Tracking movement of released insects

From within the crop, into a flower strip, and back towards the crop (strawberry)

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1. Content

Figure 1: overview of the experimental setup during this study.

2. Preface and objective

Recent evolutions in restrictions on phytosanitary products, and pest resistance against conventional ways of pest control are pushing growers to look for alternative solutions.
A possible way forward might be to rely on services that are provided by the ecosystem surrounding the fields (natural pest control), under the assumption that the landscape is not eroded to such a degree that the ecosystem can no longer function properly.
Man made provisions such as flower strips and mixed hedges might compensate the lack of natural resources.

The installation of flower strips on or near agricultural fields is being advised to meet several purposes: 1) supporting the pollinator community, 2) supporting the insect community of natural enemies that keep the community of pest insects in check, 3) supporting biodiversity in the more general sense, and 4) supporting the reputation of the sector.

Beneficial insects need extra resources, especially at times when the crop itself provides too little flowers or prey/hosts. Additional resources will be needed even during the flowering season to meet the wider nutritional requirements of the different life stages of both pollinators and natural enemies. Floral margins might be able to deliver these resources.

Floral resources (either nectar, pollen, prey or hosts) extend the life span of natural enemies, increase the number of eggs that can be produced, and improve the dispersal capacities affecting their pest control potential to a large extent.

Despite the advantages that are provided by floral margins, uptake has been low.

One of the main reasons growers give for not installing floral margins is that the flower strip might also act as a source of pest insects.

This case study builds upon a previous, similar, study in 2021 and enabled us to gain experience in using a promising method of tracking insect movement and insect resource usage.

In the 2021 study, we focussed on movement of winged aphids and some of their freely occurring natural enemies (aphid parasitoids and hoverfly species with aphidophagous larvae) between a flower strip and the adjacent strawberry field.
In the 2022 study however, we are looking into the added value of a flower margin for pest control strategies that are using commercially reared natural enemies in the crop.

Commercially reared natural enemies are still a rather expensive way of controlling pests. If it can be shown that the flower strip supports these released individuals, it might help in lowering the number or the frequency with which these natural enemies have to be released, resulting in a lower cost for the farmer.

3. Materials and methods

3.1. USING RUBIDIUM TO TRACK INSECT MOVEMENT

Rubidium is an element that generally occurs in living organisms, but in very low concentrations and can partly replace potassium in plant and insect tissue.

When applied on plants, the element gets built in into the plant tissues and even is redistributed into the nectar and pollen, raising the concentration in the plant above that of the natural background concentration of Rubidium in untreated plants. Insects that then feed on the plant (either nectar or pollen, plant tissue or plant juices) ingest these sources with a raised concentration of Rubidium, and build the element into their own tissues, raising the concentration of the element above the background concentration in their bodies too.

Rubidium is not toxic when present in elevated concentrations, doesn't affect individual behaviour of insects, and insects are internally marked through feeding. Thus, it is an ideal marker to investigate the feeding behaviour and movement of insects from one place to another.

In this study Rubidium was applied to a flower strip as a 5000ppm RbCl solution that was poured over the plants at about 1,5L/m² using a watering can with an adapted nozzle (see fig. 2).

Individuals are assigned the status of "Rb-positive", and thus fed on plants in the flower strip when the concentration of the element in their tissue exceeds the threshold level that is determined by the average background concentration in the insect functional group (either "winged aphid Reff", "Parasitoid Reff", "Ephedrus cerasicola Reff", or "Aphidius Reff") + 3 times the standard deviation of that group average. This makes that there is only a 1% chance of wrongly assigning an individual the status "Rb-positive".

Using this method we get a clear answer on the question "did the analysed specimen (captured either at any of the 3 distances into the crop, or in the flower strip) feed on resources in the flower strip?".

3.2. THE SETUP IN THE FIELD

The flower strip (mixture: "IPM Bloemen en nuttige insecten", by Jorion-Philip-seeds) was sown on September 13th 2021 at the premises of Inagro, perpendicular to the head of the cropping rows of a strawberry field (strawberry plants on raised gutters. See fig. 1 on the page with table of content for a view on the general setup).
The crop was installed in the gutters on April 11th 2022 (everbearer variety: "Verity",)

No insecticides were allowed from the start of the season onwards, and no commercial natural enemies were released to control aphids (although commercially available natural enemies against trips and spider mites were released at the following dates: 20/05: *Orius laevigatus* (against trips); 24/05: *Amblyseius swirskii* (against trips); 24/05: *O. laevigatus* (against trips); 31/05: *O. laevigatus* (against trips); 28/06: *A. swirskii* (against trips) + *Phytoseiulus persimilis* (against spider mites).

Insects moving around were collected using transparent sticky plates that were set up both in the flower strip and at 3 fixed distances from the field border into the crop (5m, 10m, 15m), in 6 parallels (see fig. 2). Coloured sticky plates are not desirable as the colour would act as an attractant, biassing the natural movement patterns of insects in the field.

Each trapping location comprised 2 sticky surfaces (each surface 17cm wide by 20cm high): 1 surface facing the flower strip, and 1 facing away from the flower strip (see fig. 2 for a view on the trap).

The reference insect community was sampled continuously using these sticky traps, during 2 weeks (07/06/2022 – 21/06/2022).

The flower strip was treated with RbCl at 2 times throughout the cropping season: June 21st and July 1st 2022.

Target insect functional groups were winged aphids and different groups of aphid parasitoid wasps, and were sampled during 3 subsequent weeks (23/06/2022 – 15/07/2022), using transparent sticky traps.

Commercially available aphid parasitoid wasps (supplier : Viridaxis) were released at 1m into the crop on june 21st 2022.
Released species were *Aphidius ervi*, *Aphelinus abdominalis* and *Ephedrus cerasicola*.
A. ervi is a known effective parasitoid of *Macrosiuphum euphorbiae* (this is by far the dominant aphid species at the trial location), *A. abdominalis* has a completely different dispersal strategy then *A. ervi* which would be interesting to follow in the trial, and reared *E. cerasicola* has a distinct antennal pattern which makes it possible to differentiate them from wild individuals. When identifying a "Rb-positive" *E. cerasicola* specimen in the field, we would be sure that it originated from the released population (which would always remain uncertain for *A. ervi* and *A. abdominalis* since these are species which are free living in the environment as well).
A few hundred specimen of each species were released in each of the 8 parallels, using cardboard tubes (see fig 5).

At the 7th of July, the flower strip was mown (the mown vegetation stayed in the flower strip) to find out if this could be a method to push parasitoids out of the flower strip, into the crop.

Sampled specimen were individually stored dry in Eppendorf tubes at -20°C until the end of the season when they were processed in the lab. This method of collection and storage was validated beforehand and had no effect on the results from the lab analysis.

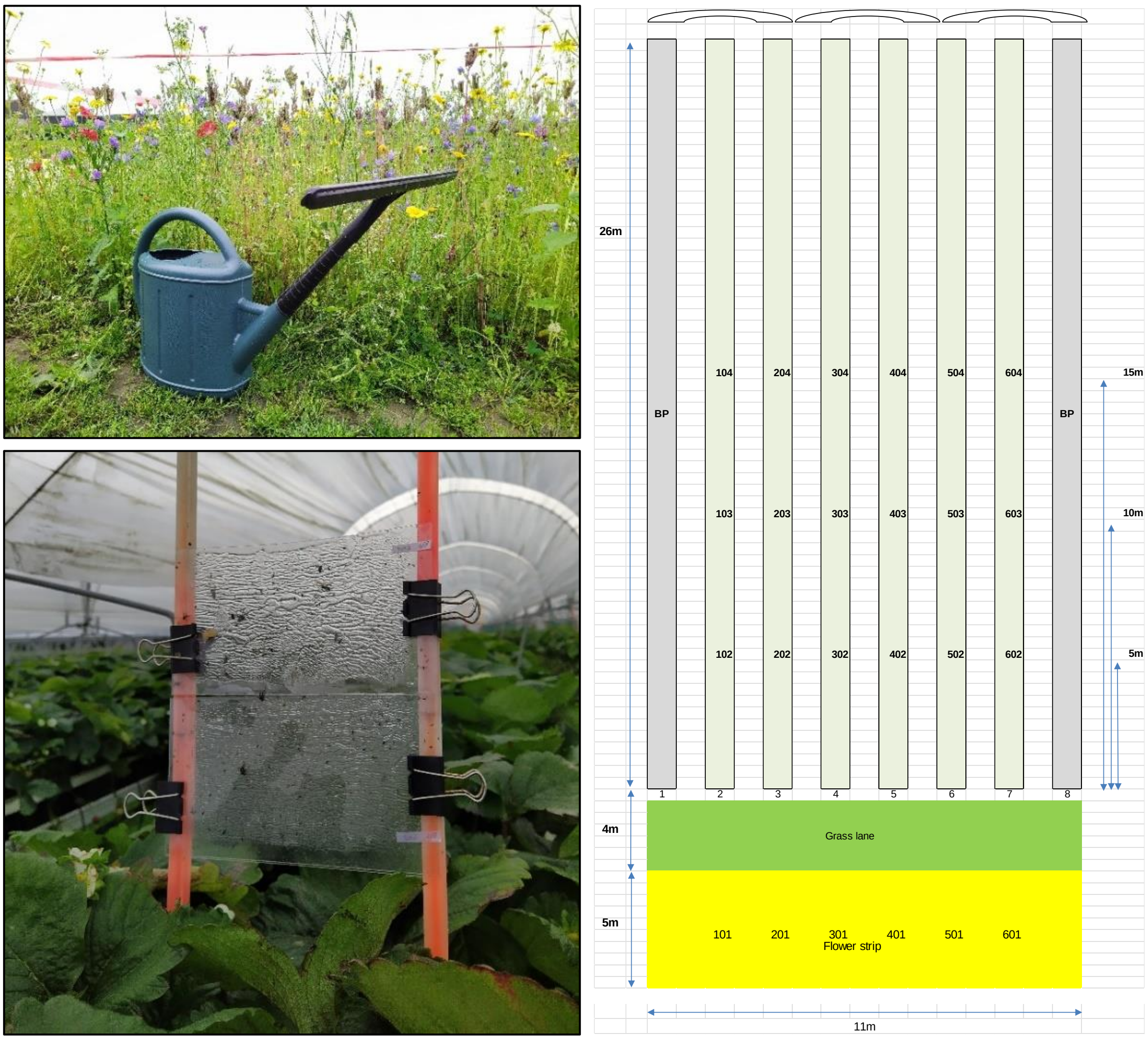


Figure 2 (clockwise):
nozzle used to evenly distribute the RbCl-solution over the flower strip (picture from the study in 2021 so the flower strip is not representative); scheme of the experimental setup; setup of a sticky trap in the crop

3.3. LAB ANALYSIS

Rubidium (Rb) was analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, see fig. 3). A technique capable of measuring at ppb (parts per billion) level. Besides the low detection limit, the analysis speed and a large linear dynamic range are important advantages.

Figure 3: ICP-MS used to analyse the Rb-content in the individual insects.

Through destruction in acids a liquid containing the analyte was prepared. That liquid is first pumped in an ICP-MS through a plasma (appr. 6000 K). That atomizes and ionizes the elements, so they can be steered into the mass spectrometer, where they can be separated due to their different mass to charge (m/z) ratio.

Normal destruction requires approximately 0,5 grams of sample. As the insects are a lot lower in mass, and a combination of insects to produce analysis results were no option, the destruction method was down-sized in volume of destruction fluids (5/1) and equipment. The analysis on the ICP-MS was unchanged.

4. Results and discussion

4.1. DEVELOPMENT OF THE FLOWER STRIP

During the winter, after the flower mixture was sown, heavy rainfall compacted the topsoil heavily, and the subsequent exceptional drought during spring and summer impacted the development of the flower strip further. It thus is safe to say that the environmental conditions had a substantial impact on the flower strip.

The flower strip in 2022 looked very different from the other flower strip from 2021 (2021 was a rather wet year). Many species that were dominant in the flower strip in 2021 were almost or completely absent in the new flower strip in 2022. Unfortunately, we did not perform a vegetation survey, as we did in 2021, making it hard to communicate findings. Generally speaking, where the flower strip in 2021 was divers, with subsequent flowering of different species, the flower strip in 2022 didn't grow very well, with few species really performing as expected. E.g. *Lobularia maritima* was nearly absent in 2022, whereas it was present in large quantities in 2021. Other species that were present in 2022 remained very small and occurred only sporadically. *Ammi majus* however was dominant in 2022. See fig 1 for an overview of the flower strip, at the time when the flower strip was treated with RbCl for the first time. The density of plants per m² however was probably about the same in 2021 as in 2022. In 2021, it was probably just more hidden in the overall vegetation, and with it's deep-rooting root it endured the drought in 2022 better than all of the other species.

4.2. BACKGROUND CONCENTRATION OF RUBIDIUM IN INSECTS

Table 1 shows the background concentration of Rubidium in each focal group, the standard deviation, the number of specimen sampled for the group average, and the obtained cut-off value to differentiate "Rb-positive" (elevated concentration) from "Rb-negative" individuals (concentration not significantly different from natural background concentration).

Table 1: average and stdev of the naturally occurring background concentration of Rb in the insect focal groups, the number of sampled individuals that were used to calculate this average value, and the resulting cut-off value used to identify Rb-positive individuals.

Reff group guild	Number of individuals sampled as Reff	Average concentration of Rb (mg/kg insect)	Stdev	Cut-off: Group average + 3 x stdev
Aphidius Reff	48	0,128	0,157	0,598
Ephedrus cerasicola reared Reff	25	0,105	0,115	0,451
Parasitoid Reff	51	0,082	0,118	0,436
Winged aphid Reff	89	0,122	0,185	0,678
Aphelinus abdominalis reared Reff	*25*	*0,109*	*0,144*	*0,541*
Aphidius ervi reared Reff	*24*	*0,196*	*0,185*	*0,749*
Aphidius wild Reff	*23*	*0,054*	*0,078*	*0,287*

Only the first 4 lines in table 1 are maintained as reference groups further on in the study: when sampling individuals in the trial after the release of the commercial parasitoid wasps (these have "_ reared _" in their names in table 1) *E. cerasicola* is the only species which can be identified as surely originating from the released population. The other 2 reared species can be found in the wild in the proximity of the crop as well and therefore cannot be used as separate populations regarding the background concentration of Rubidium.
Furthermore, we couldn't always find enough individuals for all wild reference groups in 2022, and therefore (together with the previous argument) "wild" and "reared" are pooled together into a reference population.

See table 2 for the composition of each of the 4 remaining reference groups.
"Aphidius wild Reff" is also included in the group "Parasioid Reff" since the "Parasitoid Reff" group would otherwise exist nearly exclusively out of reared individuals (see table 2), and from the difference in background concentration in "Aphidius wild Reff" VS "Aphidius reared Reff", we know that values from a wild population can differ quite a lot. See table 1.

The large proportion of reared individuals in all of the defined reference groups might have resulted in an overall higher background concentration in these reference groups (e.g. compare the background concentration of Rb in wild VS reared *Aphidius ervi*). This might have resulted in higher overall cut-off levels, and thus making it harder to identify "Rb-positive" individuals in the trial.
In the 2021 study, the cut-off for the -all wild- parastoid group was 0.03186 VS 0.436 in 2022.

Table 2: Composition of the reference groups that are used to define cut-off values which are used to differentiate Rb-positive from Rb-negative individuals in the trial.

Reff group guild	Number of individuals in the reference population	Which species or groups make up the reference population? (wild or reared)
Aphidius Reff	47	24 A. ervi reared 23 Aphidius wild
Ephedrus cerasicola Reff	25	25 E. cerasicola reared
Parasitoid Reff	51	23 Aphidius wild 2 Binodoxys angelicae 1 Aphidiinae 25 Aphelinus abdominalis reared
Winged aphid Reff	89	89 Winged aphid

4.3. THE FLOWER STRIP

We assume that the condition of the flower strip, in combination with the summer heat and draught impacted the observations from this study. The composition of the vegetation in the flower strip was clearly different from the first flower strip back in 2021, with nearly all species clearly suffering.
The process of Rubidium uptake into the plants tissue and flowers occurs when the plant grows and in doing so incorporates the element into its tissue. As plant growth was restricted during the survey, this might have impacted the outcome of this study. Furthermore, the lack of water in the soil might have impacted the production of floral resources in the flowers that where present, reducing the potential of building up Rb concentrations in parasitoid wasps that visit these flowers.

4.4. PARASITOID WASP AND WINGED APHID MOVEMENT DURING THE TRIAL

Table 3 shows the number of the different species or groups which were sampled from the sticky traps, in the 3 weeks of continuously sampling insect activity using transparent sticky traps, and respectively shows which reference group was used to compare the measured Rb-content to.

Table 3: Number of encountered specimen and the reference groups to which their Rb-content was compared.

Sampled groups after Rb treatment	Number of sampled specimen	Reff group for cut-off
Aphidius ervi	11	Aphidius Reff
Ephedrus cerasicola	16	Ephedrus cerasicola Reared Reff
Aphelinus	4	Parasitoid Reff
aphelinus ?	4	Parasitoid Reff
Binodoxys angelicae	1	Parasitoid Reff
Praon volucre	3	Parasitoid Reff
Trioxys auctus	1	Parasitoid Reff
Winged aphid	328	Winged aphid Reff

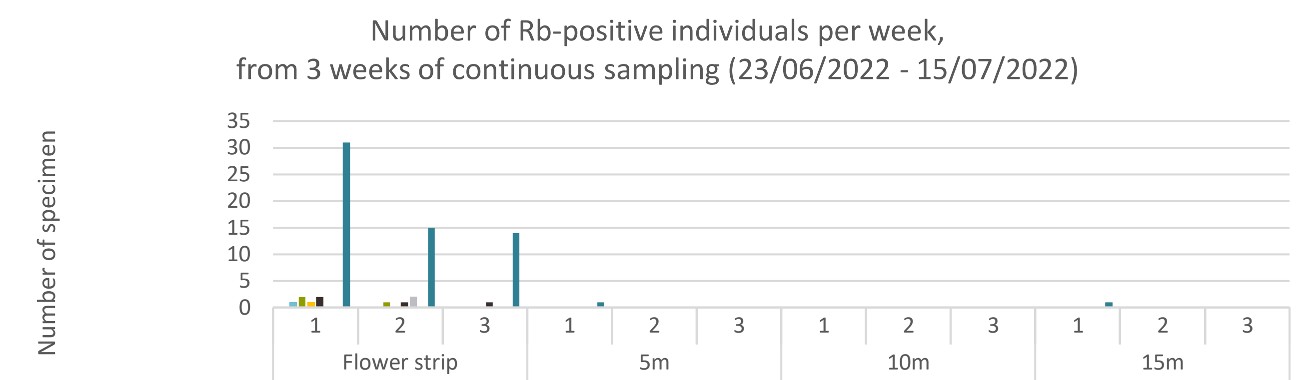
Figure 4 and table 4 show a very low level of movement between the flower strip and the crop, even at the very edges of the cropping field. Most Rb-positive individuals are found within the flower strip.

From separate observations at the level of individual plants in the field (not shown), we know that aphids were present in the crop in low to moderate levels, at 10m and 20m in the crop, whereas overall activity of parasitoid wasps (observed as the presence of mummies) was absent at both distances. This reassures that there was effectively not much activity from parasitoids in the crop (regardless the observations concerning migration from the flower strip into the field).

The observations also show that *E. cerasicola* (the only species for which we are sure that it originated from the releasing event) moved from the crop into the flower strip. This might well be because of a lack of hosts or nutritional resources in the crop. The species stayed in the flower strip for the rest of the duration of the trial, despite the rather bad conditions in the flower strip. Nectar in flowers of *Ammi majus* however, being a member of the Apiacea family and dominantly present in the flower strip is probably easily accessible by species without long tongues such as parasitoid wasps, making the plant suitable for parasitoid wasps such as *E. cerasicola* to feed on the nectaries.

In the study from 2021 however, using the same technique with Rb, we could show that wild parasitoid wasps effectively migrate from the flower strip into and across the strawberry field.

In future studies, it might be an idea to also install additional flowering structures within the cropping field (e.g. hanging under the raised gutters). Parasitoid wasps that have ended up in those small islands of flowers in times when they needed alternative resources might disperse more easily back into the crop instead of into the wider environment from a flower strip which is outside of the cropped area.



	Flower strip 1	Flower strip 2	Flower strip 3	5m 1	5m 2	5m 3	10m 1	10m 2	10m 3	15m 1	15m 2	15m 3
Aphelinus	0		0				0				0	
aphelinus ?	1			0			0					
Aphidius ervi	2	1		0	0		0	0				
Binodoxys angelicae	1											
Ephedrus cerasicola	2	1	1	0			0	0				
Praon volucre		2										
Trioxys auctus										0		
Winged aphid	31	15	14	1	0	0	0	0	0	1	0	0

Figure 4: Number of Rb-positive individuals that were collected using 6 transparent sticky traps at each distance in the field, during 3 subsequent weeks.
Before the start of the 3rd week, the flower strip was mown leaving the clippings in place. The number only show the actual "Rb-positive" individuals, and not the total number of individuals that was sampled. A "0" indicates that individuals were found, but none were "Rb-positive". Table 4 shows the total number of individuals that were found on the sticky plates, and how many of those were "Rb-positive"

Table 4: Number of Rb-positive specimen VS the total number of sampled specimen per focal species or group, at each distance, during the 3 week period of continuous sampling, using sticky traps (23/06/2022 - 15/07/2022).

Sampled species or group	Flower strip		5m		10m		15m	
	Rb-positive	Sampled total	Rb-positive	Sampled total	Rb-positive	Sampled total	Rb-positive	Sampled total
Aphelinus	0	2			0	1	0	1
aphelinus ?	1	1	0	2	0	1		
Aphidius ervi	3	6	0	3	0	2		
Binodoxys angelicae	1	1						
Ephedrus cerasicola	4	6	0	2	0	8		
Praon volucre	2	3						
Trioxys auctus							0	1
Winged aphid	60	150	1	28	0	30	1	31

In the 2022 trial, only 40% of encountered winged aphids in the flower strip were marked as "Rb-positive". In the 2021 trial however, this was nearly 70%. Here again, the suboptimal growing conditions for most of the plants in the flower strip in 2022 might have had an impact on the transfer of Rb into insect body-tissues, and thus on the overall findings of this study.

As in the 2021 trial, we find very little movement of winged aphids from the flower strip into the crop. Only 2 out of 89 winged aphids, during 3 weeks of continuous sampling can be identified as having used resources in the flower strip. On the other hand, we found 60 "Rb-positive" individuals in the flower strip. So, in spite of the potential implications from the weather conditions on the flower strip, it looks like there indeed was little movement of winged aphids from the flower strip into the crop during these 3 weeks of sampling. This means that the origin of most of the winged aphids that colonise the crop can be found outside the flower strip, as was shown in the study in 2021.

4.5. THE PUSH-EFFECT FROM MOWING THE FLOWER STRIP

Between week 2 and week 3 of sampling, the flower strip was mown, leaving the clippings on the flower strip.

Figure 4 shows no Rb-positive individuals in week 3. Mowing thus had no effect on insect dispersal into the crop. This however probably is impacted by the conditions in the flower strip: at the time of mowing, the flower strip had already almost completely dried out.

5. Conclusions

- Commercial parasitoid wasps that are released in the crop effectively use an adjacent flower strip as a food source.
- Migration of winged aphids into the crop is marginal, with the vast majority of aphids ariving in the crop not being linked to the flower strip.
- Mowing the flower strip had no effect on insect movement towards the crop.
- Weather conditions (severe draught and heat) severely impacted the growing conditions for the flower strip.
- Further research -under better weather conditions- on the movement of beneficial insects that are released in the crop, move towards the flower strip, and back towards the crop is needed.

6. Acknowledgements

Special thanks goes to Thierry Hance (professor at the Earth & Life institute, at UCLouvain, and lead project coordinator of the Interreg-project "Proverbio") for pointing out an alternative processing technique which enabled us to process individual specimen in the Rubidium-analysis, instead of having to pool individuals in order to obtain a certain minimal mass per sample; to Viridaxis for the reared commercial parasitoid wasps; to the lab team at Inagro for their dedication and everlasting enthusiasm; to the strawberry team at Inagro to make it possible to perform this study and especially to Ruben Mistiaen for support on the actual observations in this study.

7. Report from the trial in 2021, on wild insects

https://inagro.be/sites/default/files/media/files/2022-04/Report%20tracking%20insect%20movement%20using%20Rubidium_Provebio%202021.pdf

Figure 5: Commercially bred parasitoid wasps emerging from the cardboard tubes.