Pollination deficits in field beans

Validating an alternative protocol to assess pollination deficits in field beans

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01/03/2022

ondernemen in west-vlaanderen

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ONDERZOEK & ADVIES IN LAND- & TUINBOUW

Samenvatting

Bestuiving door insecten is een belangrijk fenomeen bij veel bloeiende gewassen, waaronder veldbonen.

Toch is er tot op heden geen eenvoudig protocol beschikbaar waarmee landbouwers mogelijke bestuivingstekorten in hun veldbonenvelden kunnen onderzoeken.
De mogelijkheid om dit te doen zou de boeren nuttige informatie geven om hun productie te optimaliseren en hen ertoe aan te zetten om eventuele tekorten in hun gewassen aan te pakken.

In een eerdere poging om een dergelijk protocol op te stellen bleek dat de resultaten niet voldeden aan de vereiste herhaalbaarheid om dat protocol te kunnen gebruiken. Bovendien was dat protocol afhankelijk van het tijdrovende en delicate element van manuele bestuiving.

In de studie die hier voorligt stellen we een alternatieve methode voor, en valideren deze.

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Summary

Insect pollination is an important fenomenon in many flowering crops, including field beans.

Yet, to this day, there is no farmer friendly protocol available for farmers to look into potential polination deficits in their field bean fields.
Being able to do so would give usefull information to farmers that would allow to optimise production, and trigger them to adress deficits in their crops if they would occur.

A previous attemt to produce such a protocol did not provide the repeatability in results that was needed to be able to use that protocol. Furthermore, it relied on the timeconsuming and delicate element of manual polination.

In the present study, we propose and validate an alternative method.

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Inhoud

1. 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. Previous work within this project, together with partners from the Netherlands and the UK had shown that an earlier protocol to assess pollination deficits in field beans seemed not to be useful.

The main drawback of that protocol was that the positive reference in the assessment was based on man-made manipulations that were trying to achieve the best possible levels of pollination, whilst fruit set in this positive reference turned out to be lower than in the fruit set under natural conditions in the open fields. Furthermore, the manipulations were only being performed on a limited number of flower trusses per plant because of practical issues that are related to the availability of time, and the speed with which the plants produce new flowers. Manual pollination was only being performed once per flower, on all of the flowers that were open at the time of the visit. This implies that some flowers will have been more susceptible to pollination, whilst others might not have been susceptible yet (or, were no more susceptible).

This report describes the findings from an attempt to come up with an alternative protocol to assess pollination deficits in field beans. A protocol that assesses whole stems, without manual intervention on pollination.

2. Materials and methods

1.1 LOCATION OF THE EXPERIMENTAL FIELD

The survey was performed within a larger field of a mixed crop of winter field beans (variety: Irena) together with triticale.
The field is located within a very open landscape that is dominated by arable farming, near Lo-Reninge.
The dimensions and the experimental setup can be seen in Figure 1.

Figure 1: Experimental set-up on the field. Stars indicate sampling locations on pollinator activity and on fruit set from plants in open field conditions. Blue rectangles indicate the location of exclusion cages.

1.2 OBSERVATIONS ON POLINATOR ACTIVITY

Pollinators were monitored every week as long as the flowering season lasted.
The dates were April 25th 2022, may 6th, 13th and 19th 2022, and where the same dates on which observations of flowers with holes were made (see further).

Observations were made on each of the 15 sampling locations in the field, by standing still for 5 minutes and registering bee-activity within a 1m radius: species, and behavior (regular flower visit, robbing nectar, feeding on extrafloral nectar, or passing by) were noted.

1.3 OBSERVATIONS ON FRUIT SET AND THE YIELD DEFICIT

20 Stems of similar posture were labelled at the start of the study, on each sampling location in the field, using a "Maxtang" (Figure 2).

In each parallel, an exclusion cage (1m x 1m x 1,8m; mesh size 0,95 x 1,35mm) was installed, prohibiting pollinators to visit the flowers (Figure 3).
Inside the cage, stems were chosen that were not in contact with the netting, to minimise chances of pollination by the wind beating on the nets and thus on the plants.
Because of the size of the exclusion cages, only 10 stems of similar posture per cage could be labelled.

Figure 3: Exclusion cages in the middle of the field.

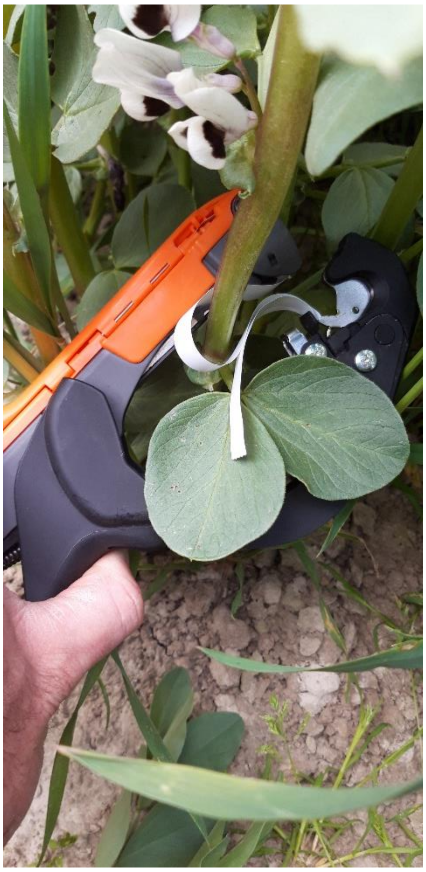

Figure 2: Using a "Maxtang" to label individual plants stems.

Plants were left in the field, and harvested well past the end of the flowering period, but when the plants were still green, leaving enough time for the youngest beans to grow into the stage where we could distinguish well-formed pods from less well formed pods on the one hand, and "big beans in big pods" from less well formed beans in big pods on the other hand. This was done for all pods on each labeled stem. Per stem, the total number of "big pods" was noted, as well as the total number of "big beans".

We chose "big beans in big pods" as a proxy upon a suggestion from our Dutch colleagues in the project. It is a proxy that was found to be the simplest representation of fruit set that still gained as much insight as other, more detailed, proxies. Observations using this proxy could easily be done by farmers when they would like to learn more on fruit set in their fields.

There was no rigid protocol to distinguish “big beans in big pods” from any other form of beans and pods, but the difference is rather clear when processing the stems: “big pods” are entirely green, standing up, and contain at least 1 “big bean”. “Big beans” must both have grown to their full size inside the pod ánd feel hard when squeezed. These observations can generally be made without opening the pods, by squeezing every potential bean in the pod to find out whether it is there ore not, and to find out if it is hard or not. Pods higher up the stem become smaller and smaller, but can still be “big pods”.
Figure 4 shows a pod with 4 fully grown beans (“big beans”) in a “big pod”.

Figure 4: 4 "big beans" in a "big pod".

On each labeled stem, the total number of flowers was counted as well.
This year we did this in the field during the flowering season, since we were making observations on the proportion of flowers with holes in their corolla tubes from bees that were robbing nectar. But counting flowers can also easily be done at harvest time as well: as long as the whole plants are still green, the scars from wilted flowers that did not turn into pods can still be counted (Figure 5).

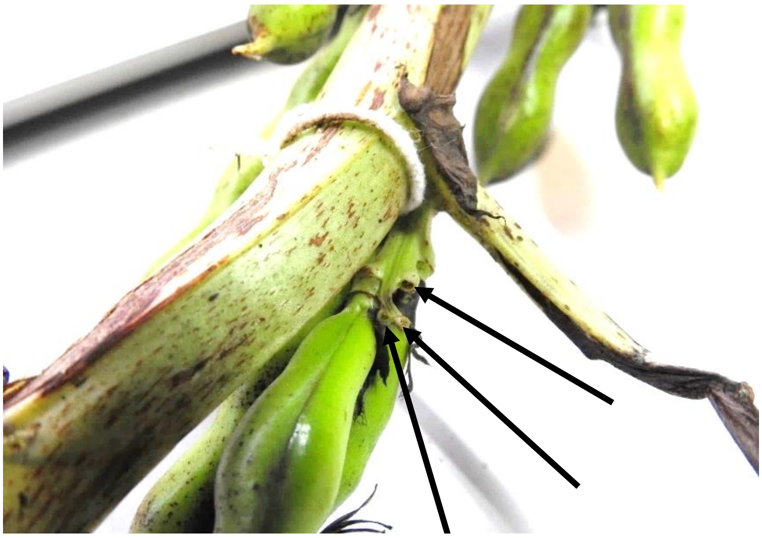

Figure 5: Scars where flowers used to be can easily be counted upon harvest (when harvesting the plants green). In this example, the 3 arrows indicate 3 scars at the top of the flowering stem, but the scars can be anywhere on the flowering stem. When counting the number of flowers: add the number of scars to the number of pods.

The absolute bean production at different locations in one and the same field can effectively be compared one to another, as the variety is the same and the soil and growing conditions are assumed to be the same across the field.
When comparing different fields however, it is necessary to use a measure that is based on proportions rather than on absolute values, because many other elements than pollination alone can influence the absolute bean production of a field (sowing date, soil conditions, crop variety, …).
That, in combination with our search to find a protocol that can be used to investigate yield deficits, leads us to the use of a measure that is based on percentual differences rather than proportional differences in absolute number of beans.

The yield deficit is based on the proportional average difference between the best performing plant stem at a certain sampling location and the other plant stems from that same sampling location (in this study, we had 20 plants stems in total on each sampling location, in 3 parallels on each distance-treatment).
This average difference then is expressed as a percentual difference from the best performing plant of that sampling location.
Finally, these percentual differences are then averaged per distance-treatment.

1.4 OBSERVATIONS CORRELATING FRUIT SET TO A PROXY OF BEE-ACTIVITY

On each plant stem, in each week during the flowering period, the proportion of flowers with holes in their corolla, to the total number of flowers of the truss was observed on the flower trusses with fully opened flowers.
To keep track of where we left of, we again used the "Maxtang" to label the highest (and thus youngest) flower truss that was scored during each survey (Figure 6).

Previous studies in this area showed that *Bombus terrestris* is by far the most abundant pollinator species, together with honey bees. *B. terrestris* bites holes in the corolla tubes of field bean flowers to be able to access the nectar of the flowers, because their tongues are to short to access it during a regular flower visit. The species does however also perform regular flower visits, presumably collecting pollen, and in doing so effectively acts as a pollinator. The same is true for honeybees and other bumblebee species, although only *B. terrestris* was observed making the holes, whereas honeybees only used holes that had already been made before. Figure 7 shows such a honeybee in action.

The hypothesis that was posed by our Dutch colleagues was that in landscapes where *B. terrestris* is among the most important pollinator species in number of individuals, with very few individuals from species with much longer tongues present in the field, (like the landscape where this survey was performed) the presence of holes might give some indication on pollinator activity, and so the proportion of flowers with holes could potentially be used as a proxy for pollinator activity. And thus it might be used as a proxy for pollination in simple landscapes.

Figure 6: Labels on the stem showed the last flower truss that had been inspected the week before.

Figure 7: A honeybee that is going to rob nectar through a hole that was made in the corolla tube by a bumblebee (*B. terrestris* complex).

3. Results and discussion

1.1 POLLINATORS

In general, very few pollinators were encountered during the monitoring events, and even less were observed visiting flowers in one way or another (Figure 8). On 2 of the 4 dates, no pollinators were found at all (Table 1).

Furthermore: whilst being present in the field for the other work in this study (we were around the whole day), very little pollinators were observed as well. On 1 occasion, 1 specimen of *Bombus hortorum* was observed visiting flowers.
This is worth noting because the tongue length of this species is sufficient to reach the nectar in field bean flowers during a regular flower visit. This species thus is considered to be a more efficient pollinator (per capita) than all of the other species that were observed. But since it was around in such low numbers, the impact can be on neglected in the study where we try to find correlations between the presence of holes in corolla tubes, and pollination of flowers

Table 1: Average number of bees encountered per sampling location (N=3) within a 1m radius.

Date	Distance	B. terrestris-group	B. lapidarius	B. pascuorum	Honeybee	Unknown	B. hypnorum
25/apr	10						
	40						
	80						
	120						
	150						
6/mei	10	0,13			0,19	0,38	
	40	0,06			0,06	0,31	
	80	0,13				0,44	
	120	0,06			0,13	0,50	
	150	0,13			0,06	0,63	
13/mei	10	0,06		0,06	0,06	0,19	
	40	0,13		0,06	0,13	0,06	
	80	0,13	0,06	0,13	0,06	0,13	0,06
	120	0,06	0,06	0,06	0,06	0,13	
	150	0,13			0,06	0,25	
19/mei	10						
	40						
	80						
	120						
	150						

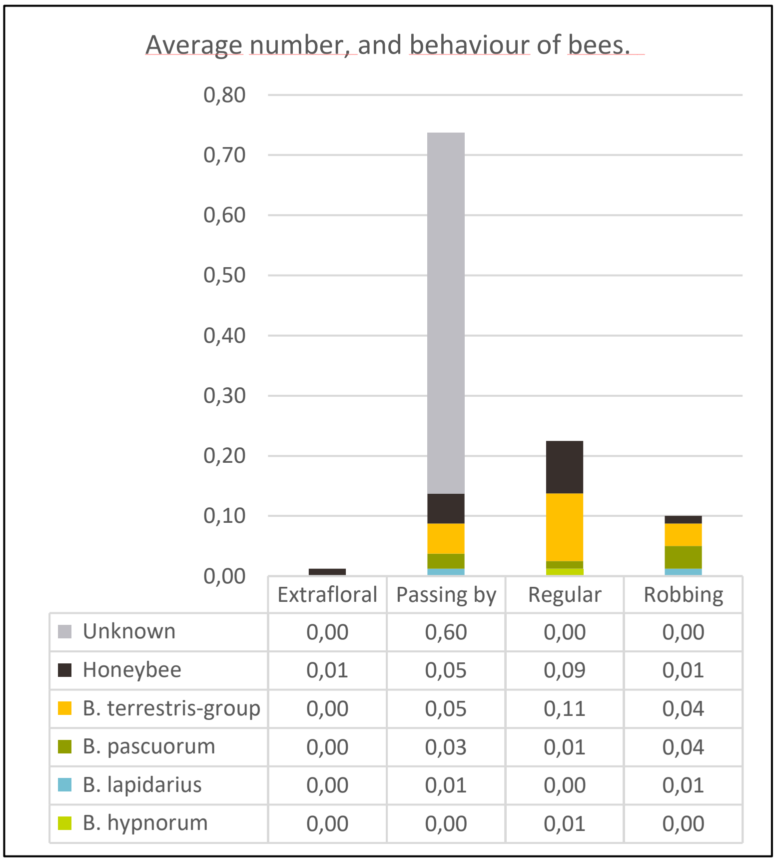

Figure 8: Average number and behaviour of bees. Averaged across all sampling dates and all sampling locations in the field (N=60).

1.2 FRUIT SET

Figure 9shows that plants that are deprived of visits from pollinators on average produce more flower clusters. The number of flowers that was produced per truss doesn't differ (Figure 10).
This could either be a compensation behavior where the plant tries to still be able to produce more pods and thus continuous flowering longer, or it could be induced by another stress factor, or a combination of stress factors.

This confirms the idea that it is best to investigate whole plants, rather than only a couple of flower trusses on an arbitrarily chosen location on the plant stems. When only inspecting a couple of flower trusses, the plant could not be the right condition to produce pods at that time, but it still might be able to keep up with the average total pod production per plant stem, at the end of the season.

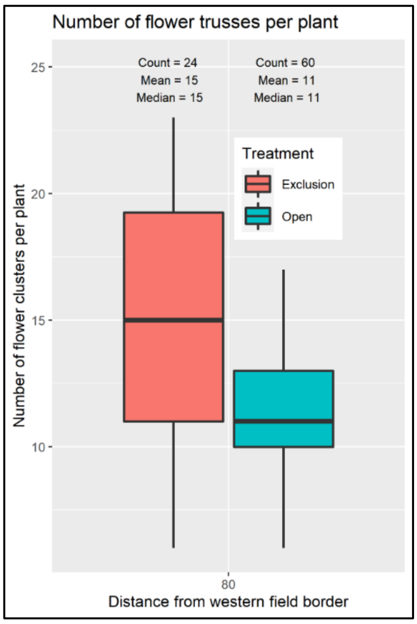

Figure 9: Number of flower trusses per plant, in the exclusion and the open field treatment.

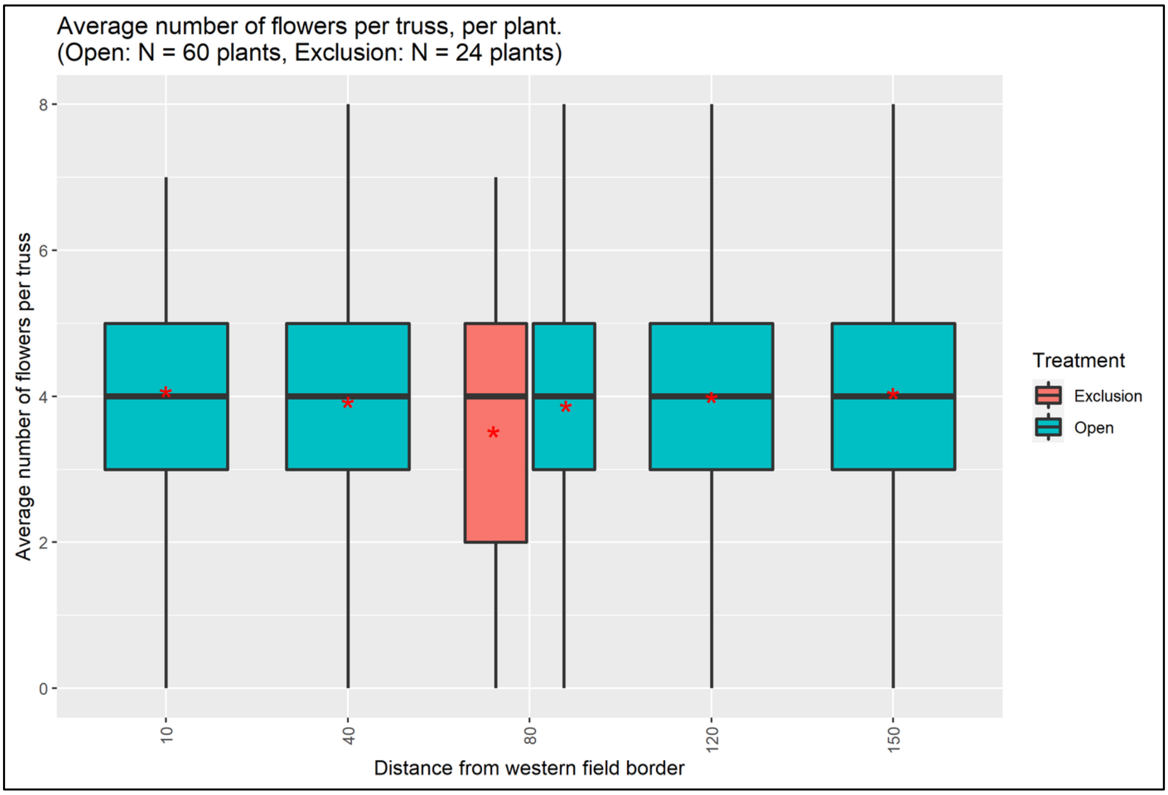

Figure 10: Average number fo flowers per truss, per plant.

Compared to open field conditions, the “big bean in big pod” production in exclusion cages is much lower, and the variability is much higher (Figure 11), emphasizing the importance of insect pollination. The results from the exclusion cages also demonstrate that field beans effectively can produce some beans, even when they are not pollinated by insects. A previous study showed that the ability to produce fruits in absence of insect pollinators is dependent on the crop variety (Thomas Van Loo, 2022).
That study however should be revisited, because observations were based on a very limited number of flower trusses per plant, instead of flower trusses from whole plants.
Bean production in the open field on the other hand is equal (both the average as well as the variability) at any of the sampled distances from the field border.

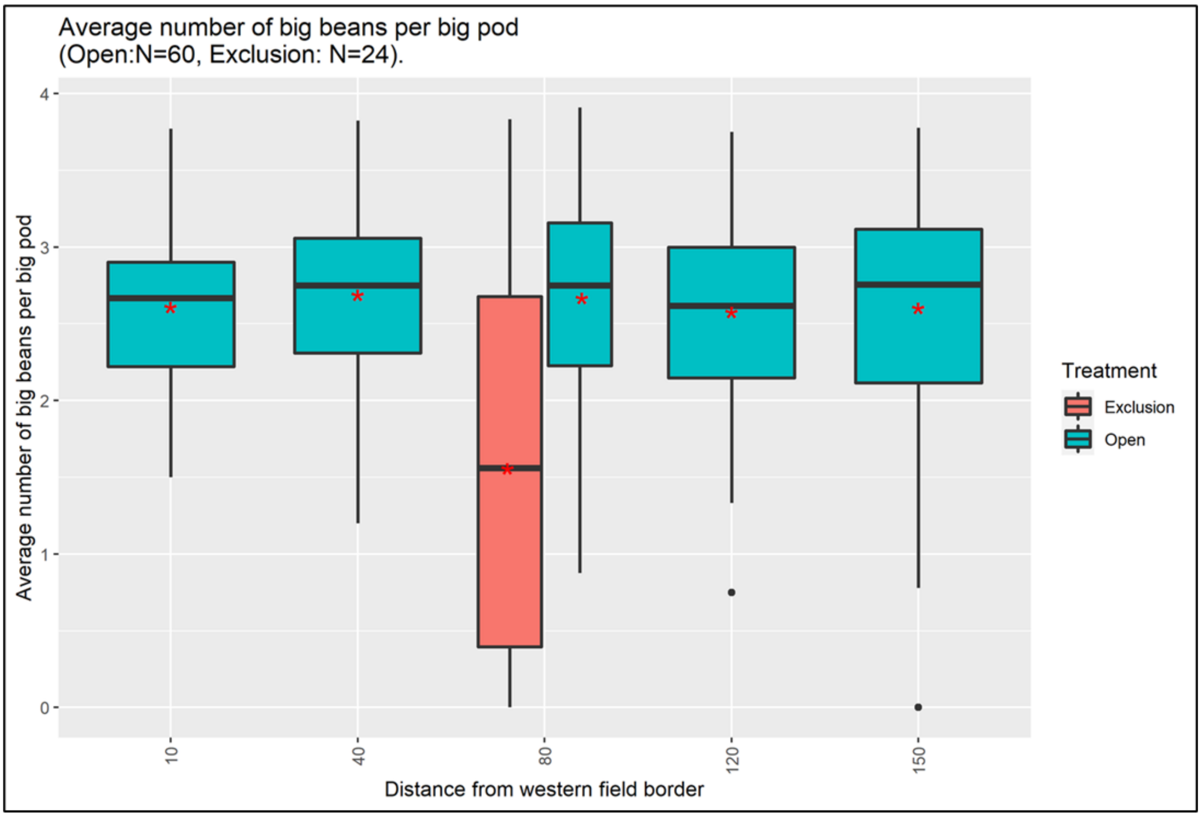

Figure 11: Average number of big beans per big pod, at each sampling distance in the field.

Finally, when calculating the yield deficit, it is clear that the yield deficit in the exclusion cages is significantly higher than in the open field (Figure 12). As in the previous graph, the variability within the exclusion-group is much higher as well compared to any of the other treatment-groups.

In the exclusion cages, the yield deficit is about 50%, whereas the yield deficit in the open field is about 25%, with very little variation within or between the open field groups. The presence of pollinators thus seems to act as a buffer against variability as well.

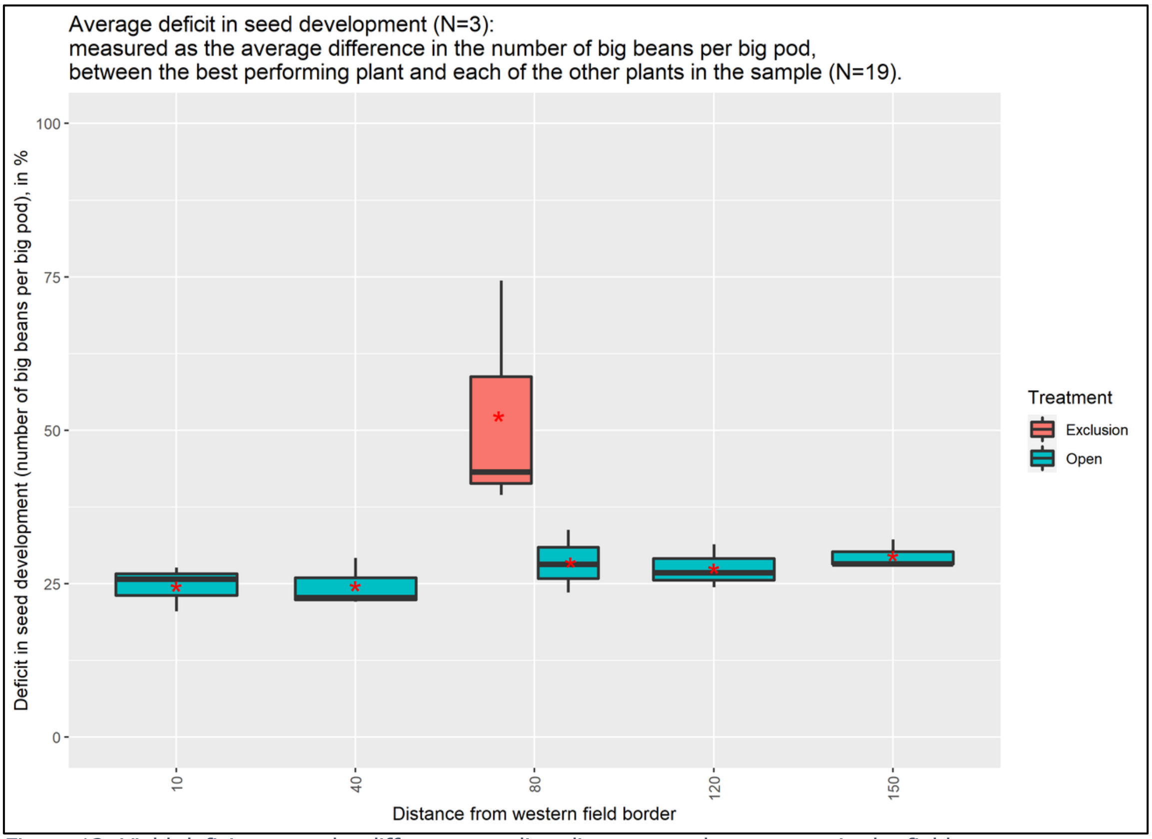

Figure 12: Yield deficit across the different sampling distances and treatments in the field. Expressed as the average difference in the number of big beans per big pod, between the best performing plant, and each of the other plants on each sampling location.

We assume that the percentual yield deficit would be smaller in fields with more pollinator visits, and/or with pollinator species that have longer tongues.

Because of the way the yield deficit is calculated, the yield deficit is never going to be "0" (it will never be the case that all the plant stems perform exactly as well as one another). So, the question remains what the minimal yield deficit, or what a 'normal' yield deficit would be.

When investigating more and more fields, it would be possible to come up with a benchmarking system to be able to compare the yield deficit of any newly investigated field against.
Farmers would then be able to see where the yield deficit of their own field is situated on this benchmark-range.

1.3 CORRELATION BETWEEN POLLINATION AND FLOWERS WITH HOLES

We could not find a correlation between the proportion of flowers with holes in their corolla tubes and bean production (Figure 13).

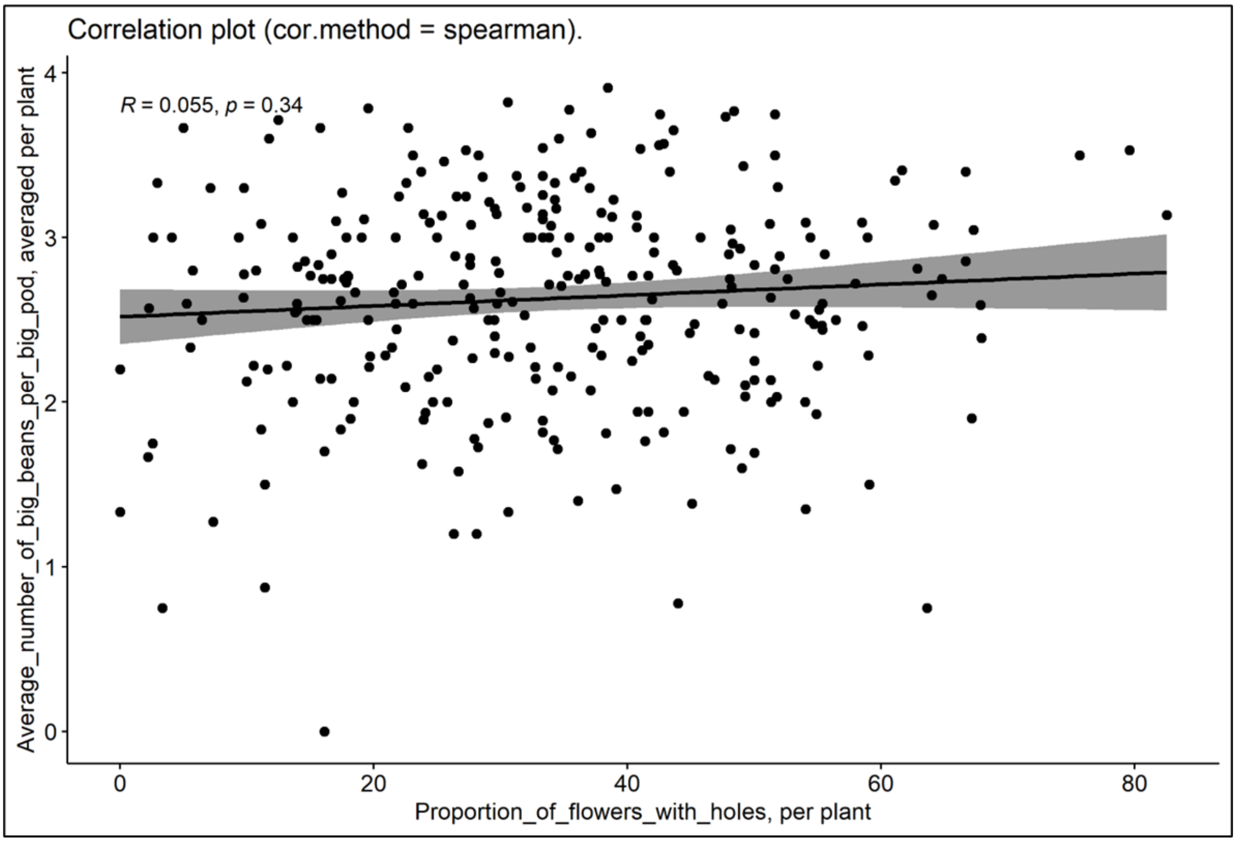

Figure 13: Correlation between the proportion of flowers with holes in their corola tubes (as aproxy of pollinator activity), and seed development in bean pods per plant.

4. Conclusions

- Very few pollinators were observed in the field during this study
- We were able to come up with an easy, not time consuming, farmer-friendly protocol to investigate yield deficits in open fields of field beans.
  However, for the results of the protocol to be meaningful, it would be necessary to be able to compare the outcome of individual fields against a benchmark.
- Therefore, for the future: it is necessary to collect data from multiple fields so that the dataset on which the benchmarking system would be based can be populated.
- Varietal trials on levels of autogamy should be revisited, looking into the performance of whole plants in stead of just a couple of flower trusses per plant.
- We could not find a correlation between the proportion of flowers with holes in their corolla tubes and bean production.

5. References

Thomas Van Loo. (2022). *Winter field beans: Assessing autogamy in 7 varieties.*