. level
Augusta	Exclusion	Open	-2,914488873	3,74E-01	ns
Augusta	Exclusion	Open+trigger	-1,544694581	1,00E+00	ns
Augusta	Open	Open+trigger	1,369794292	1,00E+00	ns
Axel	Exclusion	Open	-2,844064421	4,68E-01	ns
Axel	Exclusion	Open+trigger	-3,858640871	1,20E-02	*
Axel	Open	Open+trigger	-1,01457645	1,00E+00	ns
Diva	Exclusion	Open	0,149361751	1,00E+00	ns
Diva	Exclusion	Open+trigger	1,763706888	1,00E+00	ns
Diva	Open	Open+trigger	1,614345138	1,00E+00	ns
Hivena	Exclusion	Open	-3,99252472	6,86E-03	*
Hivena	Exclusion	Open+trigger	-4,038958425	5,64E-03	*
Hivena	Open	Open+trigger	-0,046433705	1,00E+00	ns
Irena	Exclusion	Open	-1,143816929	1,00E+00	ns
Irena	Exclusion	Open+trigger	-3,289827987	1,05E-01	ns
Irena	Open	Open+trigger	-2,146011058	1,00E+00	ns
Nebraska	Exclusion	Open	-6,78628596	1,21E-09	***
Nebraska	Exclusion	Open+trigger	-6,558760807	5,70E-09	***
Nebraska	Open	Open+trigger	0,227525154	1,00E+00	ns
Tundra	Exclusion	Open	-1,933189911	1,00E+00	ns
Tundra	Exclusion	Open+trigger	-3,892692255	1,04E-02	*
Tundra	Open	Open+trigger	-1,959502344	1,00E+00	ns

dunn.test, p.adj= bonferroni				
Groep 1	Groep 2	Z-score	P.adjusted	sign. level
Augusta_Exclusie	Axel_Open	-5,935643692	0,00000	***
Augusta_Exclusie	Diva_Exclusie	-3,635625812	0,01262	*
Augusta_Exclusie	Diva_Open	-3,406563766	0,02993	*
Augusta_Exclusie	Hivena_Open	-4,977107129	0,00003	***
Augusta_Exclusie	Irena_Open	-4,298143731	0,00078	**
Augusta_Exclusie	Nebraska_Open	-6,901228318	0,00000	***
Augusta_Open	Nebraska_Open	-3,787159166	0,00693	*
Axel_Exclusie	Nebraska_Open	-3,911674945	0,00417	**
Axel_Open	Hivena_Exclusie	5,12981516	0,00001	***
Axel_Open	Nebraska_Exclusie	5,955613204	0,00000	***
Axel_Open	Tundra_Exclusie	4,943041492	0,00003	***
Diva_Exclusie	Nebraska_Exclusie	3,655595324	0,01167	*
Diva_Exclusie	Nebraska_Open	-3,265602506	0,04970	*
Diva_Open	Nebraska_Exclusie	3,426533277	0,02782	*
Diva_Open	Nebraska_Open	-3,494664553	0,02160	*
Hivena_Exclusie	Hivena_Open	-4,171278597	0,00138	**
Hivena_Exclusie	Irena_Open	-3,492315198	0,02179	*
Hivena_Exclusie	Nebraska_Open	-6,095399786	0,00000	***
Hivena_Open	Nebraska_Exclusie	4,997076641	0,00003	***
Hivena_Open	Tundra_Exclusie	3,984504929	0,00308	**
Irena_Exclusie	Nebraska_Open	-3,77306304	0,00734	*
Irena_Open	Nebraska_Exclusie	4,318113242	0,00072	**
Irena_Open	Tundra_Exclusie	3,30554153	0,04313	*
Nebraska_Exclusie	Nebraska_Open	-6,92119783	0,00000	***
Nebraska_Open	Tundra_Exclusie	5,908626118	0,00000	***
Nebraska_Open	Tundra_Open	3,830622221	0,00582	*

Table 3 shows the percental increase in the number of beans per flower cluster, from excluding flowers from any external pollination in exclusion cages, to flowers that are both insect and manually pollinated in the open field.
Note the large amount of variability within treatments, in Figure 7 and table 2, resulting in few statistically significant differences. This makes that from the table below, only the increase for varieties Axel, Hivena, Nebraska and Tundra are actually the result of significant differences between the 'exclusion', and the 'open + trigger' treatment.

Table 3: Increase in the number of beans (=seeds) per flower cluster from the treatment "Exclusion" to the treatment "insect pollination in the open field".

Variety	Increase from "Exclusion" to "Open"
Augusta	1380%
Axel	203%
Diva	104%
Hivena	617%
Irena	144%
Nebraska	6000%
Tundra	286%

Figure 9 and table 3 show two important lessons: 1) external (insect) pollination has a massive, but unequal, impact on the proportional increase in yields, and 2) varieties that have very low self-pollination capabilities can outperform less pollinator dependent varieties, when compared under open field conditions. This can clearly be observed from figure 10: variety Nebraska produces no seeds when excluded from pollinators, but produces significantly more seeds than most other varieties (Augusta, Diva, Irena, Tundra) under open field conditions.

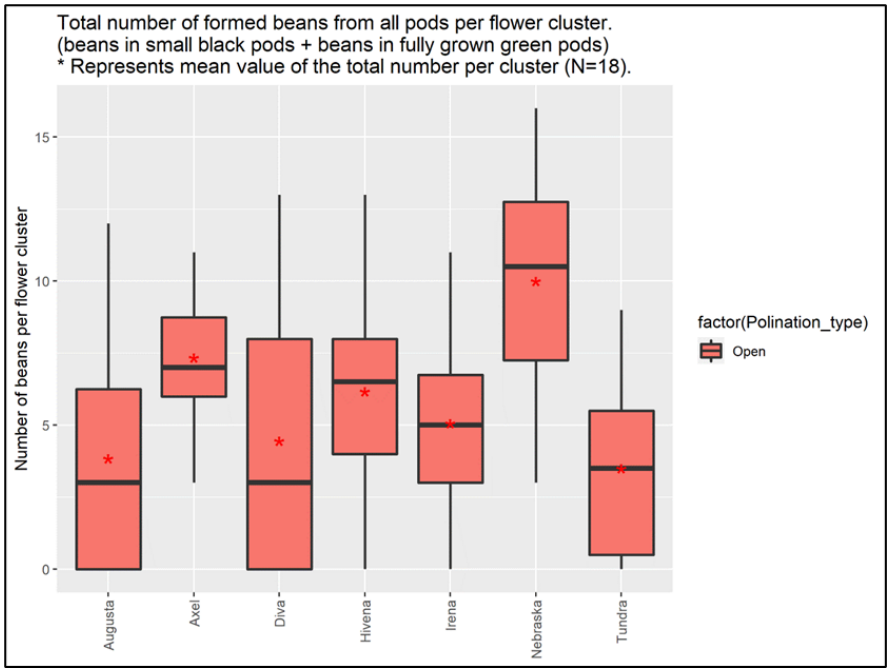

Figure 10: Total number of seeds per flower cluster under open field conditions (no extra "trigger" pollination)

5. Conclusions

- In general, pollination in winter field bean varieties is dependent on pollinators to a very large extent.
- Differences in the level of self-pollination among winter field bean varieties can be observed.
- Varieties that know a (very) low level of self-polination can be more productive under open field conditions than varieties that are able to self-polinate their flowers to a certain extent.
- The number of observed bees in a mass flowering crop such as field beans seems to decline with increasing distance to the field border.
- There is no certainty that varieties that perfom better than others when deprived from pollinators also perform better in the open field.