A wireless, user-friendly, and unattended robotic flower system to assess pollinator foraging behaviour

Kamiel Debeuckelaere[1,2], Dirk Janssens[3], Estefanía Serral Asensio[4], Hans Jacquemyn[1], María I. Pozo[1*]

[1] Plant Conservation and Population Biology, KU Leuven, Kasteelpark Arenberg 31 - box 2435, 3001 Leuven, Belgium

[2] Laboratory of Socioecology and Social Evolution, KU Leuven, Naamsestraat 59 - box 2466, 3000 Leuven, Belgium

[3] ICTS - Central Infrastructure, KU Leuven, Willem de Croylaan 52a - box 5580, 3001 Leuven, Belgium

[4] Research Centre for Information Systems Engineering (LIRIS), KU Leuven, Warmoesberg 26 - box 15101, 1000 Brussels, Belgium

Running headline: "Robotic flowers for behavioural experiments"

*Correspondence author: kamiel.debeuckelaere@kuleuven.be

Abstract

1. Pollinator foraging is a complex behaviour that has major importance for gene dispersal in plant populations. The precise behaviour of foraging insects is subject to both external (light and temperature, floral reward characteristics, flower colour and shape, flower density…) and internal factors such as recognition capabilities, age and foraging experience or health of insects. How exactly these different factors affect foraging behaviour remains a subject of study, which often requires data collection.
2. In this study, we present a wireless and cost-effective robotic flower that automatically offers nectar to visiting insects while monitoring visitation time and duration. To achieve this, the robotic flower is equipped with Internet of Things (IoT) technology, making it possible to get real-time data without interfering or even getting close to the experimental system. This makes more remote experiments without internet and power grid connection possible. Compared to previous robotic systems, the setup can easily be changed (also during a trial). Moreover, the nectar refilling rate can be altered remotely as well. Being completely wireless and autonomous, this system is mobile and easy to fix or clean.
3. With the settings used in the prototype, one battery charge can cover an experimental period of approximately two weeks. Discrete amounts of nectar (volume can be altered by changing the 3D-design of the nectar-cup) are offered to visitors. Data for more than 1500 visits per day per flower can be registered and are presented to the user in a CSV-file.
4. The robotic flower solves some issues commonly associated with manual censuses and observations while it provides accurate and reliable data on insect behaviour at the same time. Although the system was designed for bees, it could be extended to other insects commonly visiting flowers, such as flies, with minor changes to the design. The robotic flower is user friendly and can be easily adapted to address a wide range of research questions related to pollinator foraging behaviour, including the effect of nectar traits, flower colour and shape, and pollutants on foraging behaviour.

Keywords

Arduino, automatization, bees, behavioural experiments, data collection, electronics, floral rewards, foraging behaviour, infrared sensor, Internet of Things, IoT, LoRaWAN, nectar, microcontroller, pollinators, robotic flower, wireless system.

Introduction

"*If insects were to vanish, the environment would collapse into chaos.*" - E.O. Wilson

Pollinators are essential to ensure the sexual reproduction of ~90% of flowering plants (Ollerton et al., 2011), including important agricultural species (Klein et al., 2007). Although the group of species that are considered pollinators consist of both vertebrates and invertebrates, insects and especially bees are the primary pollinators (Potts et al., 2010). The transfer of pollen from female to male plant organs is mediated by complex interactions between plants and insects and largely depends on the foraging behaviour of individual insects, which in turn depends on both internal and external factors. While flower handling abilities, foraging experience, reward recognition capabilities and insect (or colony) health are the main internal factors affecting pollinators´ foraging decisions (Lunau, 2000; Lunau et al., 1996; Minahan & Brunet, 2018; Schmid-Hempel & Stauffer, 1998; Spaethe & Weidenmuller, 2002), maximization of energy gain via the collection of floral rewards is also affected by abiotic features such as light and temperature, landscape features, flower density, colour and shape of the flowers, and the quantity and quality of floral rewards (Nicolson, 2011; Spaethe et al., 2001; Sun et al., 2018; Vicens & Bosch, 2000; Waddington & Holden, 1979; Werner et al., 2016). The relative importance of these factors and how they interact with each other is not yet completely understood, especially for non-model organisms (e.g. flies; Hannah et al., 2019).

Behavioural experiments with pollinators have proven to be challenging as they often imply collecting data from fast moving animals and multiple individuals simultaneously (Dankert et al., 2009; Dell et al., 2014). Manual collection of such behavioural data relies on continuous observation and is thus labour intensive, time consuming and subject to human limitations in observing dynamic objects (Crall et al., 2015; Simons & Chabris, 1999). Moreover, invasive human actions or equipment are often needed, which could potentially influence foraging behaviour (Calisi & Bentley, 2009). Cheap, reliable, automated, and easy to use methods can help to enhance the quality and quantity of data on foraging behaviour in pollinators. In the past, researchers often used simplified devices to perform manipulative research, thus being able to address different biological questions (e.g., Goulson & Cory, 1993; Gumbert, 2000; Real, 1981). Although artificial flowers have been proposed as an efficient way to disentangle factors affecting pollinators behaviour, needless to say, artificial flower systems of any kind also come with limitations (Essenberg, 2015). In these devices, mimicking nature is restricted to a specific set of characteristics and a robotic flower field is of course not equal to a natural system, in which countless biotic and abiotic factors are at play. The robotic flowers may not resemble any natural flower, by standardizing factors, they can help researchers to understand fundamental processes (and their relation to each other) that are at play in the complex web that is pollinator behaviour.

Here, we want to make a distinction between the term 'artificial' flowers and 'robotic' flowers. Robotic flowers, which have mechanical features and can function without the requirement of human intervention, are regarded as a subcategory of artificial flowers, covering all experimental systems made to resemble real flowers in a certain property and/or to a certain degree. In the past years various robotic flower systems have been constructed with many

different mechanisms of reward delivery (for an overview, see Essenberg, 2015). Although systems have been made to use in non-nectar rewarding (e.g., Switzer et al., 2019), most robotic flowers work with nectar, which is the main reward plants offer their mutualistic counterparts (Nicolson & Thornburg, 2007). Nectar can be delivered in continuous (e.g., Ings & Chittka, 2008; Makino & Sakai, 2007; Moffatt, 2001; Ohashi et al., 2010; Paldi et al., 2003; Thomson et al., 2012) or discrete doses (e.g., Cnaani et al., 2006; Essenberg, 2015; Greggers & Menzel, 1993; Keasar, 2000; Nachev & Winter, 2012; Sokolowski & Abramson, 2010). The ability to automatically detect a floral visitor was also implemented in some systems, using an infrared detection system (e.g., Greggers & Menzel, 1993; Keasar, 2000; Kuusela & Lämsä, 2016; Nachev & Winter, 2012). For example, Kuusela & Lämsä (2016) developed a low-cost robotic flower system with automatic visit detection that offers nectar in discrete doses using a servomotor. In this way, invasive, costly, and time-consuming manipulations such as manual nectar refilling are avoided, and data collection is more reliable as it is no longer depending on visual observations (Kuusela & Lämsä, 2016). However, none of these robotic flower systems mentioned above can be used wireless. The need to be connected to a central control unit (e.g., a computer) and a power supply with cables makes them not very versatile. Fixed systems are less user-friendly (e.g., cleaning and disassembling damaged parts) and often less widely deployable compared to wireless systems, which can be moved around freely.

In this manuscript we present a new version of the robotic flower that is completely wireless by making use of a battery and low power, long range wireless data transmission within the Internet of Things (IoT) concept. To maximise the possible experimental period with one battery, extra attention was given to the power efficiency of the system. The robotic flower system developed here was specifically designed for bees, but with minor adjustments (e.g., to the size of the feeding hole and/or the nectar cup) smaller or larger floral visitors, such as flies, could be investigated as well.

Description and implementation

The robotic flower automates the remote and real-time measurement of flower visitation rate and time by using IoT technology. The flower is as such connected to the internet, enriched with sensors (infrared detection system) and actuators (servomotor for nectar refilling), and provided with processing and communication capabilities.

System Architecture

Fig. 1 shows an overview of the system architecture. The system consists of a robotic flower that registers the time and duration of every visit and transfers these data to the application which makes them online available for the user. Each robotic flower is built with its own Arduino microcontroller and a transceiver based on wireless LoRa technology for communication with LoRaWAN (Long Range Wide Area Network). LoRaWAN is a low power networking protocol used for IoT applications in which a single gateway can receive data from thousands of devices in a radius of 2 to 5 km in urban areas and up to 15 km in more remote areas (Adelantado et al., 2017). The gateway is listening to all incoming LoRa signals

and translates the incoming radio waves into electrical signals. The gateway, which is connected to the internet, then forwards the received message to a LoRaWAN backend server with an MQTT (message queue telemetry transport) broker. MQTT is a protocol based on publish/subscribe transport of messages, here used for communication between the gateway and the application (Provoost et al., 2019). The MQTT broker acts like a post office: it receives all messages sent from devices (publishers) and then forwards it to the right applications (subscribers). With a regular interval the robotic flowers publish a message containing two parts: the topic and the payload. The topic identifies the message, while the payload contains the visitation data (real time clock and duration in seconds of each visit), battery level, alarms' status, and metadata (e.g., the time of sending, a list of gateways that received the transmitted message and the speed of data transmission).

Specifically, we made use of DingNet, the LoRaWAN communication infrastructure of the university of Leuven (KU Leuven), which provides the LoRaWAN backend and the gateways with antennas that ensure coverage across the whole city (Provoost et al., 2019). Alternative physical setups can be used for projects not affiliated to KU Leuven, such as the use of a gateway and The Things Network (The Things Industries B.V.), which provides a free LoRaWAN backend infrastructure globally.

Being low-power and long-range, LoRaWAN is limited in the amount of data that can be sent. Consumed airtime should be kept as small as possible for fair spectrum division (Adelantado et al., 2017). In the prototype firmware, data are sent every 10 minutes. That is the reason why our robotic flower only sends a maximum of 51 bytes each time, which corresponds to the data of 16 visits. At least for bumblebees, where a temporarily repellent effect of scent deposits left by conspecifics during a previous visit has been observed (Witjes & Eltz, 2007), this should be sufficient. If more than 16 visits take place between two sending events, the extra data are stored at the back of the sending queue, waiting for the next LoRaWAN transmission. In this way, data for more than 1500 visits per day per flower can potentially be stored in the CSV-file.

To monitor all robotic flowers that are in use and to access the gathered data, an application consisting of a backend and a frontend was developed with Node-RED (OpenJS Foundation), a browser-based programming tool that allows to create a flow of connected JavaScript functions. The program consists of two parts, one for file logging and one for communication with all the robotic flowers separately. These two parts are available on GitHub in JSON-format ready to be imported in Node-RED (see part 'Data accessibility'). The backend receives data from the LoRaWAN backend, processes them and provides them to the frontend application (Node-RED dashboard). Node-RED must run continuously when the robotic flowers are in use. Here a computer (localhost) running 24/7 was used, but there are other possibilities, e.g., with a Raspberry Pi, with a server or in the cloud. The backend stores the visitation data in a daily CSV-file (an example can be found in supporting file S1). For every visit, a new line is added in the file containing the time at the start of the visit, duration of the visit and the name of the device that registered the visit (e.g., 'flower 1').

When the backend receives a message from the robotic flower, a small message is sent back to the flower to confirm whether the data transmission was successful or not. If not

successful, the flower will try to resend the data for a limited number of times. The robotic flower receives the order to go to sleep or wake-up at a pre-selected time from the LoRaWAN backend as an answer to a sent message.

The frontend application ('dashboard') allows the user to monitor all the robotic flowers in use (Fig. S2). In the tab 'Battery', the voltage of the battery for each device is shown together with a graph presenting the progression of the voltage decline over time. The other tabs show the alarms, give the user the option to change the hours of the sleep mode, let the user choose the refill gap and give the user an overview of the created CSV-files.

Robotic Flower Design

The mechanical design of the robotic flower is based on the previous design by Kuusela & Lämsä (2016). A visit is recorded by a voltage drop of the infrared sensor when it is blocked by a bumblebee in the feeding hole (Fig. 2). This detection system has been used before and has proven to be robust, straightforward, and reliable (Keasar, 2000; Lämsä et al., 2018; Sokolowski & Abramson, 2010). Nectar is offered in discrete amounts to simulate the natural availability of nectar in flowers and to stimulate foraging in floral patches. The refilling of artificial nectar from the nectar reservoir is done with a servo-arm and a small nectar cup connected to a servomotor (Fig. 2). Making use of a servomotor for refilling the nectar cup has the advantage that overfilling is avoided, which would not be the case with a pump system. Although other systems are possible (e.g., an electromagnet as used by Keasar (2000)), a micro-type servomotor has proven to be the easiest, most fail-proof and cheapest solution till date (Kuusela & Lämsä, 2016).

The design of the robotic flower was made using Autodesk Fusion 360. Each flower consists of five 3D-printed components: a stem, background plane, central flower disk, nectar cup, and the reservoir case (see part 'Data accessibility' for the design files). The used 3D-printer was a Prusa I3 MK3S (Prusa Research a.s., Prague). The material for printing was a mixture of PLA (polylactic acid) and colourants. An animation of the robotic flower 3D-parts assembly and detailed pictures with information about the design and construction can be found in the supporting information (video S3 and file S4). All parts can be disassembled easily to facilitate cleaning, and electronics are protected either from internal spills or external rain or dirt. The weight of the total system without artificial nectar in the reservoir is approximately 370 g.

The stem serves as the housing part for the electronic components of the robotic flower. It protects them against dirt, moisture and other substances that could impair their function. Moreover, in this way the internal parts are hidden so that foraging behaviour can't be influenced by, e.g., observable motion of the servo-arm. The background plane is shaped as a square so that it is possible to form one continuous platform when grouping multiple flowers together (Fig. S5). The colour green was chosen to make a cryptic background (Chittka et al., 1994). The central flower disk represents the actual flower and was designed with a wide diameter (45 mm) and colour contrasting the green background (bright yellow and blue were selected for the prototype) so that detectability by bees is enhanced during flight (Spaethe et

al., 2001). Besides attracting pollinators, the central flower disk has other functions. It contains a feeding hole with infrared detection system designed with dimensions specifically for medium/large bees (> 10 mm long), which covers the most relevant pollinators in temperate areas such as honeybees, bumblebees, mason or leaf cutting bees (Goulson, 2003; Fig. 2). At the bottom of the feeding hole, there is a nectar hole through which the nectar cup that is pressed against the central flower disk can be probed. Transparent silicone is used to seal the central flower disk and to attach it to the background plane.

Under natural conditions, the volume of nectar has substantial inter- and intraspecific variances (L. Real & Rathcke, 1988). Here, the volume of the nectar cup can be altered to meet specific research requirements by changing the 3D-design. In this prototype, the robotic flower presents 15.73 ± 2.30 µL of artificial nectar to visitors in the central flower disk (measured with a 30% sugar solution, see supporting file S6). This discrete volume is not enough to let a visiting bumblebee fill its honey stomach (according to Goulson (2010) ranging between 60 and 120 µL, also depending on size of the worker) with only one visit, and therefore will stimulate pollinator movement among the different robotic flowers. The chosen volume is also not too small, so that the bees in an experiment don't starve to death as there is only a limited number of robotic flowers available to feed on. The nectar cup gets replenished with nectar at a standard rate (every 10 min. in the prototype firmware) when there are no pollinators visiting the robotic flower. This is a basic refilling rate to prevent evaporation of the nectar cup over time. On the other hand, when a visit to the robotic flower reaches a certain threshold duration (defined by the user in the firmware, e.g., a visit lasting at least 3 sec.), a nectar refill is scheduled to take place after the visit ends. However, the robotic flower will not refill immediately after a visit, but rather leave a lag. This waiting period was installed to avoid learning behaviour of the bees (Cnaani et al., 2006). The user can select one of the predefined (in the firmware) options for the refill-gap duration from the dashboard website for each individual robotic flower, and this can also be changed during an experiment. The refilling itself takes approximately three seconds.

The reservoir case is built to carry the nectar reservoir while also forming a protective barrier between the electronic components at the bottom and the artificial nectar located at the top. The sole purpose of the nectar reservoir itself is to keep the required artificial nectar for the specific experimental setup. For this prototype an off-the-shelf reservoir with a maximal volume of approximately 80 ml was used.

Finally, the internal hardware of the robotic flower consists of different electronic parts, of which many are soldered onto a printed circuit board (PCB). A separate battery module is added to use the battery. Hardware parts were selected so they are easy to solder manually. The supporting information contains an overview of the electrical schematic (Fig. S7) and a list of all the used parts, where they were bought, and their article number (supporting file S8). The Gerber files that are needed for PCB fabrication can be found in the GitHub repository (see part 'Data accessibility').

Firmware

The firmware that is running on the Arduino in every robotic flower is written in the programming language C++ and runs on the Arduino microcontroller of each flower. When this microcontroller is powered on, e.g., by switching on the battery power with the rocker switch, the program will start running. This always starts with a setup loop, which is only run once after powering up. This initializes the robotic flower functions and establishes the LoRaWAN connection before it continues with the main loop, which keeps running as long as power is supplied.

The flower is programmed to have two states: working and sleeping. During the working state, the infrared detection system is functional and regular data transmissions are taking place. These functions are stopped during the sleeping state to save battery life. If the targeted study species are not foraging during the night (e.g., bumblebees (Spaethe & Weidenmuller, 2002)), no data are lost doing this.

Some setting of the robotic flower system can be changed remotely from the dashboard website, where the user can simply select parameters for each flower separately. The first setting is to tell the flower when to go to sleep and when to wake up, the second is to define the gap between a visit and a nectar refill. When new parameters are selected, they will be sent to the flower in the next LoRaWAN communication.

The firmware also allows to adjust the hardcoded behaviour of the flower. This can only be done by uploading the changed firmware with a USB-cable to the microcontroller. Specifically, the following variables can be easily adjusted: time of deep sleep (i.e., how long the flower is staying in deep-sleeping mode), sensor sensitivity (i.e., how big the voltage drop in the infrared sensor needs to be to detect a visitor), automatic nectar refilling frequency (to prevent drying out), the different options for the refill-gap duration that can be chosen from the dashboard website, data transmission frequency, the maximum visit duration, the battery voltage at which a warning is given, the minimum visit duration before a nectar refilling is triggered, and the threshold to give an obstruction alarm.

Price

A rough price estimate of one robotic flower, using only genuine parts, is € 120, see Table 1. Although, this price can be reduced by using cheaper alternatives to the parts that were used here.

Battery

The expected battery lifetime was tested to be 14 days, see supporting information (supporting file S9). If longer experimental periods are desired, empty batteries can be easily replaced.

Proof of concept

In a first test, the system was used under both laboratory and natural conditions to assess 1) the attractiveness of the mechanical design, 2) the accuracy of visit detection and nectar probing times, and 3) the obtained voltage-drop -thus detectability- by visitors of different body sizes

(from approximately 5 up to 20 mm in length - from *Eupeodes corollae* to *Bombus terrestris*). The results showed that different floral visiting species (e.g., solitary bees, syrphid flies, bumblebees, and honeybees) were attracted to the robotic flower and consumed nectar. The visit detection and probing time were accurate for the aforementioned insect groups, but less optimal to other visitors such as Bombyliidae and Lepidoptera. A voltage drop in the system was obtained for visitors with different body sizes, but we recommend calibrating the sensor sensitivity carefully prior to an experiment (e.g., Syrphidae). See supporting information S10 for more details.

Discussion

The combination of many automated flowers in an experimental setup creates a robotic flower field, in which every flower can be seen as an autonomous lab where several factors can be controlled and manipulated. As such, it could be used to test hypotheses under optimal foraging theory, where pollinators are expected to optimize net energy intake by selecting optimal flowers and/or patches, e.g., in the 'marginal value' or 'ideal free distribution' theorems (Waddington & Holden, 1979). This robotic flower field can be readily used to assess the effect of nectar composition on the behaviour of floral visitors: many manipulations can be made while keeping other factors unchanged at the level of the nectar. Not only sugar composition or concentration, but also amino acids, secondary metabolites, and trace elements in nectar can drastically influence pollinator behaviour (Afik et al., 2008; Nepi et al., 2012; Stevenson et al., 2017). The concentrations of these resources can be easily manipulated in experiments using these robotic flowers to assess the influence of nectar characteristics on pollinator behaviour. Similarly, the effect of human induced disturbances (e.g., pesticides or pollution traces; Lämsä et al., 2018) or microbial inoculation (e.g., by yeasts or bacteria; Alvarez-Perez et al., 2012; Pozo et al., 2020) can be studied using the robotic flower. A concrete example of a setup like this could be a flight cage containing a bumblebee colony and two types of flowers, one with control nectar and one with nectar containing pesticide traces (see supporting picture S5). The discrete amount of nectar that is offered to insects can also be manipulated by changing the volume of the nectar cup, and in this way also flowers from tropical or temperate regions can be mimicked. What is new in the robotic flower system presented here, is that every robotic flower making up the robotic flower field is wireless and completely independent. This implicates that the setup can easily be changed during an experiment: robotic flowers can be moved, added, or removed during an experiment and the rate of refilling nectar can be altered remotely. This can be useful in research questions dealing with ephemeral food sources or changing patchy availability of resources like is the case with flowers.

The design of the robotic flower can be easily adapted to fit requirements for specific research topics. A range of floral visitors besides bees can be studied, or a comparison between species can be made. In the proof of concept, it was already proven that non-bee species, specifically syrphids flies, are attracted to the robotic flower as well. But not only insects can be studied: with the right hardware changes also bigger animals such as mammals (e.g., flower visiting rodents) and birds (e.g., hummingbirds) can be targeted with the robotic flower.

We aimed at creating a study system as versatile as possible but still accessible and easy to construct using the information available here. The firmware was created in such a way that

many hardcoded parameters in the firmware can be easily changed in the adjustable variables' header file. Besides software changes, also hardware can be adapted, e.g., extra batteries and sensors could be used. Measuring temperature, humidity, light intensity, or other factors could give useful information. For example, the foraging behaviour of some bees depends on temperature, solar radiation, humidity, and wind speed (Vicens & Bosch, 2000). One alternative possibility could be to use one device containing these extra sensors as measuring hub to provide centralized data for all surrounding robotic flowers. These data could also be used to adjust parameters in the robotic flower functioning, e.g., going in sleep when it is getting dark or refilling nectar less frequently in dry conditions.

Other flower characteristics (e.g., flower disk size, shape, or colour) can be easily altered, e.g., to change search time or handling time. This can be used to investigate associative learning behaviour (Menzel, 1993; Werner et al., 2016), opening the possibility to test the learning abilities of bees associated to stimuli that may not be advertised before collecting the reward (e.g., cues that do not affect palatability or odour). For example, damaged flower parts could be a sign of smaller nectar awards and can invoke avoidance behaviour in pollinators (Goulson et al., 2007). Robotic flowers can be constructed to mimic floral defects for investigating the selectivity of floral visitors. Moreover, not only single but also combinations of manipulations are possible. Another possibility offered by the robotic flower is the design of dynamic experiments where the system can respond to pollinator behaviour or environmental factors. For example, the robotic flower can be programmed to stop offering nectar for the day after a threshold number of visits.

Although our robotic flower can be used to address a wide range of research questions related to pollinator foraging behaviour, it still has limitations and leaves room for improvement. The robotic flower is made of hard plastic, which does not resemble natural plant tissue in feeling and in appearance. It currently also lacks UV-patterns that are present in some flowers to guide pollinators to the reward (Hansen et al., 2012). This will not keep floral visitors away, but it might slightly reduce the salience of the robotic flower (Chittka et al., 1994). Similarly, floral scents could be provided to further approximate natural flower systems or to test the influence of plant volatiles on foraging behaviour.

At present it is not yet possible to automatically discriminate between individual floral visitors and the measured foraging pattern only gives a global view and does not provide information on individual choices. When used in open field, also the different species identities of visitors are unknown. Taking a picture of the feeding hole at the beginning of a visit could help tackle this issue, with the downside that pictures are too big to send over LoRaWAN and would thus need to be stored locally for later analysis. In addition, it increases the price of the system and could decrease the battery life. Another option is using a combination of information from different sensors such as weight and buzz vibration to deduce the visitor identity, maybe by making use of artificial intelligence software, which might then again require a more powerful (and more expensive) processor in the robotic flower.

The measured probing time serves as a good proxy for nectar volume that is consumed during a visit. That is why the maximum visit duration was set at 250 seconds in this prototype, giving the visitors enough time to complete nectar depletion. Visits that take longer have been

proven not to be foraging related, but rather grooming, food pump from crop to ventriculus, or rest (Pozo et al., 2020). Regrettably, all these processes are not subjected to an unambiguous act of crossing the infrared sensor and can be only detected by direct observation.

Data communication with LoRaWAN is very well suited for this kind of application, but, like all (wireless) communication technologies, it also has its limitations and concerns. Packages that are lost during transmission cannot be recovered. This has been compensated in the flower design by asking the backend for a receive-confirmation, and if necessary, repeat the message. Using an external memory (which was already provided in the hardware of this prototype) to store data as a local back-up is also a possibility.

Besides nectar, pollen is often collected by some pollinator groups to fulfil their nutritional requirements (Simpson et al., 1983; Simpson & Neff, 1981). For example, buzz pollination that can be found in some very important crop species such as tomatoes is an interesting topic for study (De Luca & Vallejo-Marin, 2013). Therefore, this field of study could make good use of robotic flowers able to present pollen to visitors (e.g., the system made by Switzer et al., (2019)). The function of presenting pollen in this robotic flower was not implemented yet, but by combining both rewards in the same robotic flower system information on another level might be obtained. An idea could be to add artificial anthers fitted with the necessary electronics to release (artificial) pollen when a certain vibration frequency is registered.

Conclusion

The presented robotic flower advances the state of the art by providing a full wireless data transmission mechanism to automatically monitor foraging behaviour at real time. The absence of cables or external control units makes it possible to use the robotic flowers in more remote experimental setups. The system is versatile and offers many parameters that can be changed easily. A dashboard website allows the user to monitor all robotic flowers deployed in a setup and change the nectar refilling rate remotely for every flower individually. These capabilities make the flower a versatile tool for behavioural studies with pollinators. Moreover, the flower is robust, relatively easy and cheap to construct, and offers the appearance of a commercial product, while being still completely customizable.

Acknowledgements

The development of this robotic flower would not have been possible without the inspiration and the technical ideas of Prof. Jeff Rozenski. We want to acknowledge Marc Lambaerts and Thomas Pilkington from FabLab KU Leuven for their help with 3D-printing and Kasper Van Acker for his help with the assembly of the robotic flower. We are also grateful for the help received by Hanna Schallauer, Jana Offenberger, Alejandro Arbelaez and Alfredo Benavente Martinez in carrying-out the proof of concept.

Data accessibility

The firmware, the Node-RED flows, files for 3D printing and PCB design files are available at GitHub: https://github.com/Kamiel-debeuckelaere/One-Robotic-Flower-Field-To-Study-Them-All.git.

Conflict of Interest statement

None declared.

Authors' contributions statement

M.I.P. and H.J did the conceptualization and proposed the project. K.D. developed the robotic flower system with the help of D.J. for the selection of hardware parts and PCB design. K.D. did the software development with substantial input from D.J. and E.S. The assembly and testing of the robotic flowers were done by K.D., D.J. and M.I.P. The writing of the manuscript was led by K.D. and all authors contributed critically to the drafts and gave final approval for publication.

References

Adelantado, F., Vilajosana, X., Tuset-Peiro, P., Martinez, B., Melia-Segui, J., & Watteyne, T. (2017). Understanding the Limits of LoRaWAN. *IEEE Communications Magazine*, *55*(9), 34–40. https://doi.org/10.1109/MCOM.2017.1600613

Afik, O., Dag, A., & Shafir, S. (2008). Honeybee, Apis mellifera, round dance is influenced by trace components of floral nectar. *Animal Behaviour*, *75*(2), 371–377. https://doi.org/10.1016/j.anbehav.2007.04.012

Alvarez-Perez, S., Herrera, C. M., & de Vega, C. (2012). Zooming-in on floral nectar: a first exploration of nectar-associated bacteria in wild plant communities. *FEMS Microbiology Ecology*, *80*(3), 591–602. https://doi.org/10.1111/j.1574-6941.2012.01329.x

Billiet, A., Meeus, I., Van Nieuwerburgh, F., Deforce, D., Wäckers, F., & Smagghe, G. (2016). Impact of sugar syrup and pollen diet on the bacterial diversity in the gut of indoor-reared bumblebees (Bombus terrestris). *Apidologie*, *47*(4), 548–560. https://doi.org/10.1007/s13592-015-0399-1

Calisi, R. M., & Bentley, G. E. (2009). Lab and field experiments: Are they the same animal? *Hormones and Behavior*, *56*(1), 1–10. https://doi.org/https://doi.org/10.1016/j.yhbeh.2009.02.010

Chittka, L., Shmida, A., Troje, N., & Menzel, R. (1994). Ultraviolet as a component of flower reflections, and the color-perception of Hymenoptera. *Vision Research*, *34*(11), 1489–1508. https://doi.org/10.1016/0042-6989(94)90151-1

Cnaani, J., Thomson, J. D., & Papaj, D. R. (2006). Flower choice and learning in foraging bumblebees: Effects of variation in nectar volume and concentration. *Ethology*, *112*(3), 278–285. https://doi.org/10.1111/j.1439-0310.2006.01174.x

Crall, J. D., Gravish, N., Mountcastle, A. M., & Combes, S. A. (2015). BEEtag: A Low-Cost, Image-Based Tracking System for the Study of Animal Behavior and Locomotion. *PLOS ONE*, *10*(9). https://doi.org/10.1371/journal.pone.0136487

Dankert, H., Wang, L., Hoopfer, E. D., Anderson, D. J., & Perona, P. (2009). Automated monitoring and analysis of social behavior in Drosophila. *Nature Methods*, *6*(4), 297–303. https://doi.org/10.1038/NMETH.1310

De Luca, P. A., & Vallejo-Marin, M. (2013). What's the `buzz' about? The ecology and evolutionary significance of buzz-pollination. *Current Opinion in Plant Biology*, *16*(4), 429–435. https://doi.org/10.1016/j.pbi.2013.05.002

Dell, A. I., Bender, J. A., Branson, K., Couzin, I. D., de Polavieja, G. G., Noldus, L. P. J. J., Pérez-Escudero, A., Perona, P., Straw, A. D., Wikelski, M., & Brose, U. (2014). Automated image-based tracking and its application in ecology. *Trends in Ecology & Evolution*, *29*(7), 417–428. https://doi.org/https://doi.org/10.1016/j.tree.2014.05.004

Essenberg, C. J. (2015). Flobots: Robotic Flowers for Bee Behaviour Experiments. *Journal of Pollination Ecology*, *15*(1), 1–5. https://pubmed.ncbi.nlm.nih.gov/25722755

Goulson, D. (2003). Conserving wild bees for crop pollination. *Journal of Food, Agriculture & Environment*, *1*(1), 142–144.

Goulson, D. (2010). *Bumblebees: behaviour, ecology, and conservation.* (Second Edi). OXFORD UNIV PRESS INC.

Goulson, D., & Cory, J. S. (1993). Flower constancy and learning in foraging preferences of

the green-veined white butterfly Pieris napi. *Ecological Entomology*, *18*(4), 315–320. https://doi.org/10.1111/j.1365-2311.1993.tb01107.x

Goulson, D., Cruise, J. L., Sparrow, K. R., Harris, A. J., Park, K. J., Tinsley, M. C., & Gilburn, A. S. (2007). Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees. *Behavioral Ecology and Sociobiology*, *61*(10), 1523–1529. https://doi.org/10.1007/s00265-007-0384-4

Greggers, U., & Menzel, R. (1993). Memory Dynamics and Foraging Strategies of Honeybees. *Behavioral Ecology and Sociobiology*, *32*(1), 17–29.

Gumbert, A. (2000). Color choices by bumble bees (Bombus terrestris): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology*, *48*(1), 36–43. https://doi.org/10.1007/s002650000213

Hannah, L., Dyer, A. G., Garcia, J. E., Dorin, A., & Burd, M. (2019). Psychophysics of the hoverfly: categorical or continuous color discrimination? *Current Zoology*, *65*(4), 483–492. https://doi.org/10.1093/cz/zoz008

Hansen, D. M., der Niet, T., & Johnson, S. D. (2012). Floral signposts: testing the significance of visual `nectar guides' for pollinator behaviour and plant fitness. *Proceedings of the Royal Society B- Biological Sciences*, *279*(1729), 634–639. https://doi.org/10.1098/rspb.2011.1349

Ings, T. C., & Chittka, L. (2008). Speed-Accuracy Tradeoffs and False Alarms in Bee Responses to Cryptic Predators. *Current Biology*, *18*(19), 1520–1524. https://doi.org/10.1016/j.cub.2008.07.074

Keasar, T. (2000). The spatial distribution of nonrewarding artificial flowers affects pollinator attraction. *Animal Behaviour*, *60*, 639–646. https://doi.org/10.1006/anbe.2000.1484

Klein, A.-M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B- Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Kuusela, E., & Lämsä, J. (2016). A low-cost, computer-controlled robotic flower system for behavioral experiments. *Ecology and Evolution*, *6*(8), 2594–2600. https://doi.org/10.1002/ece3.2062

Lämsä, J., Kuusela, E., Tuomi, J., Juntunen, S., & Watts, P. C. (2018). Low dose of neonicotinoid insecticide reduces foraging motivation of bumblebees. *Proceedings of the Royal Society B- Biological Sciences*, *285*(1883). https://doi.org/10.1098/rspb.2018.0506

Lunau, K. (2000). The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution*, *222*(1–4), 89–111. https://doi.org/10.1007/BF00984097

Lunau, K., Wacht, S., & Chittka, L. (1996). Colour choices of naive bumble bees and their implications for colour perception. *Journal of Comparative Physiology A - Sensory, Neural and Behavioral Physiology*, *178*(4), 477–489.

Makino, T. T., & Sakai, S. (2007). Experience changes pollinator responses to floral display size: from size-based to reward-based foraging. *Functional Ecology*, *21*(5), 854–863. https://doi.org/10.1111/j.1365-2435.2007.01293.x

Menzel, R. (1993). Associative Learning in Honey-Bees. *Apidologie*, *24*(3), 157–168. https://doi.org/10.1051/apido:19930301

Minahan, D. F., & Brunet, J. (2018). Strong Interspecific Differences in Foraging Activity

Observed Between Honey Bees and Bumble Bees Using Miniaturized Radio Frequency Identification (RFID). *Frontiers in Ecology and Evolution*, *6*. https://doi.org/10.3389/fevo.2018.00156

Moffatt, L. (2001). Metabolic rate and thermal stability during honeybee foraging at different reward rates. *Journal of Experimental Biology*, *204*(4), 759–766. https://doi.org/10.1242/jeb.204.4.759

Nachev, V., & Winter, Y. (2012). The psychophysics of uneconomical choice: non-linear reward evaluation by a nectar feeder. *Animal Cognition*, *15*(3), 393–400. https://doi.org/10.1007/s10071-011-0465-7

Nepi, M., Soligo, C., Nocentini, D., Abate, M., Guarnieri, M., Cai, G., Bini, L., Puglia, M., Bianchi, L., & Pacini, E. (2012). Amino acids and protein profile in floral nectar: Much more than a simple reward. *Flora*, *207*(7), 475–481. https://doi.org/10.1016/j.flora.2012.06.002

Nicolson, S., & Thornburg, R. (2007). Nectar chemistry. In *Nectaries and Nectar* (pp. 215–264). https://doi.org/10.1007/978-1-4020-5937-7_5

Nicolson, S. W. (2011). Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two. *African Zoology*, *46*(2), 197–204. https://doi.org/10.3377/004.046.0201

Ohashi, K., D'Souza, D., & Thomson, J. D. (2010). An automated system for tracking and identifying individual nectar foragers at multiple feeders. *Behavioural Ecology and Sociobiology*, *64*(5), 891–897. https://doi.org/10.1007/s00265-010-0907-2

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Paldi, N., Zilber, S., & Shafir, S. (2003). Associative olfactory learning of honeybees to differential rewards in multiple contexts - Effect of odor component and mixture similarity. *Journal of Chemical Ecology*, *29*(11), 2515–2538. https://doi.org/10.1023/A:1026362018796

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, *25*(6), 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Pozo, M. I., van Kemenade, G., van Oystaeyen, A., Aledon-Catala, T., Benavente, A., Van den Ende, W., Wäckers, F., & Jacquemyn, H. (2020). The impact of yeast presence in nectar on bumble bee behavior and fitness. *Ecological Monographs*, *90*(1). https://doi.org/10.1002/ecm.1393

Provoost, M., Weyns, D., & IEEE. (2019). DingNet: A Self-Adaptive Internet-of-Things Exemplar. In *2019 IEEE/ACM 14th International Symposium on Software Engineering for Adaptive and Self-Managing Systems (SEAMS 2019)*. https://doi.org/10.1109/SEAMS.2019.00033

Real, L. A. (1981). Uncertainty and Pollinator-Plant Interactions - The Foraging Behavior of Bees and Wasps on artificial flowers. *Ecology*, *62*(1), 20–26. https://doi.org/10.2307/1936663

Real, L., & Rathcke, B. J. (1988). Patterns of individual variability in floral resources. *Ecology*, *69*(3), 728–735. https://doi.org/10.2307/1941021

Schmid-Hempel, P., & Stauffer, H. P. (1998). Parasites and flower choice of bumblebees.

Animal Behaviour, *55*(4), 819–825. https://doi.org/10.1006/anbe.1997.0661

Simons, D. J., & Chabris, C. F. (1999). Gorillas in our midst: sustained inattentional blindness for dynamic events. *Perception*, *28*(9), 1059–1074. https://doi.org/10.1068/p2952

Simpson, B. B., Neff, J. L., & Seigler, D. S. (1983). Floral Biology and Floral Rewards of Lysimachia (Primulaceae). *American Midland Naturalist*, *110*(2), 249–256. https://doi.org/10.2307/2425266

Simpson, B., & Neff, J. (1981). Floral Rewards: Alternatives to Pollen and Nectar. *Annals of the Missouri Botanical Garden*, *68*, 301. https://doi.org/10.2307/2398800

Sokolowski, M. B. C., & Abramson, C. I. (2010). From foraging to operant conditioning: A new computer-controlled Skinner box to study free-flying nectar gathering behavior in bees. *Journal of Neuroscience Methods*, *188*(2), 235–242. https://doi.org/10.1016/j.jneumeth.2010.02.013

Spaethe, J., Tautz, J., & Chittka, L. (2001). Visual constraints in foraging bumblebees: Flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(7), 3898–3903. https://doi.org/10.1073/pnas.071053098

Spaethe, J., & Weidenmuller, A. (2002). Size variation and foraging rate in bumblebees (Bombus terrestris). *Insectes Sociaux*, *49*(2), 142–146. https://doi.org/10.1007/s00040-002-8293-z

Stevenson, P. C., Nicolson, S. W., & Wright, G. A. (2017). Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. *Functional Ecology*, *31*(1), 65–75. https://doi.org/10.1111/1365-2435.12761

Sun, S., Leshowitz, M. I., & Rychtář, J. (2018). The signalling game between plants and pollinators. *Scientific Reports*, *8*(1), 6686. https://doi.org/10.1038/s41598-018-24779-0

Switzer, C. M., Russell, A. L., Papaj, D. R., Combes, S. A., & Hopkins, R. (2019). Sonicating bees demonstrate flexible pollen extraction without instrumental learning. *Current Zoology*, *65*(4), 425–436. https://doi.org/10.1093/cz/zoz013

Thomson, J., Ogilvie, J., Makino, T., Arisz, A., Raju, S., Rojas Luengas, V., & Tan, M. (2012). Estimating pollination success with novel artificial flowers: Effects of nectar concentration. *Journal of Pollination Ecology*, *9*, 108–114. https://doi.org/10.26786/1920-7603(2012)14

Vicens, N., & Bosch, J. (2000). Weather-dependent pollinator activity in an apple orchard, with special reference to Osmia cornuta and Apis mellifera (Hymenoptera : Megachilidae and Apidae). *Environmental Entomology*, *29*(3), 413–420. https://doi.org/10.1603/0046-225X-29.3.413

Waddington, K. D., & Holden, L. R. (1979). Optimal Foraging - Flower Selection by Bees. *American Naturalist*, *114*(2), 179–196. https://doi.org/10.1086/283467

Werner, A., Stürzl, W., & Zanker, J. (2016). Object Recognition in Flight: How Do Bees Distinguish between 3D Shapes? *PLOS ONE*, *11*(2), 1–13. https://doi.org/10.1371/journal.pone.0147106

Witjes, S., & Eltz, T. (2007). Influence of scent deposits on flower choice: experiments in an artificial flower array with bumblebees. *Apidologie*, *38*(1), 12–18. https://doi.org/10.1051/apido:2006048

Supporting information

S1: CSV-file example showing the gathered data from simulated visits in different robotic flowers on 2021/10/27

S2: screenshots of the different tabs opened in the frontend application (PNG). Top, left to right: 'Battery', 'Alarms' and 'Sleep Time'. Bottom, left to right: 'Refill Gap' and 'Files'.

S3: animation video (mp4) of 3D-assembly showing how different parts of the robotic flower fit together

S4: file (PDF) with description and figures showing the flower parts & the assembly of PCB, battery module and 3D-parts

S5: figure (PNG) of two robotic flower field setups with 6 flowers each as an example of a potential experimental setup mimicking different flower patches (blue and yellow patches)

S6: file (Excel) with measurements of prototype nectar cup volume

S7: figure (PNG) of electrical schematic

S8: file (Excel) with list of all parts that are needed to build one robotic flower (BOM)

S9: file (PDF) giving calculations and explanation for the theoretical battery life

S10: file (PDF) containing information and tables of the conducted first proof of concept

List of Tables

Table 1: Rough price estimate for the parts to build one complete robotic flower (transport costs not included).

Component	Price (€)
Arduino Pro Micro	18
LoRaWAN module + antenna	27
Servomotor	3
Infrared system	1
Other hardware parts	25
Battery + module	12
3D-print	30
Nectar reservoir	1
Assembly material	3
TOTAL:	**€ 120**

List of Figures

Figure 1: Overview of the robotic flower system. The system consists of the robotic flower, which sends data packages using LoRaWAN (Long Range Wide Area Network) to the LoRaWAN-backend through a gateway. The backend subsequently transfers the data using the internet network to the application. The application also has a backend, which is constantly running to receive and store data in the database, and a frontend that enables the user to overlook all deployed robotic flowers and retrieve the daily created CSV files. These files contain all the data that were gathered by the robotic flowers.

Figure 2: Mechanical design of the robotic flower based on Kuusela & Lämsä (2016) with A) the feeding hole, B) Infrared Emitter, C) Infrared sensor and D) the nectar cup. The servo-arm is used to refill the nectar cup from the nectar reservoir (rotation of 100°) and press it to the central flower disk, where floral visitors can reach the nectar using their proboscis.

Fig. 1

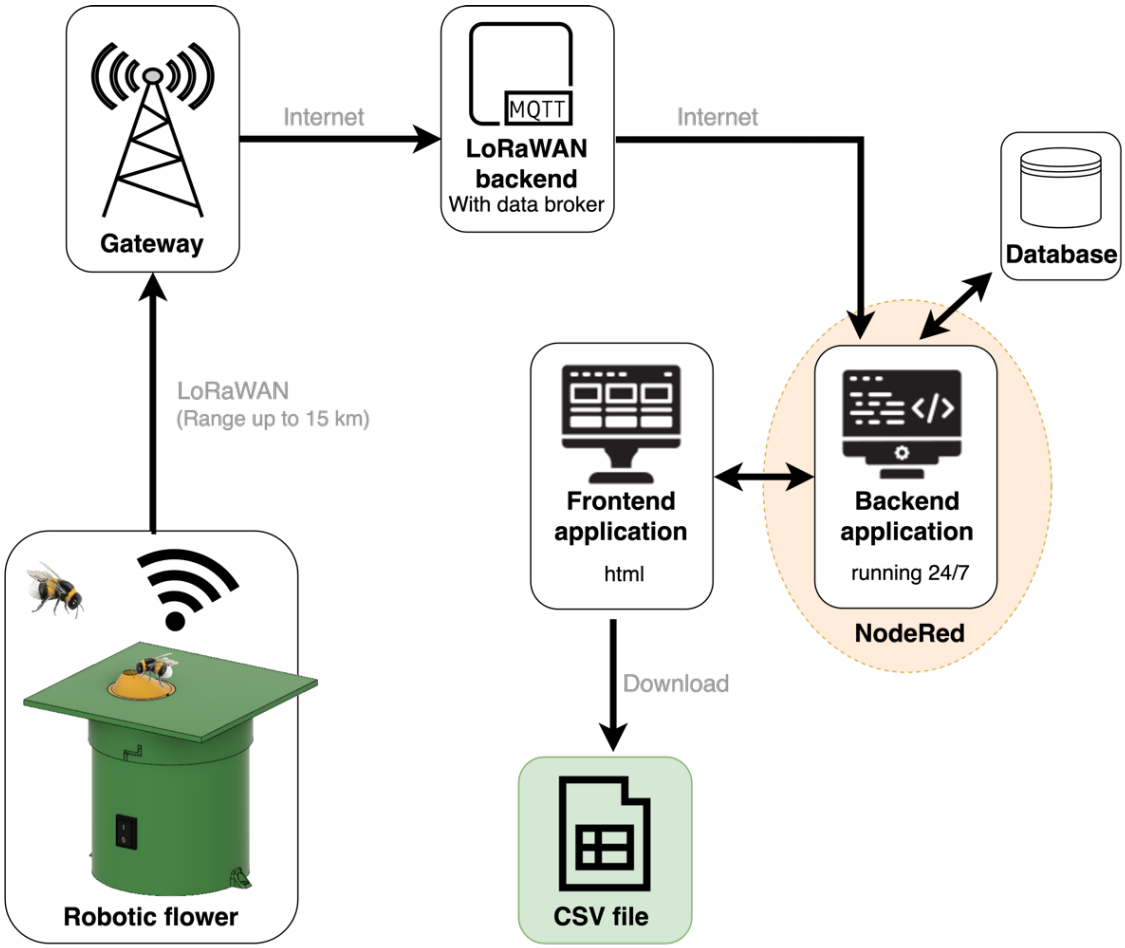
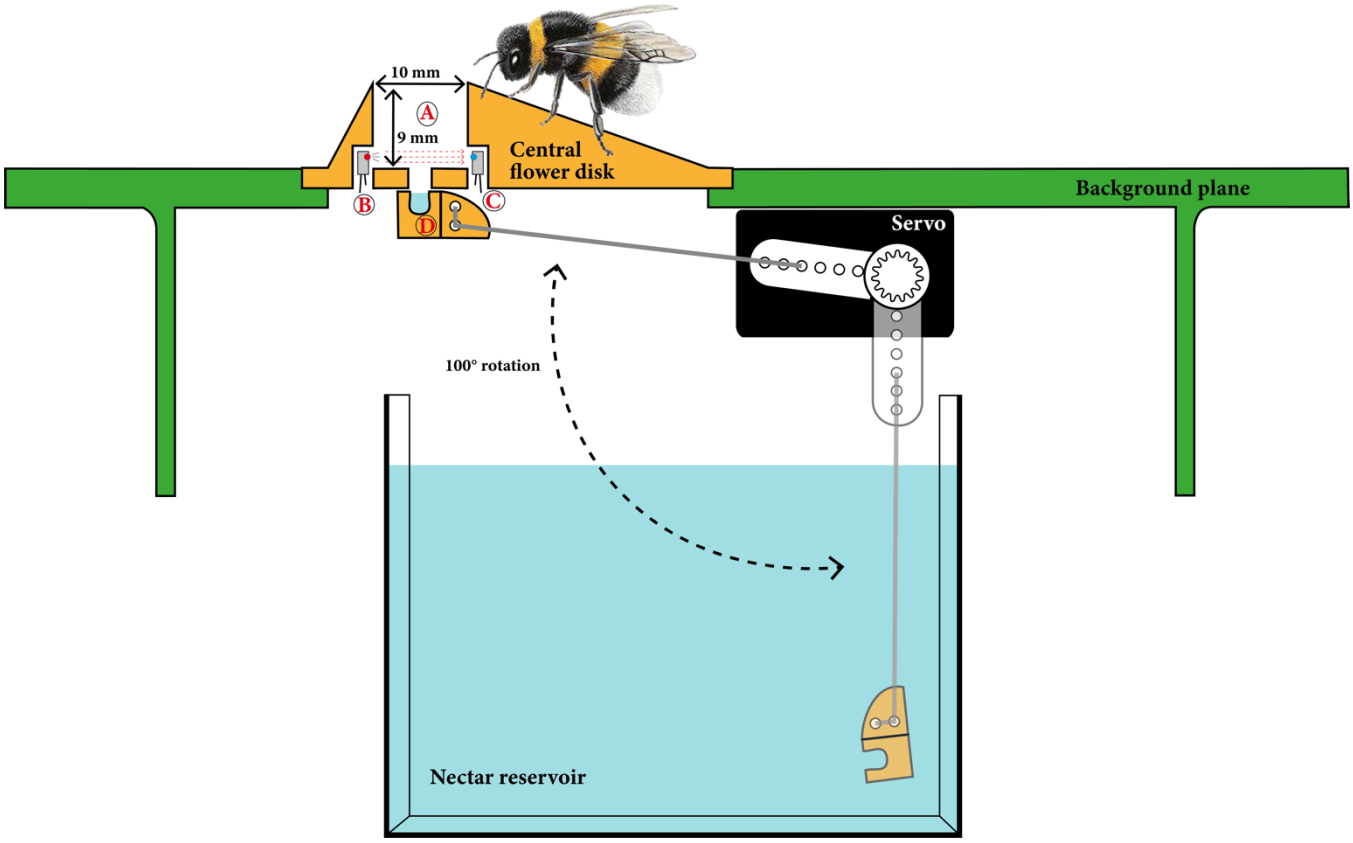

Gateway
Internet
MQTT
LoRaWAN
backend
With data broker
Internet
Database
LoRaWAN
(Range up to 15 km)
Frontend
application
html
Backend
application
running 24/7
NodeRed
Download
Robotic flower
CSV file

Fig. 2


10 mm
A
9 mm
Central
flower disk
B
C
D
Background plane
Servo
100° rotation
Nectar reservoir