Master's thesis

Effect of heather flavonoids on the buff-tailed bumblebee

Presented by **Clément Tourbez** to obtain the degree of Master in Organisms and Ecology Biology with Research Finality

Conducted in the zoology laboratory at the University of Mons

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Résumé et mots clés (Français)

Les abeilles sont soumises à de nombreuses menaces environnementales parmi lesquelles figurent les parasites. Pollen et nectar des plantes à fleurs contiennent des métabolites secondaires qui sont transmis aux abeilles via leur nutrition et peuvent potentiellement influencer l'interaction avec le parasite. Dans le cadre de ce mémoire, nous avons questionné les effets induits par l'assimilation de pollen de bruyère (*Calluna vulgaris*) et de ses flavonoïdes sur le bourdon terrestre (*Bombus terrestris*) et les effets de son parasite (*Crithidia bombi*). En utilisant une chromatographie en phase liquide et de la spectrométrie de masse (HPLC-MS/MS) nous avons tout d'abord démontré la répartition spécialisée de 22 flavonoïdes chez la bruyère. Cette distribution des toxines semble favoriser l'interaction avec le pollinisateur puisque le pollen contient moins de flavonoïdes que les feuilles et le nectar n'en contient pas. Au contraire, les deux ressources florales contiennent un terpénoïde, probablement du callunene, connu pour contrer *C. bombi*. Afin d'évaluer l'effet des flavonoïdes sur le bourdon, nous avons réalisé des tests en microcolonies parasitées ou non et soumissent à différentes diètes polliniques : 'saule' (contrôle), 'saule avec extrait de flavonoïdes' (de feuille et de pollen) et enfin 'bruyère'. L'assimilation des extraits de flavonoïdes de feuilles et de pollen a induit des effets négatifs similaires sur les microcolonies en limitant la production de descendants et démontrant leur toxicité. En comparant ensuite les diètes 'saule' et 'bruyère', nous avons pu démontrer la plus faible qualité nutritive du pollen de bruyère. Ce dernier induit notamment une réduction de la production de descendants bien que celle-ci soit inférieure à celle observée avec les extrait pur de flavonoïdes. Nos microcolonies parasitées ont pour finir permis de mettre en évidence le très faible effet du parasite à l'échelle de la microcolonie par comparaison à celui de la diète. A l'échelle individuelle, le stress parasitaire a cependant, tout comme le stress flavonoïde, induit une augmentation de la réponse immunitaire indiquée par une augmentation de la masse des corps gras. Alors que nous n'avons pas identifié de diminution de l'effet du parasite due aux flavonoïdes, que du contraire, le pollen de bruyère a eu une influence sur cette réponse immunitaire ce qui pourrait être dû à sa charge en terpénoïde ce qui n'a pas encore pu être testé.

Mots clés : Pollinisateur, Interaction plante-pollinisateur, Herbivorie, Défense chimique, Flavonoïde, Bourdon (*Bombus terrestris*), Pollen, Bruyère (*Calluna vulgaris*), Parasite, *Crithidia bombi*.

Abstract and keywords (English)

Bees are subject to many environmental threats, one of which being parasites. Pollen and nectar from flowering plants contain secondary metabolites that are supplied to bees through their nutrition and can potentially influence the interaction with the parasite. In this master's thesis, the effects induced by the uptake of heather (*Calluna vulgaris*) pollen and its flavonoids in the buff-tailed bumblebee (*Bombus terrestris*) and its parasite (*Crithidia bombi*) effects were investigated. Using liquid chromatography and tandem mass spectrometry (HPLC-MS/MS), the specialised distribution of 22 flavonoids in heather was first demonstrated. The distribution of these toxins seems to favour the interaction with pollinators since pollen contains less flavonoids than leaves and nectar does not contain any flavonoids. On the contrary, both floral resources contain a terpenoid, probably the callunene, known to counteract *C. bombi*. In order to evaluate the influence of flavonoids on the bumblebee and its parasite effects, tests in parasitised and unparasitised bumblebee microcolonies were performed and bumblebees were subjected to different pollen diets; 'willow' (control), 'willow with flavonoid extract' (of leaf and of pollen) and 'heather'. The uptake of flavonoid extracts from leaves and pollen induced similar negative effects on the microcolonies by limiting offspring production and demonstrating their toxicity. The lower nutritional quality of heather pollen was demonstrated by comparing the 'willow' and 'heather' diets. The latter induces a reduction in the production of offspring, although this was lower than that observed with pure flavonoid extracts. Finally, parasitised microcolonies showed that the effect of the parasite at the microcolonial level was very weak compared to that of the diet. However, at the individual level, parasitic stress, like flavonoid stress, induced an increase in the immune response indicated by an increase in fat body mass. While no decrease in the parasite effect due to flavonoids was described, quite the opposite, heather pollen had a positive influence on this immune response which could be due to its terpenoid load which could not yet be tested.

Keywords: Pollinator, Plant-pollinator interaction, Herbivory, Chemical defence, Flavonoid, Bumblebee (*Bombus terrestris*), Pollen, Heather (*Calluna vulgaris*), Parasite, *Crithidia bombi*.

"Science is more than a body of knowledge. It's a way of thinking, a way of sceptically interrogating the universe."

Carl Sagan's last interview, 1996

"I have to learn by experiencing things myself, that's all I can do"

Sui Ichida

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I. Introduction

I. 1. Herbivory and pollination, the dilemma of flowering plants

During the Cambrian (541 to 485.4 mya, Paleozoic era), some of the photosynthetic organisms representing the broad monophyletic clade of Embryophyta (Plantae; Haeckel, 1866), gradually isolated from aquatic environments to reside and colonise land (Niklas 1997; Taylor et al. 2009; Morris et al. 2018; Servais et al. 2019). This clade had to adapt to living conditions on these terrestrial environments, in particular to desiccation (e.g., cuticle, stomata, desiccation tolerance, spore protection; Wood 2005; Raven & Edwards 2014) and gravity (e.g., vertical structure and robust tissues, lignin, radial symmetry; Graham & Cook 2007). Succeeding in this process, the continents swiftly became covered with plants thereby shaping new ecosystems (Shear 1991). These original environments were joined later by animals that conquered the terrestrial lands mainly during the Ordovician and the Silurian (483.8 to 419.2 mya, Paleozoic era; Wilson & Anderson 2004). The resulting plant-animal interactions in these new environments were accompanied by a primary danger for plants, namely herbivory (Rosenthal & Kotanen 1994).

I. 1.1 Herbivory defence mechanism

Terrestrial plants have developed parallel mechanisms of tolerance towards herbivores on the one hand and active defence against them on the other (Rosenthal & Kotanen 1994; Strauss & Agrawal 1999). To tolerate herbivore damages, plants have protected their meristems, have stored nutrients in the form of reserves for regrowth and/or have allocated their energy to resource uptake (Coughenour 1985; Heilmeier et al. 1986; Chapin & McNaughton 1989; Bilbrough & Richards 1993). Plants have also developed various ways for preventing and stopping these attacks. Starting with chemical defences, they have produced almost all the different groups of secondary metabolites and used them for example as toxins or repellents (Bennett & Wallsgrove 1994; Karban et al. 1997; Zeng et al. 2008; Wink 2010; Boyd 2012; Mithöfer & Boland 2012; Tiwari & Rana 2015; Aljbory & Chen 2018; Gebreziher 2018; Jain et al. 2019). Plants are also using compounds that reduce their digestibility or ease of

assimilation (e.g. resin, silica, lignin and calcium oxalate crystals, Finley 1999; Brodeur-Campbell 2006; Mithöfer & Boland 2012). They have in addition to this, dynamic mechanisms following animal aggression such as the induction of additional toxin synthesis that act on the animal once ingested (Karban et al. 1997; Zeng et al. 2008; Boyd 2012; Gebreziher 2018). Plants have also evolved numerous morphological adaptations that have prevented aggression and that could have been harmful to animals such as spiny outgrowths of different organs, scales or tougher leaves (Cooper & Owen-Smith 1986; Fernandes 1994; Young & Okello 1998). Some plants can even have developed organ adaptations that mimic other species, like herbivore predators (e.g., ants) or the presence of their eggs, which deter herbivores from approaching and feeding on the plant (Williams & Gilbert 1981; Augner 1998; Lev-Yadun & Inbar 2002).

I. 1.2 Coevolution between angiosperms and pollinators

The Angiosperms form a large group of over 370,000 species representing about 80% of the diversity of all known green plants (Royal Botanic Garden 2016). These flowering plants have a floral envelope with an ovary protecting the ovule (the female reproductive agent) and stamens releasing pollen (the male agent). The latter must be distributed to reach the pistil of another flower in order to fertilise the ovule in a process called pollination. To ensure their pollen transport and therefore their pollination and sexual reproduction, flowering plants require pollinating vectors. These agents can be abiotic (i.e. wind, water or gravity; Cox 1991; Ackerman 2000) or biotic (i.e. animals). These two modes of transmission are very unevenly distributed since 87.5% of flowering plants (308,006 according to Olerton et al. 2011) rely on animals and especially on insects to ensure their pollination (Kearns et al. 1998). With the advent of pollination interaction with insects, the need for protection from animals, and more specifically from the latter, had to be partly reconsidered.

Animal pollination, also known as zoophily, is enabled by many vertebrate taxa such as birds (Nabhan et al. 1997; Sakai et al. 1999; Krauss et al. 2017), squamates (Pérez Mellado et al. 2006), and mammals (Wooller et al. 2003; Fleming et al. 2009) even including some primates (Calley et al. 1993). Yet, most zoophilous plants are pollinated by insects, i.e. they are entomophilous (Kearns et al. 1998), and by one insect group in particular, i.e. bees (Hymenoptera) that comprises more than 20,000 species (Michener 2000). Thanks to their close relationship with flowering plants since their appearance in the Cretaceous period, bees have

developed morphological (hairs, harvesting brush) and ethological adaptations that have made them crucial pollinating agents (Michener 2000; Mayfield et al. 2001; Poinar et al. 2006; Hu et al. 2008). Pollinators and flowering plants interact with each other in complex ways, constituting organized networks of plant-pollinator interactions. Different bee species display different nutritional needs and do not equally interact in these networks (Blüthgen & Klein 2011; Dormann 2011). Some species have a very limited number of interactions, i.e. the monolectic species that interact with a single plant species and the oligolectic species that interact with a limited number of plant species. Other species have a vast number of interactions, i.e. the polylectic species that interact with a greater diversity of plant species (Michez et al. 2008; Cane 2021).

I. 1.3 Pollination induced costs to plants

For all these plants, the interaction with the pollinators and the pollen dispersal benefit they derive from them is not cost-free. Most flowers contain nectar (mainly a source of fructose, glucose and sucrose; Lüttge 1971; Bernardello et al. 2000) which are produced in order to attract the pollen carriers (Goulson 2010). Pollen itself is also a feeding resource for pollinators (source of amino acids, proteins, lipids including sterols and vitamins, Campos *et al.* 2008). Biotic pollination, although a mutualistic interaction, has therefore an important cost for flowering plants (Pyke 1991). This cost can reach 37% of the energy allocated to nectar synthesis alone which is then used to attract pollinators (Southwick 1984). Most of pollen collected from a flower is lost (i.e., used for pollinator species feeding) and only a small proportion is available for pollination (Müller et al. 2006; Hargreaves et al. 2009). Bees and other pollinators have therefore negative effects on flowering plants as they are herbivorous, i.e. they collect pollen and nectar to feed themselves and their larvae (Michener 2000; Thorp 2000). Facing this, flowering plants have developed several mechanisms to limit pollen collection such as morphological adaptations (e.g., anthers releasing pollen gradually or poricidal thus requiring buzz pollination) limiting pollen access to the most legitimate pollinators (Westerkamp & Classen-Bockhoff 2007; Pritchard & Vallejo-Marín 2010; Lunau et al. 2015). In parallel, they have evolved pollen with various chemical characteristics that could deter pollinators, such as toxins (Praz et al. 2008; de Assis Junior et al. 2011; Wang et al. 2019; Stevenson 2020), low digestibility (partly also associated with grain morphology; Bell et al. 1983; T'ai & Cane 2000)

or nutrient poverty (Vaudo et al. 2017). Therefore, with the advent of flowering plants, plant-animal interactions have become more complex and the crucial need for pollinators for their reproduction has raised a dilemma (Figure 1). Despite the need to defend themselves against herbivores, a group of plant-eaters focusing on floral resources is now providing a beneficial mutualistic interaction between plants and animals, in contrast to the other herbivores. On the one hand, they have therefore to protect themselves from herbivory, partly through chemical defences and, on the other hand, they have to attract and reward pollinating animals in order to sustain this mutualistic interaction (Howe & Westley 1988; Bennett & Wallsgrove 1994). Plants synthesise secondary metabolites that are dangerous for animals and just as dangerous for pollinators and have to use strategies to avoid this complex conflict.

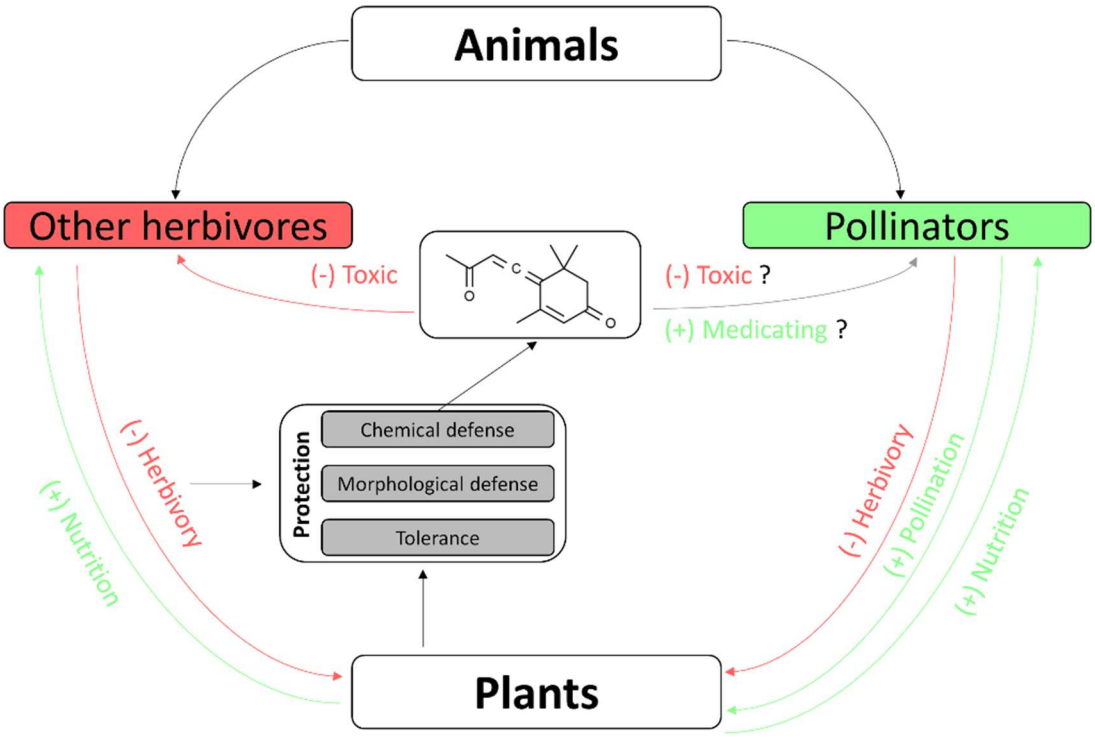


Figure 1. Diagram of the plant-animal interaction dilemma. Representation of the interactions between plants and the two types of herbivores. Among the protective strategies against herbivore damages are the synthesis of secondary metabolites (chemical defences) that are potentially also toxic to pollinators but may also have positive effect. The molecule shown here is 4-(3-oxobut-1-enylidene)-3,5,5-trimethylcyclohex-2-en-1-one (or callunene), a terpenoid (Source: ChemSpider, October 2021).

Green arrows and annotations (+) indicate a beneficial effect for the group pointed to by the arrow. Red arrows and annotations (-) indicate a deleterious effect.

I. 2. Plant secondary metabolites and their link with pollinator immunity and parasites

I. 2.1 Secondary metabolite, the protection and communication pathway of plants

In order to modulate all their interactions, plants synthesise "secondary" metabolites, i.e. metabolites that are not involved in the general development of an organism (*vs.* amino acids, lipids, proteins, nucleic acids for example), but which provide benefits by mediating interactions with the environment and ensuring the continued existence of an organism in its ecosystems (Seigler 1998). They can be divided into several large molecular groups (Table 1 based on Mithöfer & Boland 2012; Verpoorte & Alfermann 2000; Pagare et al. 2015). These secondary metabolites have very diversified roles in plant organisms. As said before, they can participate in defence against herbivores (toxins) but also pathogens (e.g. fungal infections; Bennett & Wallsgrove 1994; Pusztahelyi et al. 2015; Zaynab et al. 2018) or against abiotic environmental stresses such as UV radiations (Li et al. 1993). It is even possible that pollinators also benefit from the bioactive and pharmaceutical character of these molecules (e.g. Manson et al. 2010; Richardson et al. 2015). Pollinators do have access to these metabolites as several studies support a vast array of secondary metabolites in nectar and pollen that vary between plant species (Adler 2000; Irwin et al. 2014; Palmer-Young *et al.* 2019; Stevenson et al. 2020). First, they vary in terms of diversity of molecules, with pollen being on average 63% richer in metabolites than nectar (Cook et al. 2013; Palmer-Young et al. 2019). Second, they vary in terms of concentration which may be highly variable between the different parts of the plant (Price et al. 1980) and with metabolites generally being more concentrated in pollen (Cook et al. 2013; Gosselin et al. 2013; Palmer-Young et al. 2019). The diversity of metabolites found in pollen is numerous and about 200 metabolites have already been counted (Denisow & Denisow-Pietrzyk 2016). Among them are bioactive secondary metabolites from a plethora of different molecule families such as carotenoid pigments, polyphenols, flavonoids, terpenes, alkaloids and saponins (Tomás-Lorente 1992; Wadhawan & Rao 1993; Campos et al. 1997; Szczesna 2006; Pascoal et al. 2014; Barlow et al. 2017).

While many of these compounds in leaves and other tissues have been linked to herbivore *sensus stricto* defences (e.g. Hoffmann-Campo et al. 2001; Chen et al. 2004; Thoison et al. 2004), the role of these metabolites in plant floral resources is still debated and several hypotheses are emerging (Rivest & Forrest 2020). The first suggests that the presence of these compounds in nectar and pollen may simply be a by-product of their synthesis in other organs (i.e. pleiotropy) where they act as a repellent to herbivores (Adler 2000; Strauss & Whittall 2006; Kessler & Halitschke 2009; Adler et al. 2012). The second hypothesis supports an adaptive phenomenon of pollen and nectar protection, in which pollen is protected against overcollection by pollinators (Wang et al. 2019). Both pollen and nectar are also protected against various pathogens that could grow in them, the metabolites acting as antimicrobial agents (Aizenberg-Gershtein et al. 2015; Atsalakis et al. 2017; Schmitt et al. 2021). These different hypotheses are not mutually exclusive and it is likely that their respective importance varies interspecifically (Kessler & Kalske, 2018).

I. 2.2 Bumblebee parasites

Bumblebees suffer from a very large number of extremely diverse parasites at the different levels of their social system (See appendix A for illustrations). The condensed colony system of the bumblebees induces the presence of large food quantities in one place. As a result, this attracts many other parasitic organisms (T. B. Hasselrot 1960; A. Pouvreau 1967; Husband & Brown 1976). Besides, bumblebees themselves can constitute the food resource and can be parasitised extra corporally (Koulianos & Schwarz 1999). Bumblebee internal resources are also a possible source of food. First, there are insects (e.g. flies of the genus *Brachicoma* laying eggs in bumblebee larvae (Vaidya et al. 2018) or of the Conopidae family and several wasps) that lay eggs directly in bumblebees (Schmid-Hempel 1990; Shykoff & Schmid-Hempel 1991; Müller 1994; Moure-Oliveira et al. 2019). Bumblebees can also be parasitised by nematodes (e.g. *Sphaerularia bombi*; Poinar & Van der Laan 1972) as well as a wide range of single-celled organisms such as microsporidia (e.g. *Nosema bombi*; Otti & Schmid-Hempel 2007 & 2008; Van Der Steen 2008) and are also subject to viruses (e.g. Deformed wing virus; Tehel et al. 2016; Wells et al. 2016).

Table 1: Diversity of the main plant secondary metabolite families. The table includes the general structure definition and a non-exhaustive list of the properties of the three largest families of molecules found in secondary plant metabolites. Diversity is the number of different molecules in groups according to Mithöfer & Boland, 2012.

	STRUCTURE	PROPERTIES	DIVERSITY	REFERENCES
Alkaloids	Highly variable but always contain at least one nitrogen atom and often heterocyclic.	• Antimicrobial • Herbivore repellent • Anti-malaria • Anti-cancer • Psychotropic drugs • Analgesic	> 12.000	• Pelletier 1983 • Zhou et al. 1995 • Ziegler et al. 2005 • Achan et al. 2011 • Pagare et al.2015
Phenolic compounds	Metabolites with a phenol group.	• Anti-nematode • Anti-fungal • Insecticide • Rhizobia formation • Colours of many flowers • Anti-oxidant • Anti-inflammatory • Anti-mutagenic • Treatment of cancer • Treatment of Alzheimer	> 9.000	• Dangles & Deluzarche 1994 • Burak & Lmen 1999 • Sreevidya et al. 2006 • Wuyts et al. 2006 • Vermerris & Nicholson 2007 • Brooker et al. 2008 • Lee et al. 2009 • Panche et al. 2016 • Rotariu et al. 2016
Terpenoids	Based on one or more modified isoprene molecules. General formula $(C_5H_8)_n$	• Plant hormonal role • Insecticides • Response to water stress	> 30.000	• Jones 1973 • Duke et al. 1988 Macrae et al. 1993 • Riguera 1997 • Francis et al. 2002 • Hostettmann & Marston 2005 • Campbell 2012 • Pagare et al.2015 • Ludwiczuk et al. 2017 • Kuromori & Shinozaki 2018
Others	Apart from the three families mentioned above constituting the major families, flowering plants possess numerous classes of minor molecules (Pagare *et al.* 2015): (1) **cyanogenic glucosides** (e.g. Fabaceae and Rosaceae), (2) **glucosionates** (e.g. Brassicaceae), (3) **cardenolides** (e.g. Plantaginaceae), (4) **sulphur molecules** (Saito 2004), (5) **modified amino acids** (Pagare *et al.* 2015), (6) **Polyketides (**variable assembly of methylene and carbonyl groups (McNaught 1997) and (7) **steroids** in Ranunculaceae (Mithöfer & Boland 2012). Plants may also contain **silica** (Poaceae) or different **latex** (Papaveraceae and Euphorbiaceae; Mithöfer & Boland 2012).			

I. 2.3 Immune and defence ability of bees

Insects possess an internal immune response to defend themselves from threats (Brown et al. 2003a; Mallon et al. 2003; Fowler et al. 2020). This system consists of a strong innate and constitutive part composed of two response pathways, the cellular (phagocytosis, nodulation and encapsulation by haemocytes) and the humoral (phenoloxidase and antimicrobial peptide (AMP) production systems) responses (Kaslow & Welburn 1996; Söderhäll & Cerenius 1998; Rosales 2017). This system is mainly related to one organ, the fat body, whose mass is associated with the immune response since this organ produces and releases AMPs for example (Wilson-Rich et al. 2008; Rosales 2017). Beyond their immune system, bumblebees are able to modify their behaviour and their consumption of resources when parasitised. Floral choices could be modified towards species with secondary metabolites of positive interest in their nectar and pollen. A phenomenon of self-medication could therefore be employed in order to decrease infection level of the parasite (Lozano 1988; Baracchi et al. 2015). The assimilation of such compounds could then prevent the contamination by the parasite (i.e., prophylactic effect; Abbott 2014; Koch et al. 2019; De Roode et al. 2013; De Roode & Hunter 2019) or participate in the decrease of the parasite presence when it is already installed and thus post-infection (i.e., therapeutic effect; Raberg et al. 2007; Abbott 2014; De Roode et al. 2013; De Roode & Hunter 2019). Some of these compounds have no effect on the parasites but may still have positive effects on pollinators leading to parasite tolerance (De Roode & Hunter 2019). This phenomenon of self-medication is also expressed at the colony level this time for social bees leading to social-medication. The whole resource harvesting of the colony by workers can be shifted to more interesting plant species in the presence of infected members (Gherman et al. 2014; Baracchi et al. 2015; Spivak et al. 2019). Excluding infected members (Arathi et al. 2000) or relocating the colony (Drees et al. 1992), are other behaviours that can also contribute to colony recovery (reviewed in Cremer et al. 2007).

I. 2.4 Plant metabolites and parasitic infections in bumblebees

Some studies have highlighted different molecules that have an effect on bumblebee parasites (Manson et al. 2010; Richardson et al. 2015). Exposing bumblebees to a diet of nectar of one species rich in alkaloids (i.e., gelsemine, anabasine or nicotine) can reduced the parasite load by up to 81% (Tadmor-Melamed et al. 2004; Manson et al. 2010; Richardson et al. 2015). Apart from alkaloids, two other molecules, namely thymol, a terpenoid for example found in *Tilia europea* (Guyot et al. 1998) and catapol, a glycosic iridoids (e.g. from *Chelone glabra,* Plantaginaceae), have also shown a significant impact on parasitic load in bumblebees (Richardson et al. 2015). In addition to metabolites found in nectar, it has also been shown that metabolites present in pollen can have an influence against infestation (Giacomini et al. 2018). This phenomenon has also been observed for other pollinators since a parasite of the honeybee (*Apis mellifera*), *Nosema ceranae*, is also negatively affected by the metabolites present in the nectar (Gherman et al. 2014) and the pollen (Giacomini et al. 2018) of sunflowers (*Helianthus annuus*). It suggests that this medicinal phenomenon might be more spread among apoid pollinators and might be effective against other types of parasites, which may result in a key mechanism for the health of pollinators.

The plant-derived secondary metabolites may have damaging effects on parasites. The mechanisms underlying these effects are however much less known. According to Manson et al. 2010 and Richardson et al. 2015, several hypotheses can be considered:

(1) The molecule could directly affects the parasite, inducing cell death or a delay in development associated with the parasite degree of tolerance to the molecule (Manson et al. 2010). This mechanism is already known in *Trypanosoma sp.* for several molecules belonging to the phenol, alkaloid and terpenoid groups (Merschjohann et al. 2001; Rosenkranz & Wink 2008). Similarly, these metabolites could affect the metacyclogenesis of the parasite blocking its development (Cardoso & Soares 2010).

(2) Consumption of the molecule could alters the living environment (e.g. the bumblebee gut for gut parasite). The pH or other physiochemical properties of the living environment could be modified by these molecules inducing the loss of the parasite normal ability and its elimination or weakening (Stiles & Paschke 1980; Logan et al. 2005). The microbiota may also be affected

and play a stronger role in the protection against the pathogen (Koch & Schmid-Hempel 2011; Cariveau et al. 2014; Näpflin & Schmid-Hempel 2018; Castelli et al. 2020).

(3) The physiology of the bumblebee in the presence of the secondary metabolite could be affected. The presence of potentially toxic molecules such as alkaloids leads to an increase in excretion rate, which may increase the ejection of parasites (Tadmor-Melamed et al. 2004; Despres et al. 2007). The immune system of bumblebees could also be improved in the presence of these metabolites participating in the fight against the parasite (Schmid-Hempel 2005).

I. 3. Biological models

I. 3.1 *Bombus terrestris* (L., 1758)

Bumblebees are insects in the order of Hymenoptera, which includes bees, ants and wasps. More specifically, bumblebees belong to the family Apidae, one of the seven families (i.e., Colletidae, Halictidae, Andrenidae, Melittidae, Megachilidae, Stenotritidae and Apidae) of bees in the world (Michener 2000, Danforth et al. 2013). The Apidae family is distinctive since it contains the three most eusocial genera of bees (i.e. living in colonies with a division of labour and several castes) namely *Melipona*, *Apis* (including *A. mellifera*, the European honeybee) and *Bombus*, the latter including all the bumblebees (Michener 2000). The *Bombus* genus comprises more than 250 species (Williams 1998; Michener 2000) with a distribution covering almost the entire northern hemisphere and up to South America but relatively absent from Africa and Oceania (Michener 2000). These bees as the others are declining worldwide (Williams & Osborne 2009). Bumblebees are very efficient pollinators (Willmer et al. 1994; Kameyama & Kudo 2009; Kudo et al. 2011) and even one of the most economically important pollinator groups in temperate regions (Potts et al. 2010). They are mostly generalist (e.g. *B. terrestris* studied in this project, Goulson 2010) but some are highly specialised species such as *B. gerstaeckeri* or *B. consobrinus* who can only be found on the plant genus *Aconitum* (Loken 1973; Jeppsson 2004; Dellicour et al. 2015). *Bombus terrestris* was selected for this study because of its ease of rearing due to its great adaptability (Kleijn & Raemakers 2008). It is also an easily available species since it is commonly exploited commercially and used by farmers to promote pollination in their fields or in greenhouse crops such as tomatoes, melons and

cucumbers to increase their yield (Lee et al. 2012; Sirali et al. 2012). Some companies are thus specialised in breeding and selling these bumblebees (e.g. Biobest) to farmers and researchers. More information on bumblebee colony life cycle and organisation see Appendix B and C.

I. 3.2 *Crithidia bombi* Lipa & Triggiani, 1980

Several species of the *Crithidia* genus are known to be detrimental to bees in general; e.g. *C. mellificae* or *C. bombi* (Baer & Schmid-Hempel 2001; Runckel et al. 2014; Strobl et al. 2019; Ngor et al. 2020). *Crithidia bombi* (Euglenozoa; Lipa & Triggiani 1980) is a protozoan parasite of bumblebee intestines (but may also occurs in other bee genera, Figueroa et al. 2021). *C. bombi* has three forms including two mobile flagellated forms, the promastigotes, a genus-specific form called choanomastigote and a sessile and anchored form called amastigote (Olsen 1986; Logan et al. 2005; Schmid-Hempel & Tognazzo 2010). The life cycle of *C. bombi* oscillates between these three forms. It begins when a bumblebee ingests nectar contaminated on flowers or in colonies by the faeces of another contaminated bumblebee or by direct contact with another contaminated individual (Durrer & Schmid-Hempel 1994; Otterstatter & Thomson 2007; Ruiz-González et al. 2012; Deshwal & Mallon 2014). The development and infection cycle of *C. bombi* is still poorly understood and more studies will be needed in the future to fully describe it. Nevertheless it is known that the parasite stabilises in its amastigote form in the gut probably by embedding itself via its flagellum (Koch et al. 2019). It then takes there the nutrients from the host and reproduces, producing new infecting cells 3 to 4 days after infection (Olsen 1986; Schmid-Hempel & Schmid-Hempel 1993). *C. bombi* is a very common and widespread parasite in bumblebee populations (Cordes et al. 2012). Moreover, it has a high infestation capacity and contaminated individuals can quickly lead to a general contamination of their colony of up to 80% of the individuals (Imhoof & Schmid-Hempel 1999; Erler et al. 2012). Fortunately, in contrast to other parasites such as *Apicystis bombi* and *Nosema bombi*, whose infestations can lead to rapid death of colonies (Schmid-Hempel 1998; Schmid-Hempel 2001), *C. bombi* is thought to be less dangerous and to cause problems only in stressful situations (Schmid-Hempel 2001; Brown et al. 2003a; Deshwal & Mallon 2014) However, *C. bombi* infestations can still lead to highly variable and diverse adverse effects on individuals and on the colony as a whole (Table 2).

Table 2: Detrimental effects of *C. bombi* on *B. terrestris*.

CATEGORY	*C. BOMBI* EFFECT	REFERENCES
WORKER	• Pollen collected less effectively • The choice of best floral resource species is disturbed • Difficulty to handle complex flowers • Risk of starvation • Increased mortality in stressful conditions • Reduced lifespan	• Schmid-Hempel and Schmid-Hempel 1991 • Schmid-Hempel 1998b • Schmid- Hempel & StauVer 1998 • Brown et al. 2000 • Brown et al. 2003a • Otterstatter et al. 2005 • Gegear et al. 2006
QUEEN	• Weakness and weight loss • Less efficient ovaries • Workers reproductive activity is less suppressed and they quickly compete with the queen	• Brown et al. 2003a • Erler et al. 2012
COLONY	• Lower growth rate • Shorter life span • Globally weaker and quickly infected • Sexuals produced later • Less energy stored to get through the hibernation phase and resulting colonies the following spring are poorer in workers	• Schmid-Hempel 1998b • Shykoff & Schmid-Hempel 1991 • Schmid-Hempel 2001 • Erler et al. 2012 • Schmid-Hempel 2001

I. 3.3 *Calluna vulgaris* (L.) Hull, 1808

Calluna vulgaris, commonly called heather, is a plant species of the Ericaceae family (Figure 2A; Gimingham 1960). The stamens of this species are poricidally dehiscent, requiring vibratory pollination, also known as buzz pollination, to extract the pollen. Only a few pollinators, including bumblebees, are able to extract pollen efficiently from these flowers (Buchmann et al. 1983). It is a species establish from Spain to Scandinavia in Europe as well as in North America (Diemont et al. 2013). This species is characteristic of communities called

heathlands which have been in serious decline for two centuries as a result of anthropogenic activities and are getting increasingly rare (Figure 2B and 2C; Gimingham 1972; Webb 1998; Piessens & Hermy 2006; Diemont et al. 2013). These communities are home to a wide variety of pollinators, mainly bees and syrphids (Descamps et al. 2015). The number of insect species reported to use *C. vulgaris* for feeding has been reported in different studies to be 61 in The Netherlands (Beijerinck 1940), 50 in Belgium (Mahy et al. 1998; Bacchetta 2014) and 57 in the south of France (Descamps et al. 2015), showing the very generalist character of this species and its involvement in many insect feeding and especially summer insect species (Gimingham 1960). They are also the unique habitat of some species such as *Bombus jonellus* and *Bombus monticola*, bumblebees that are endangered and decreasing with their habitats (Rasmont et al. 1993). *C. vulgaris* is also known for its pharmaceutical effects and is used as a medicinal herb in traditional medicine (Tunon et al. 1995; Calliste et al. 2001; Kumarasamy et al. 2002; Kraus et al. 2007). This plant contains indeed high concentrations of secondary metabolites (reviewed in Monschein et al. 2010; Jalal et al. 1982). This species was chosen for the studies because Koch *et al.* demonstrated in 2019 the inhibitory effect on the parasite of the 4-(3-oxo-1-butynyl)-3,5,5-trimethylcylohex-2-en-1-one or callunene (Guyot et al. 1999; Dimitrova et al. 2006; Koch et al. 2019) a terpenoid naturally found on heather nectar. Bumblebees are naturally in contact with this molecule at a concentration higher than the determined parasite LD50 (23ppm or 113 µM, Koch et al. 2019) and may profit of its effect. This molecule is responsible for the loss of the parasite flagella which could therefore no longer complete its cycle and duplicate itself (Koch et al. 2019).

I. 3.4 Flavonoids

Among the many secondary metabolites, the group of flavonoids from *C. vulgaris* was chosen. This choice is based on the pre-screening of metabolites found in pollen and leaves where were found these molecules in large quantities. Callunene was not selected due to its absence in other tissues and resources than nectar and only presence in low concentration in pollen (Koch et al. 2019) which were confirmed here. Flavonoids belong to the group of phenolic compounds (i.e., including metabolites with a phenol group in their molecular structure; Vermerris & Nicholson 2007; Panche et al. 2016) and are composed of two aromatic rings connected by three carbon atoms, the latter most often forming a heterocycle. There are

several classes of flavonoids, including the anthocyanins (Dangles & Deluzarche 1994), which are responsible for the colours of many flowers which can be pH-affected (Rotariu et al. 2016). Flavonoids are also highly bioactive compounds with numerous medicinal applications such as anti-oxidant, anti-inflammatory and anti-mutagenic properties (Burak & Lmen 1999; Panche *et al.* 2016) as well as cancer and Alzheimer's disease treatment (Burak & Lmen 1999; Lee et al. 2009). The action mechanisms of these flavonoids are varied. They are able to chelate metals or stabilising peroxide radicals and reactive oxygen species (Sarni-Manchado & Cheynier 2006). They can also interact with enzymes inducing their inhibition and various consequences (e.g. interactions with cyclins and cell cycle disruption; Casagrande 2000).

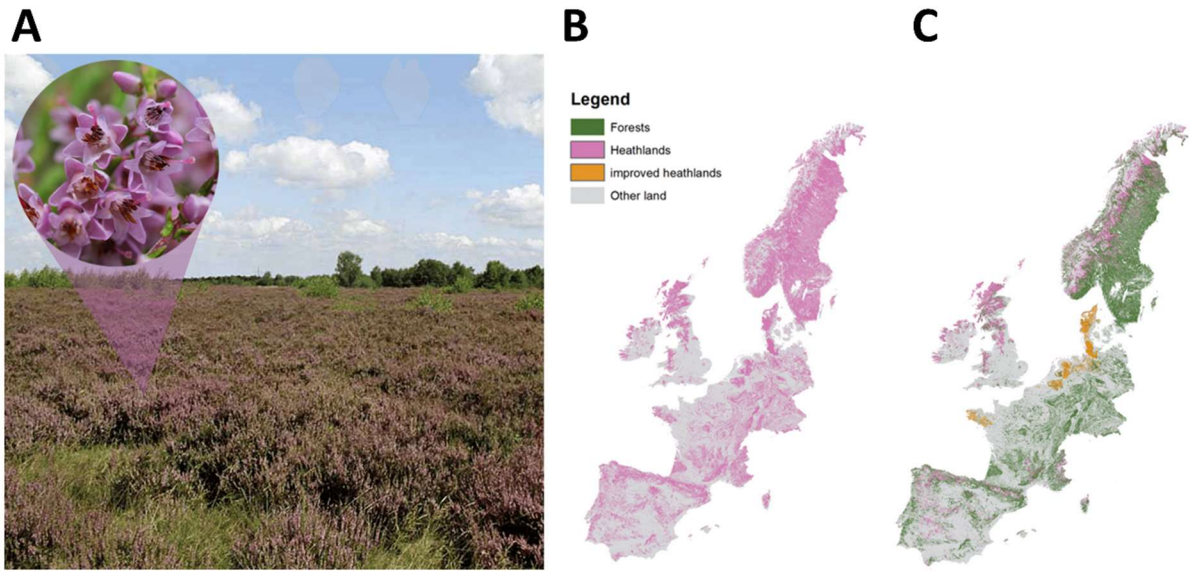


Figure 2. A. Photo of a typical Heathland, here in the Drover Heide natural reserve in Germany (Photo: R. Mause, Source: Bruce et al. 2010). The inset image shows a detail of the flower of *Calluna vulgaris*. **B.** Assessment of the extent of heathland in the early 19th century and **C.** in present showing the rapid decline of this ecosystem type in recent centuries (Source: Diemont et al. 2013).

II. Biological questions

This study is part of the METAFLORE project carried out by a cooperation of three UMONS laboratories; the Laboratory of Zoology (Prof. P. Rasmont and Prof. D. Michez), the Laboratory of Organic Synthesis and Mass Spectrometry (Prof. P. Gerbaux) and the Laboratory of Therapeutic Chemistry and Pharmacology (Prof. P. Duez). The METAFLORE project aims to identify plants with beneficial properties to pollinators, thereby improving their general health. In the previous academic year, two students had already carried out studies in this project: A. Gekière studied the phenolamides of sunflower (*Helianthus annuus*) and M. Begou studied the flavonoids of hawthorn (*Crataegus monogyna*). This year, through this master's thesis focusing on heather (*C. vulgaris*) and A. Michel's one working on the cherry tree (*Prunus avium*), we continued to seek to identify plant secondary metabolites potentially interesting for the health of pollinators and we carried out bioassays to test the effects of their secondary metabolites on them.

In order to better understand the plant-pollinator-parasite interaction, the study was divided into four distinct biological questions (Figure 3):

1. Does the secondary metabolites profile vary within the tissues and floral resources?

We aim to identify secondary metabolites in heather and to test for variation in the diversity and concentration of these compounds across its tissues and floral resources. We hypothesize that these compounds are specialized across the plant to promote pollinator interaction and repel other herbivores.

2. How do different flavonoid profile affect the pollinator?

We want to identify the impacts and potential differences of pollen and leaf flavonoid uptake for bumblebees at the individual and microcolony level. As for others molecules we expect flavonoids to act as toxins for bumblebees.

3. What is the impact of heather pollen on the pollinator?

We will determine, in comparison to a control willow pollen, how heather pollen will influence the bumblebee at the individual and semi-social (microcolony) level. We aim to evaluate the impact of its nutritional quality and its secondary metabolite profile on the

bumblebee. We presumed a different nutritional quality between these pollens due to their different molecular composition.

4. Can heather pollen and its flavonoids influence parasitised pollinator?

We first describe again the impact of *C. bombi* on bumblebee at the individual and microcolony level. We then studied how bumblebees fed with different diets evolve in the presence of the parasite and we hypothesise that the secondary metabolites present in our diets could affect the extent of the parasite effects.

If we do find a positive effect of these metabolites on the health of *B. terrestris* (good nutritive properties or effect against the parasite), we will be able to promote the addition of *C. vulgaris* in bee conservation strategies, where, together with other plants, it could participate in the creation of natural pharmacies for wild or domesticated bumblebees, and by extension, maybe also for other apoids. Furthermore, we will add an argument to promote the conservation of the increasingly threatened heathlands.

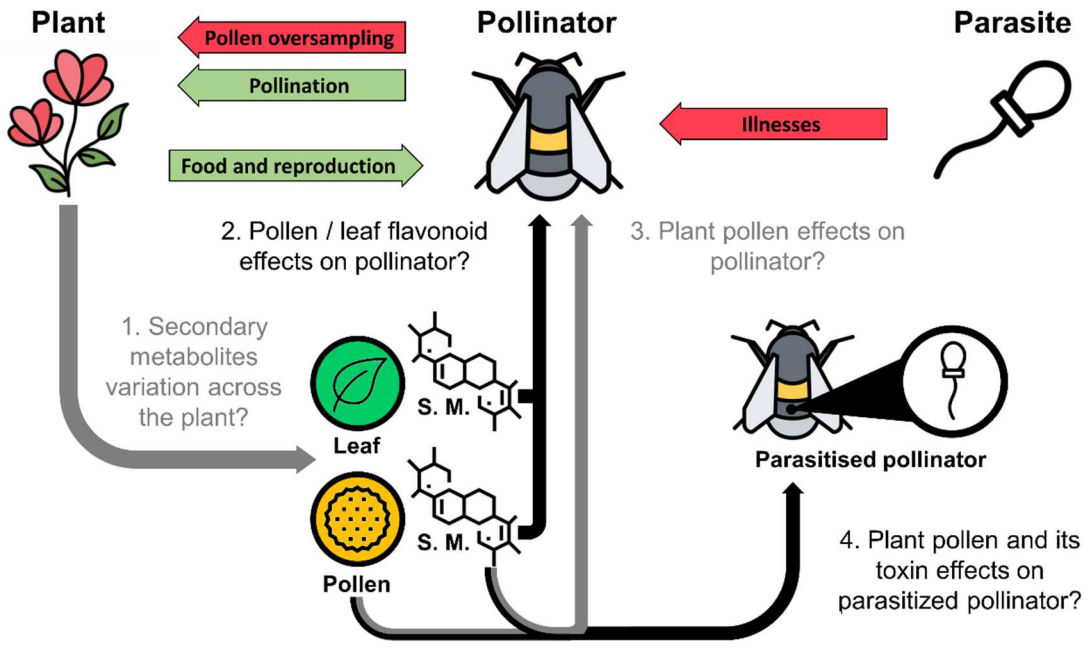


Figure 3. Summary of the biological questions of the project. Red arrows indicate a negative impact, green arrows a positive impact of one organism on the other. S. M. refers to secondary metabolites.

III. Materials and Methods

III. 1. Sampling and screening of secondary metabolites

III. 1.1 Sampling of plant material

To perform sampling for metabolite screening, five distinct heather individuals were selected on the same station (Be., Brabant Wallon, Virginal-Samme, Bois de la Houssière, WGS84: 50° 37' 54.67" N 4° 11' 26.14" E, 135m altitude). Two inflorescences per individual were isolated in a tulle to prevent pollinators from collecting nectar and pollen (following Koch et al. 2019). The sampling was carried out several days from 25 August to 2 September 2021 to allow sufficient amount of plant material. Sampling was always performed in the following order: nectar, pollen, corolla and finally leaf. Therefore following an ascending order of destruction of the individuals in order to limit the activation of the plant defence mechanisms against herbivory, which could influence the results. Nectar was collected early in the morning with 1µl microcapillaries inserted at the base of the corolla in the flower (sampling of around 30min for a total of 10µl). Pollen was collected by gently tapping the flowers on a glass screen and then scraping the pollen into an eppendorf with a scalpel. To obtain pollen in larger quantities, stamens were also dissected out and then placed in an incubator at 26°C for several days (see Vaudo et al. (2020) for further details). Petals were collected through one hour long dissection of the flowers. Large-scale sampling of leaves (400g) and pollen (400g) was also conducted in order to perform mass flavonoid extraction. Heather pollen was obtained from a private beekeeper (Dittlo François, France, Gironde, Le Nizan) and collected massively with pollen load traps set on beehives in heathlands. Pollen loads were sorted by hand on the basis of the colour of the pollen loads after identified heather corresponding colour thanks to palynological analysis (Sawyer 1981; Dafni et al. 2005, Figure 4). Petals and leaves (for screening and large scale sampling) were placed directly in liquid nitrogen after sampling to prevent any degradation of the molecules and then stored at -80°C. They were next freeze-dried during one week (CHRIST® Alpha 1-2LDplus). The 400g of leaves (large-scale sampling) were also crushed (Polymix® PX-MFC 90 D; 6000 rpm).

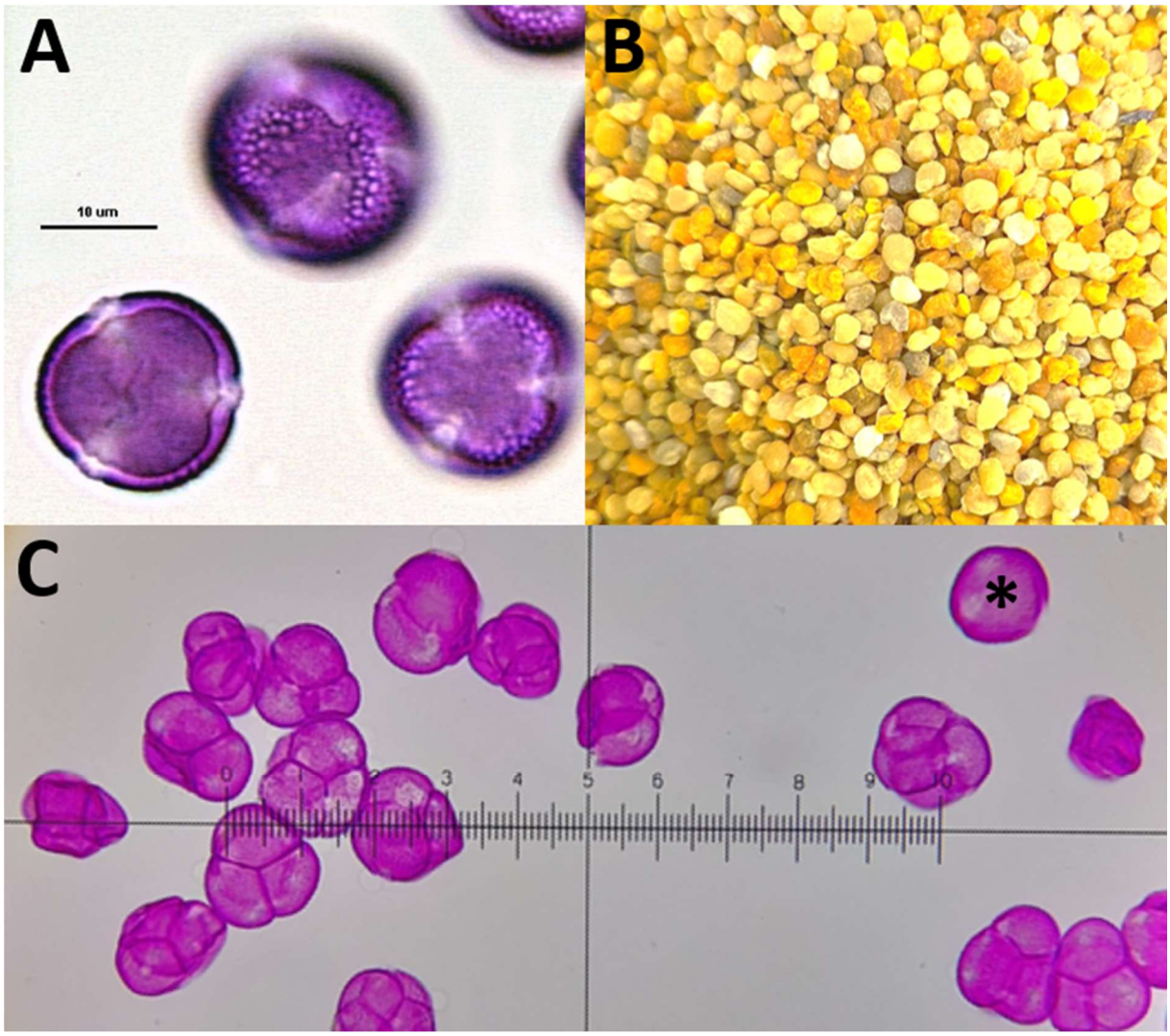


Figure 4. Palynological analysis carried out on the pollen loads used as diet basis. A. Pollen grains of willow (*Salix sp*.), from pollen loads get at the association *Ruchers de Lorraine*. **B.** Pollen batch to be sorted containing partly heather pollen loads. **C.** Pollen grains of the heather (*C. vulgaris*) defining pollen loads to be used in the experiment (grey colour). (*) Example of other pollen: here *Hedera helix*. One unit on the scale (i.e., 10 graduations) is equivalent to 25µm.

III. 1.2 Metabolite screening

The dry samples (i.e., leaves, pollen and petals) for the metabolite screenings were first ground (Retsch® Mixer Mill MM 400; 30Hz; 2x1 min) and then suspended in a 70% methanol solution before being centrifuged (Sigma 2-16P; 4500rpm; 10min). The supernatant was collected and filtered (Pall Acrodisc Syringe Filter 0.2µm) and 500µl of the filtrate was dried before being resuspended in 1ml of 70% methanol solution. For the nectars, the samples were

centrifuged (Sigma 2-16P; 1000rpm; 1min), 8 to 10mg of nectar was then diluted in 1ml of a similar 70% methanol solution. These solutions are then used for compound identification. High performance liquid chromatography associated with tandem mass spectrometry (HPLC-MS/MS) was used to identify the metabolites present in samples. Liquid chromatography was performed on a Waters™ Alliance 2695 (flow rate: 0.25mL/min; column temperature: 40°C; autosampler temperature: 20°C) using a Phenomenex® Kinetex C18 EVO column (150 × 2.1mm i.d., 100Å particle size) for separation and with a mobile phase composed of methanol (A) and water + 0.01% formic acid (B) in the following gradient: A = 10%, B = 90% at t = 0min; A = 30%, B = 70% at t = 6min; A = 35%, B = 65% at t = 11min; A = 50%, B = 50% at t = 18min; A = 90%, B = 10% at t = 23min; A = 100%, B = 0% at t = 25min; A = 100%, B = 0% at t = 27min, A = 10%, B = 90% at t = 30min. All solvents are of HPLC grade and from VWR. The spectrometer used for pollen, leaves and corollas was a Waters Q-ToF US (positive and negative electrospray (ESI) modes) targeting a mass range of 50 to 2,000Da. The parameters were as follows; source temperature: 120°C; desolvation gas temperature and flow 300°C and 500L/h; capillary voltage 3.1kV, cone voltage 30V and scan time 0.5sec. For nectar, the mass spectrometer was the Synapt G2-S*i* (ESI) coupled to the Acquity UPLC H-Class system. The parameters were the same, except for the capillary voltage which was 2.5kV, and the cone voltage which was 40V. The identified molecules were finally quantified in triplicates by LC-MS (in ESI-) using quercetin (Sigma-Aldrich) as standard for flavonoids.

III. 1.3 Massive flavonoid extraction

In order to supplement the diets with flavonoid extracts, mass extractions of these molecules were performed on the 400g of leaves and pollen collected. The extraction was performed by a Soxhlet extractor (temperature: 100°C; solvent: methanol; duration: 30h), the extracts were then vacuum filtered and evaporated (rotavapor IKA RV8). Metabolites in these extracts were first screened (20-40mg dissolved in 1ml methanol) using the similar HPLC-MS/MS protocol to allow quantification of flavonoid content. The remaining extracts were then dissolved via a mixture of 50% ethanol and 50% water and reduced to a concentration similar to the natural concentration to be used as a supplement in the treatments (see Appendix D).

III. 2. Bumblebee bioassays

III. 2.1 Experimental design

To assess the biological effect of secondary metabolites present in the different tissues and floral resources of heather on bumblebees and its parasite effects, several bioassays were performed. Queen-less microcolonies were exposed to specific diet treatments (protocol widely used to study the effects of pollen and its metabolites on healthy or infected bumblebees, e.g. Richardson et al. 2015; Vanderplanck et al. 2018; 2019 and 2020; Brochu et al. 2020). The different treatments are the following: microcolonies fed with control pollen (i.e., willow pollen) containing either (i) parasitised or (ii) non-parasitised bumblebees*;* microcolonies fed with heather pollen containing either (iii) parasitised or (iv) non-parasitised bumblebees; microcolonies fed with control pollen supplemented with extracts of flavonoids from heather pollen containing either (v) parasitised or (vi) non-parasitised bumblebees; as well as microcolonies fed with control pollen supplemented with extracts of flavonoids found in heather leaves containing (vii) non-parasitised bumblebees. Diets (i) and (ii) allowed us to establish the reference baseline of the microcolonies with or without the presence of the parasite and to check for parasite effects. Diets (iv) and (vi) allowed us to assess the effects of heather pollen and its metabolites on microcolonies. Diets (iii) and (v) allowed us to characterise whether the influence of the parasite is modulated by the consumption of heather pollen and its metabolites. Finally, diet (vii) allowed us to highlight the potentially detrimental impacts of heather leaf flavonoids on microcolonies. Although a secondary metabolite-free artificial diet would be ideal as a control treatment, a previous pilot study highlighted the absence of such a diet (Gekière 2021). Willow pollen (*Salix* sp.) was therefore used as control, which is known to be used by queens and early spring workers, as well as for its quality in terms of nutrient supply (Aupinel et al. 2000; Vanderplanck et al. 2014).

III. 2.2 Rearing condition

For each of the seven treatments, a set of ten replicates (i.e., ten microcolonies) was set up and analysed (total number of microcolonies = 70). Each microcolony was initiated with five workers from one of five different queen right colonies (colonies from Biobest *bvba*; i.e., 14 microcolonies derived from each queen right colony), so that among the ten microcolonies

per treatment came from different queen right colonies (i.e., two microcolonies derived from every queen right colony). If one of the workers died over the course of the experiment, it was replaced by another from the same queen right colony and marked with a colour dot for recognition. The microcolonies were kept in plastic boxes (10 x 16 x 16 cm) with only a few apertures to allow ventilation but to prevent the passage of bumblebees (Figure 5; Regali & Rasmont 1995). The microcolonies were reared at the University of Mons (Belgium, Mons, Campus de Nimy, WGS84 50°27'54.9"N 3°57'24.9"E) in the dark and handled under red light (allowing the bumblebees to be handled without them seeing us). The rearing room had a constant temperature of 26-28°C and a relative humidity of 65%.

As a surrogate for nectar, bumblebees were provided with sugar syrup (water/sugar 35:65 w/w) *ad libitum* placed under the microcolony in a container connected by a capillary (Figure 5). The microcolonies were fed with their treatment-corresponding pollen diet for 35 days. The pollen candies given to microcolonies were between 1 and 4g depending on the size of the microcolony. The willow (*Salix* sp.) pollen was supplied by the company *Ruchers de Lorraine*. The two sets of pollen (i.e., willow and heather) were individually homogenised, crushed and mixed with a 65% sugar solution. For the supplemented diets, willow pollen was mixed with solutions containing flavonoid extracts from either heather pollen (see treatment (v) and (vi) in section 2.a) or leaves (see treatment (vii) in section 2.a). In order to determine the concentrations to be supplemented, a screening of the metabolites present in the two pollen sets (i.e., willow and heather) was also performed via HPLC-MS/MS as described previously. The results of this screening and the one describing the metabolites present in the leaves (see Appendix E), allowed us to define the concentrations to be added to willow pollen in these treatments in order to reflect the concentrations found *in natura* (see Appendix D). For each treatment, the pollen and nectar candies were changed and weighted every two days to avoid bacterial development and degradation of the metabolites studied.

III. 2.3 Parasite pool and inoculation

In order to have a supply of *Crithidia bombi* for experiments, wild queens of *B. terrestris* were collected in March 2021 (Mont Panisel, Mons, Belgium). The faeces of these queens were observed under a light microscope (400X magnification; BA210, Motic) in order to screen for the presence of *C. bombi*. The faeces of the infected queens were collected, mixed in a 50%

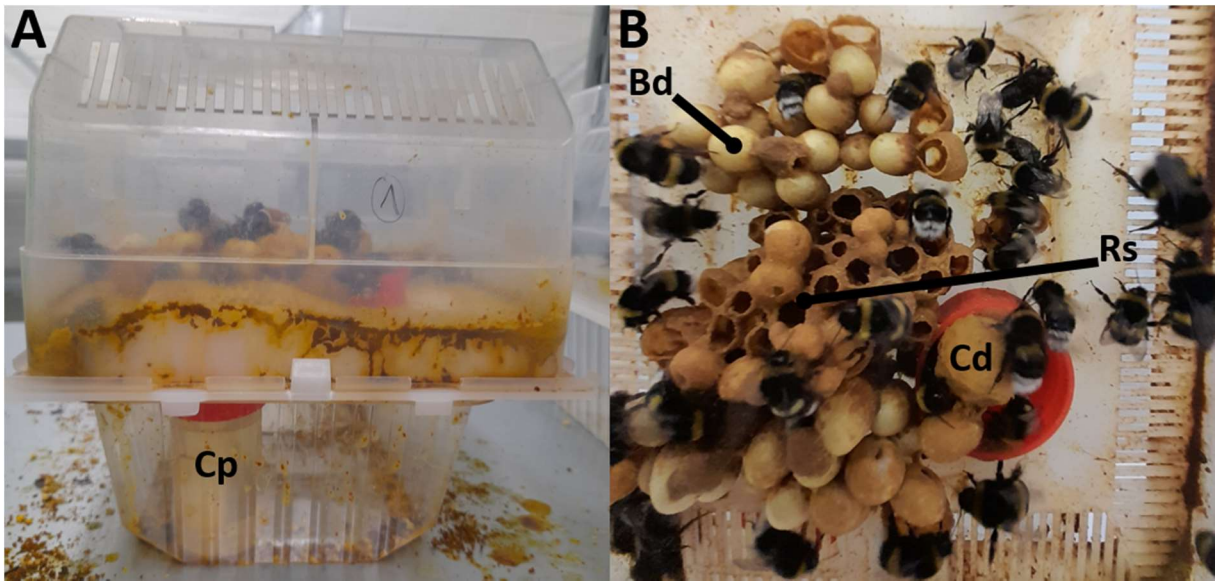


Figure 5. Microcolony used in bioassays. All microcolonies are composed of a box (10 X 16 X 16 cm) where 5 workers develop their colony. The box is connected by a capillary available for bumblebees to syrup and pollen candies are provided every two days. **A.** Side view of the microcolony structure. **B.** Top inside view showing the microcolony development. **Cp.** capillary for syrup. **Cd.** pollen candy. **Bd.** Brood containing offspring. **Rs.** Honeypot and pollen used as a reserve.

sugar solution and placed in a recipient in colonies from Biobest *bvba* every two days, for 12 times. Three colonies developed a high level of infestation and thus constituted the *C. bombi* reservoir for Antoine Gekière's and Martin Begou's Master's theses. New Biobest *bvba* colonies were repeatedly inoculated with contaminated faeces from these reservoir colonies throughout the year in order to ensure a turnover of the available *C. bombi* pool. From the three primary colonies, *C. bombi* infestation was transferred six times until this research. To implement the parasitised microcolonies, the faeces of a total of 45 workers from these reservoirs were collected and pooled (to ensure multiple-strain *inocula*), homogenised and brought to 1ml with 0.9% NaCl solution. The *inocula* were then purified by a triangulation method following Baron et al. (2014) and Martin et al. (2018). Briefly, this method is based on the principle of countercurrent distribution chromatography and consists of a succession of centrifugation and resuspension to separate the *C. bombi* cells from the rest of faecal content. The concentration of *C. bombi* in the purified solution was then estimated by counting with a Neubauer chamber (see protocol below) and diluted to 2,500 *C. bombi* cells/µl with a 40% sugar solution. Workers allocated to the infected microcolonies were placed in individual

Nicot® cages and given 10µl of the inoculum meaning 25,000 *C. bombi* cells (Logan et al., 2005) after a 5-hour starvation period. Workers allocated to non-infected microcolonies were also placed in these cages and starved to ensure a similar stress among between treatments.

III. 2.4 Monitoring and measured parameters

III. 2.4.1 Microcolony development

To investigate the impacts of the molecules, the diets and the parasite, several developmental parameters in microcolonies were measured (Tasei et al. 2008b). In microcolonies, the lack of a queen and its pheromones makes oviposition possible by workers (Röseler 1970). One of the workers then matures the ability to lay eggs and initiates the development of the microcolony by producing offspring (only males due to the absence of fertilisation, Génissel et al. 2002). The parameters measured were the following: (i) worker mortality and (ii) the number of ejected larvae. These parameters were recorded every two days. In addition, at the end of the 35-day period, all microcolonies were dissected to establish and compare offspring production (i.e., number and total mass of eggs, non-isolated larvae, pre-defecating larvae, post-defecating larvae, pupae, non-emerged and emerged males, Goulson 2010).

III. 2.4.2 Resource collection

The collection of (i) pollen and (ii) syrup was measured every two days. Each new syrup pot and pollen candy was weighed before being introduced into the microcolony and reweighed when replaced to calculate the resource uptake by the microcolony. In order to consider the mass loss due to evaporation and apply a correction factor on analyses, a microcolony without bumblebees with a syrup pot and every pollen diets was used. It was also monitored every two days. With these last parameters, the ratio between the collection of syrup and pollen was calculated to define (iii) the pollen dilution. Similarly, the ratio between the mass of offspring produced and pollen collection was calculated to define (iv) the pollen efficiency (Tasei et al. 2008b).

III. 2.4.3 Parasite load

The parasite load of microcolonies was measured continuously during the 35 days of the experiment (Figure 6). The first measurement was made 3 days post-inoculation (D4), to enable

C. bombi to multiply and ensure its presence in the faeces (Logan et al. 2005). A total of seven further measurements were then taken, first every other day (D6, D8, D10 and D12) to best describe the infestation curve of *C. bombi*, and then at larger intervals during the plateau phase (D16, D20 and D35). The parasite load by microcolony was measured for a single marked worker. In case of death of the marked worker (happened eight times), a second marked worker was used and avoiding the replacing workers. The individual to be analysed was held in a 50ml Falcon in the light until the faeces were expelled. The same manipulation was performed for non-parasitised microcolonies to ensure homogeneity of stress among treatments. Faeces were collected in a 10µl microcapillary tube and diluted two to ten times with distilled water to enable rational cell counting. Parasite cells present were then counted using a haemocytometer (Neubauer) under an inverted phase contrast microscope (400X magnification, Eclipse Ts2R, Nikon; Figure 7). In order to confirm the absence of cross-contamination between microcolonies, at the end of the 35-days experiment, faeces were sampled and analysed of one individual per non-parasitised microcolony to confirm the absence of parasites. No parasite cells were detected in the faeces of individuals from uninfected microcolonies.

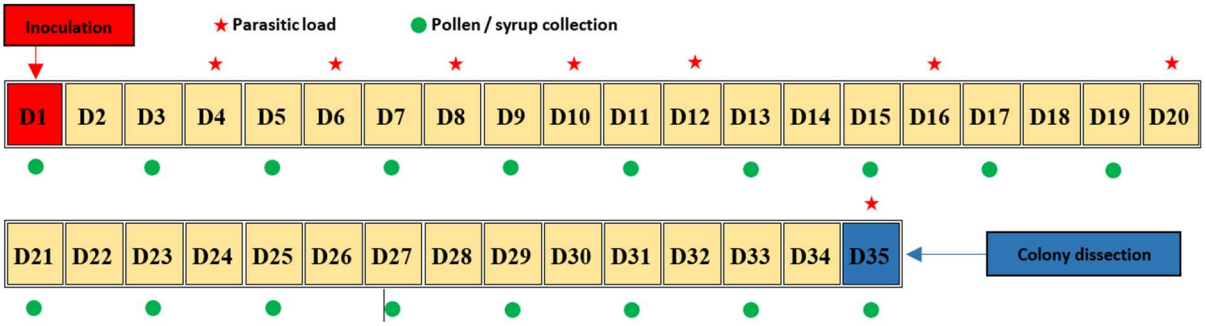


Figure 6. Organisation of microcolony monitoring. The microcolonies are subjected to the treatments for 35 days from the time of inoculation. During these 35 days pollen (candy) and syrup was changed every two days and weighed in order to monitor resource consumption. From three days post-inoculation parasite loads were measured first every two days and then at longer intervals (eight measurements in total). At the end of the treatment period, the colonies were sacrificed and dissected.

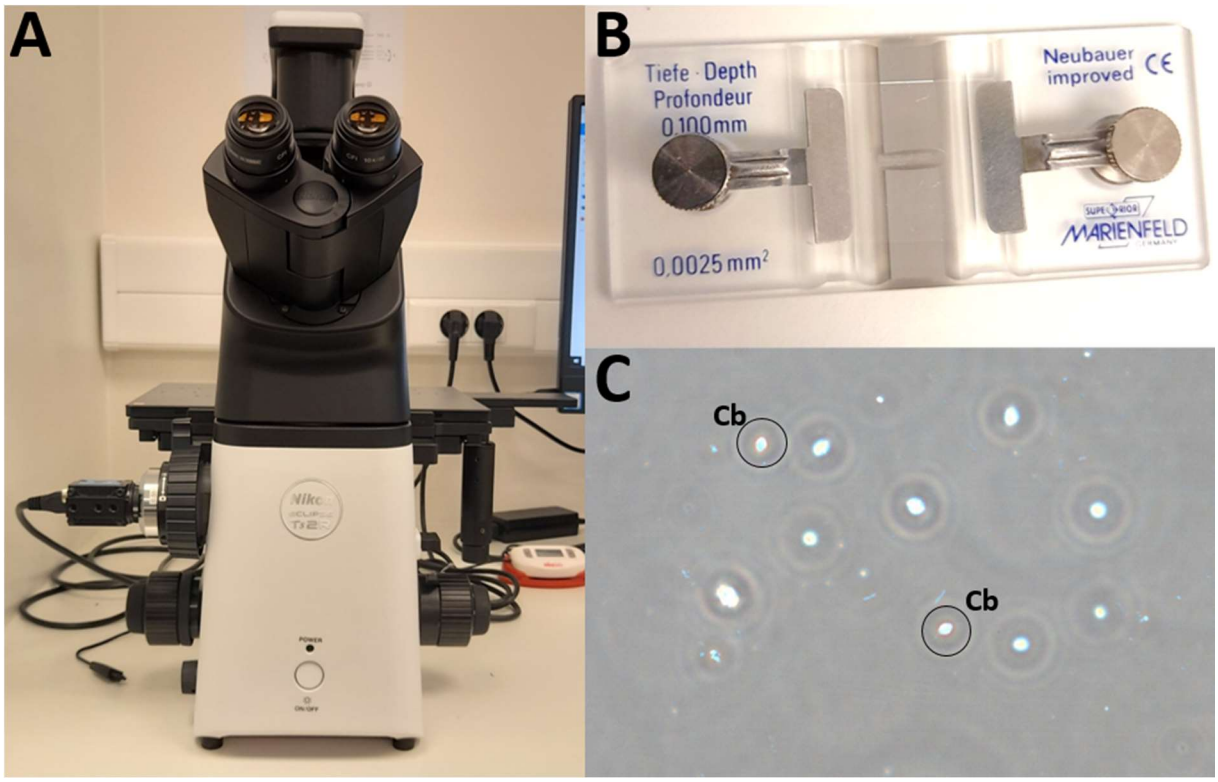


Figure 7. Device for counting the parasitic load of *C. bombi*. A. Inverted phase contrast microscope (Eclipse Ts2R, Nikon). **B.** Haemocytometer (Neubauer) **C.** *C. bombi* cells (Cb; 400X magnification).

III. 2.4.4 Individual-level parameter

The last parameter studied was the fat body mass, an organ involved in insect immune system through its function in the synthesis of antimicrobial peptide and other proteins present in the hemolymph (Arrese & Soulages 2010; Rosales 2017). The fat body can therefore be used as a good marker of immunocompetence in bioassays with *B. terrestris* (Vanderplanck et al. 2021). As this parameter is on an individual scale, results according to sex could be compared. The study of the fat body of the males shows the influence of the treatment during the growth of the bumblebees (larval stage to just emerged imago) since all their development have been processed under the specific treatment. Whereas the studies on the females show the effect of the long term exposure (35 days) of the treatment on the imagos. To study this parameter, at the end of the bioassays, 40 abdomens (i.e., two from males and two from workers per microcolony) were collected per treatment (n = 280). When a colony produced too few males, it was compensated as much as possible with individuals from another microcolony subjected

to the same treatment and originating from the same mother colony. Fat body content was measured following Ellers (1996). The abdomens were first dehydrated in an incubator at 70°C for three days and weighed. They were then placed for one day in 2ml of diethyl ether in order to solubilise the lipids constituting the fat body. The abdomens were then washed twice and incubated at 70°C for seven days before being weighed. The mass difference before and after lipid solubilisation corresponds to the mass of the fat body. The proportion of the fat body mass in the abdomen is calculated by dividing fat body content by the dry abdomen mass prior to solubilisation.

III. 3. Data analysis and statistics

All the statistical analyses were done using the R software v.4.0.5 (R Core Team 2020). For all statistical analyses, $p < 0.05$ was used as a threshold for significance. When significant models highlighted interactions between variables, only the results of the significant interactions were discussed (Berrington de González & Cox 2007). To detect precisely which treatments differed between others, estimate marginal means based on main explanatory variables or their interactions were compared (*emmeans* function from the emmeans R-package v.1.7.3; Lenth 2018). Only significant results are shown here in illustrations. Graphs and plots were all performed using the R-package ggplot2 v.3.3.6 (Wickham 2016) except the one referring to the survival probability of the workers performed with the *ggsurvplot* function of the survminer R-package v.0.4.9 (Kassambara et al. 2021).

III. 3.1 Distribution of flavonoids in *C. vulgaris* tissues

To investigate variation in flavonoid diversity in different tissues and floral resources of *C. vulgaris* a non-parametric Kruskal-Wallis test was performed (*kruskal.test* function from the stats R-package v.4.0.5; R Core Team 2018). As the test returned significant differences, a post-hoc multiple pairwise comparisons with Bonferroni correction was ran (*pairwise.wilcox.test* function from the stats R-package v.4.0.5; R Core Team 2018). The flavonoid profiles was plotted using a principal component analysis (PCA; *PCA* function from the FactoMineR R-package v.2.4; Husson et al. 2016). Further analysed were done on these data via a permutational multivariate analysis of variance (perMANOVA) with Euclidean distance and

9999 permutations as parameters (*adonis2* function from the vegan R-package vegan v.2.6.2; Oksanen et al. 2020) as well the corresponding post-hoc tests (pairwise.adonis formula from Martinez 2020), taking care to adjust *p*.values with Bonferroni correction.

III. 3.2 Resource use and development of the microcolony

To study the impact of the different treatments on the variables measured during and after microcolony bioassays, the dataset was subdivided into three subsets to answer biological questions separately:

- A first subset "*Flavonoid*" contains data from the non-parasitised pure willow and leaf/pollen extract supplemented willow treatments (n = 30 microcolonies). This subset is used to address and compare the impacts of leaf and pollen flavonoids on bumblebees in relation with herbivory defence mechanisms.
- A second subset "*Heather"* contains data from the non-parasitised pure heather and willow as well as pollen extract supplemented willow treatments (n = 30 microcolonies). This subset is used to evaluate the consumption effects of heather pollen on bumblebees and the role of its flavonoids in these effects.
- A third dataset "*Parasite"* contains data from all parasitised treatments (i.e., willow, heather and pollen extract supplemented willow) as well as from non-parasited pure willow treatment (n = 40 microcolonies). This last subset allowed us to determine the impact of the parasite as well as the impact of the diet on parasite bumblebees.

Before starting the analysis of the mass-reflective parameters (i.e., brood mass by offspring types and total, pollen collection, and syrup collection), all these data were first standardised by the total mass of workers in the microcolonies to avoid potential bias from worker activities (i.e., consumption and brood care). Because of a problem of homogeneities of variances (*leveneTest* function from the car R-package v.3.0.13; Fox & Weisberg 2019) and of normalities of residuals (*shapiro.test* function from the stats R-package v.4 .0.5; R Core Team 2018), most of these variables were analysed via generalized mixed models (GLMMs; *glmmTMB* function from the glmmTMB R-package v.1.1.3; Brooks et al. 2017) with Gamma distribution and log link. Otherwise a linear mixed models was used (LMMs; *lmer* function from the nlme R package v.3.1.157; Pinheiro et al. 2020) with classical Gaussian distribution.

LMM and GLMMs were parameterised with Treatment as fixed factor and Colony as random factors for the three subsets.

After calculating pollen dilution and pollen efficacy, LMMs were again used or GLMMs in case of a problem of homogeneities of variances or normalities of residuals linear mixed models. Again, Treatment was selected as fixed factor and Colony as random factors for all three subsets.

The number of individuals of each growth stage in the offsprings was also analysed with a GLMM (Poisson distribution, log link function). Treatment was set as fixed factor and Colony was set as random factors in all three subsets. Overdispersion and zero inflation problems were systematically checked using (*testDispersion* & *testZeroInflation* function respectively from the DHARMa R-package v.0.4.5; Hartig 2021). In case of overdispertion or zero inflation a negative binomial model was used (*glmmTMB* function from the glmmTMB R-package v.1.1.3; Brooks et al. 2017; with family = nbinom2 then family = nbinom1 if the problem persists).

For larval ejection, a GLMM (Binomial distribution, logit link function, bivariate response: ejected larvae *vs.* total number of living offspring produced) was calculated. Treatment was set as fixed factor and Colony was set as random factors for the three subsets. Overdispersion and zero inflation problems were checked using (*testDispersion* & *testZeroInflation* function respectively from the DHARMa R-package v.0.4.5; Hartig 2021) but did not apply in this case.

Lastly, to examine the influence of treatment and parasites on worker mortality, the proportionality of hazards were first checked (*cox.zph* and *ggcoxzph* functions from the survival R-package v.3.3.1; Therneau 2021) and the absence of influential observations (*ggcoxdiagnostics* function from the survminer R-package v.0.4.9; Kassambara et al. 2021) in order to perform a Cox proportional hazard (mixed-effect) model (*coxph* function from the survival R-package v.3.3.1; Therneau 2021; *coxme* function from the coxme R-package v.2.2.16; Therneau 2020). These models evaluate how variables influence the mortality rate at a given time via a hazard function (Bradburn et al., 2003). Individuals alive at the end of the 35-day treatment were assigned as censored, those who died as uncensored. The final models included again treatment as fixed factors and Colony was set as random factors.

III. 3.3 Parasitic load and individual-level parameter

The last parameter measured at the scale of the whole colony was the parasite load, only measured for the "*Parasite*" subset. The treatment were studied together via a negative binomial GLMM because of overdispersion, with Treatment and census day as fixed factors and Colony as random factor. For individual level parameters (fat body), GLMMs were performed (Gamma distribution, logit link function). For fat body weight measurements, the models were parameterised with Treatment and Sex as well as their interaction as fixed factors. Microcolony nested within Colony as a random factors was included in models of all three subsets.

IV. Results

IV. 1. Distribution of flavonoid profile of *C. vulgaris*

Of the samples analysed, only the nectar did not contain any flavonoids. However, it contained a terpenoid with a structure similar to callunene but it still had to be confirmed and quantified. This terpenoid could not be identified in the other samples except in pollen where it was weakly present. The comparison of the flavonoid concentration among the two tissues (i.e., leaf and corolla) and two floral resources (i.e., pollen and nectar) revealed significant differences (Kruskal-Wallis test, $\chi 2 = 15.067$, DF = 3, *p*.value = 0.0018, Table3). Post-hoc analysis showed that among samples, only the nectar did not contain any flavonoids, so it was significantly different from the pollen and the two types of tissues analysed. The leaves were the samples the richest in flavonoids, being significantly different from the lower concentration in pollen and corolla, the latter two not being significantly different.

Table 3. Screening of flavonoid concentrations in the different tissues and floral resources of heather. Quantification in triplicates of five heather specimens by HPLC-MS/MS analysis of flavonoid concentration per quercetin equivalent (QE). The concentrations given are the averages of the triplicates performed for each specimen. The two tissues studied (leaves and corollas) and the pollen all contain flavonoids. *No flavonoid was detected in the nectar, so the results were grouped for simplicity. The bold letters in the 'Tissue / floral resource' column refer to the result of the post-hoc test comparing the means between each tissue. Different letters indicate a significant difference between two tissues.

Tissue / floral resource	Individual	Flavonoids (mg_{QE}/g of tissue)	Mean ± SD (mg_{QE}/g of tissue)
Leaf (**a**)	1	17.859	16.155 ± 2.825
	2	11.891	
	3	15.132	
	4	16.642	
	5	19.246	
Corolla (**b**)	1	14.332	11.528 ± 2.372
	2	11.590	
	3	9.2516	
	4	13.387	
	5	9.077	
Pollen (**b**)	1	3.043	7.244 ± 6.239
	2	2.957	
	3	11.437	
	4	16.205	
	5	2.575	
Nectar* (**c**)	1 to 5	0	0

The flavonoid profile were found to be significantly different between heather sample types (perMANOVA test, $F_{(3,16)} = 160.98$, DF = 3, *p*.value = 0.0001, Figure 8). Pairwise post-hoc analyses highlighted significant differences between all of them. The PCA grouped the individuals into three clusters, isolating corolla and leaf and grouping pollen and nectar together. Although significantly different, nectar was more similar to pollen in that they lacked many flavonoids that were present in leaves and corollas. Among the 22 flavonoids identified (See Appendix E for flavonoid identification and more information), only one (i.e., kaempferol-O-coumaroylhexoside isomer A) was identified in more than one sample type (i.e., corolla and pollen), highlighting the high diversity and segregation among heather.

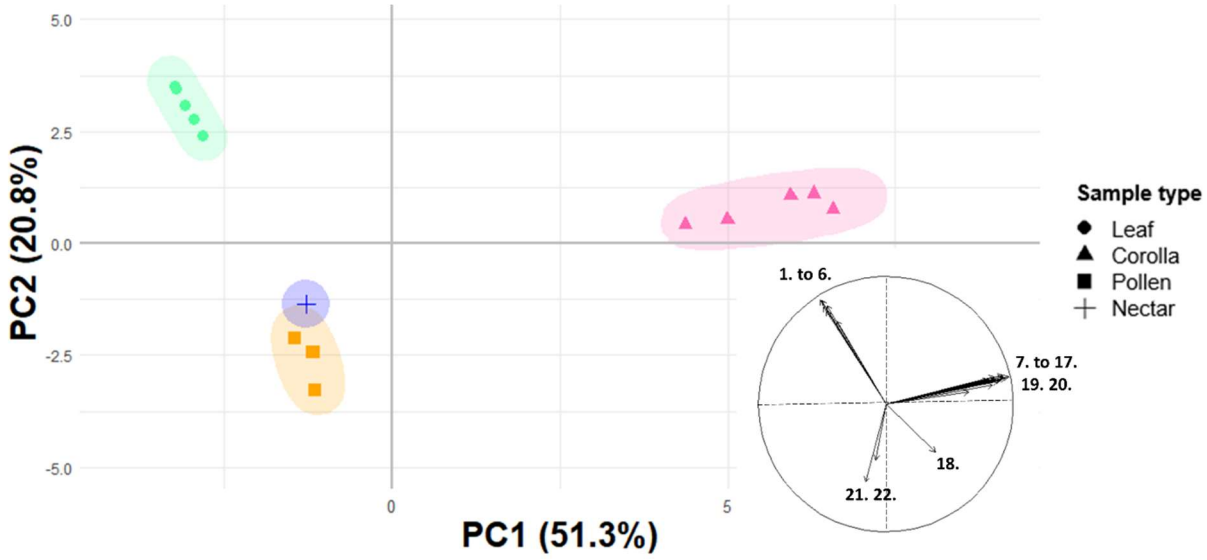


Figure 8. Principal component analysis (PCA) of flavonoids found in tissues and floral resources of heather. Main plot represents the graph of individuals while the graph of variables (inset) illustrates the correlation of the variables and their involvement in the two dimensions. PC1 explains 51.6% of the variance while PC2 supports 21% of it for a total of 72.6%. Molecular legend: 1. Kaempferol-O-caffeoyl ester; 2. Quercetin-O-feruloyl ester; 3. Unknow molecules A; 4. Kaempferol-O-caffeoylpentoside; 5. Quercetin-O-caffeoyl ester (Castillicetin); 6. Quercetin-O-caffeoyl pentoside; 7. Quercetin-O-hexoside isomer A; 8. Myricetin-O-rhamnoside; 9. Quercetin-O-hexoside isomer B; 10. Quercetin-O-hexoside isomer C; 11. Kaempferol-O-hexoside; 12. Quercetin-O-rhamnoside; 13. Glycosylated flavonoid; 14. Quercetin-O-pentoside isomer A; 15. Quercetin-O-pentoside isomer B; 16. Apigenin-O-hexuronide; 17. Kaempferol-O-rhamnoside; 18. Kaempferol-O-coumaroylhexoside isomer A; 19. Kaempferol-O-coumaroylhexoside isomer B; 20. O-substituted apigenin; 21. Kaempferol-O-coumaroyldihexoside; 22. Kaempferol-O-dicoumaroylhexoside.

IV. 2. Effects of heather flavonoids on bumblebees

Microcolony consumption

All statistical outputs of this project and their component are grouped in Appendix F (Supplementary Table 3). The presence of flavonoid extracts from the heather in the diet significantly impacted the consumption of resources by the microcolony. Pollen collection was significantly lower (GLMM, $\chi 2 = 16.294$, DF = 2, *p*.value = 0.0003, Figure 9.A) when the microcolony was subjected to a flavonoid-supplemented diet regardless of their origin (leaf vs. pollen). Syrup collection was also similarly affected, being lower in the presence of flavonoids (GLMM, $\chi 2 = 12.064$, DF = 2, *p*.value = 0.0024, Figure 9.B).

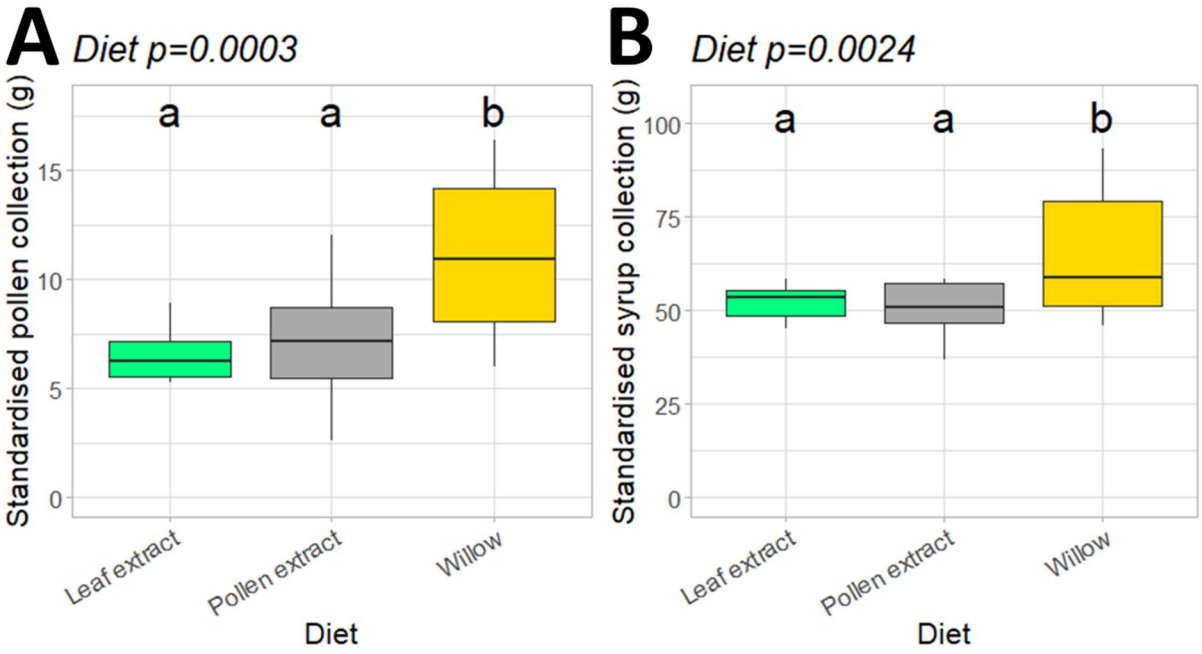


Figure 9. Effect of heather flavonoids on resource collection. Total mass of **A.** pollen and **B.** syrup collected during the 35 days of treatment. All mass values were standardised by the mass of workers present in the microcolony and adjusted for evaporation. The letters above the boxplots refer to the results of the post-hoc analyses. Different letters for two different diets indicate a significant divergence.

Offspring production

The presence or absence of heather flavonoids between treatments had little impact on offspring production. While the treatment affected the number of emerged males (GLMM, $\chi 2 = 37.997$, DF = 2, *p*.value < 0.0001, Figure 10), none of the other developmental stages were significantly affected (*p*.value > 0.05). Post-hoc analyses showed reduced production of emerged males in the presence of leaf and pollen flavonoid extracts.

Regarding the mass of offspring produced, the treatment was found to be significant (LMM, $\chi 2 = 20.335$, DF = 2, *p*.value < 0.0001, Figure 11). It was again the presence of flavonoids that decreased the mass of offspring produced with, according to the post-hoc analysis, a higher total mass in their absence (i.e. the unsupplemented willow diet).

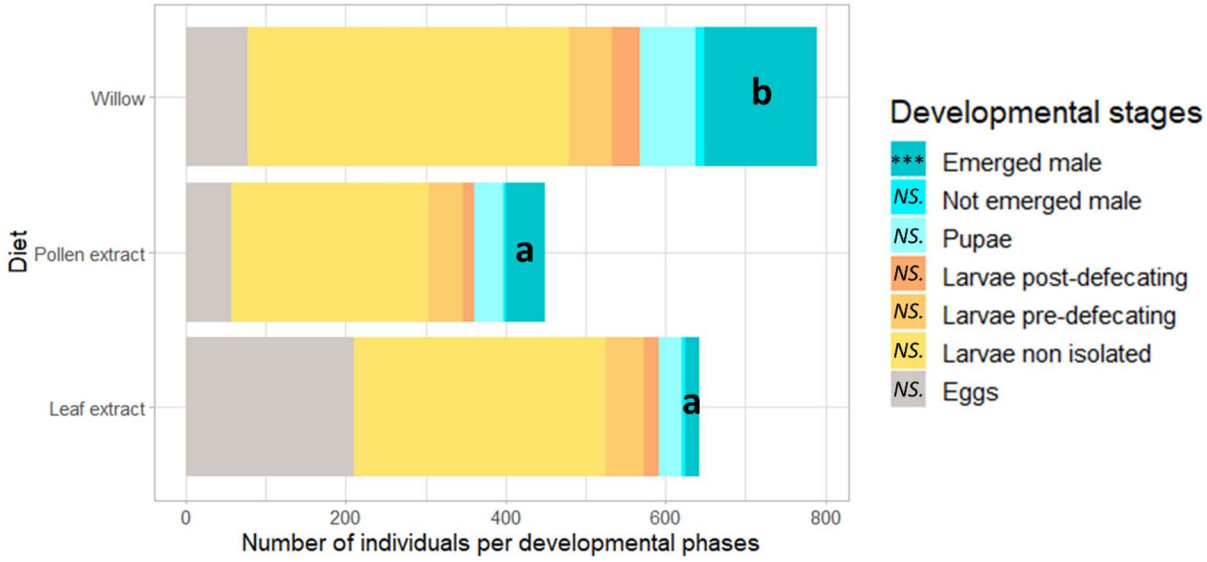


Figure 10: Influence of heather flavonoids on offspring production. Emerged males were the only stage to be significantly influenced by the diet and are marked '***' in the legend, underlining a p.value < 0.001. '*NS.*' refers to not significant. The letters refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

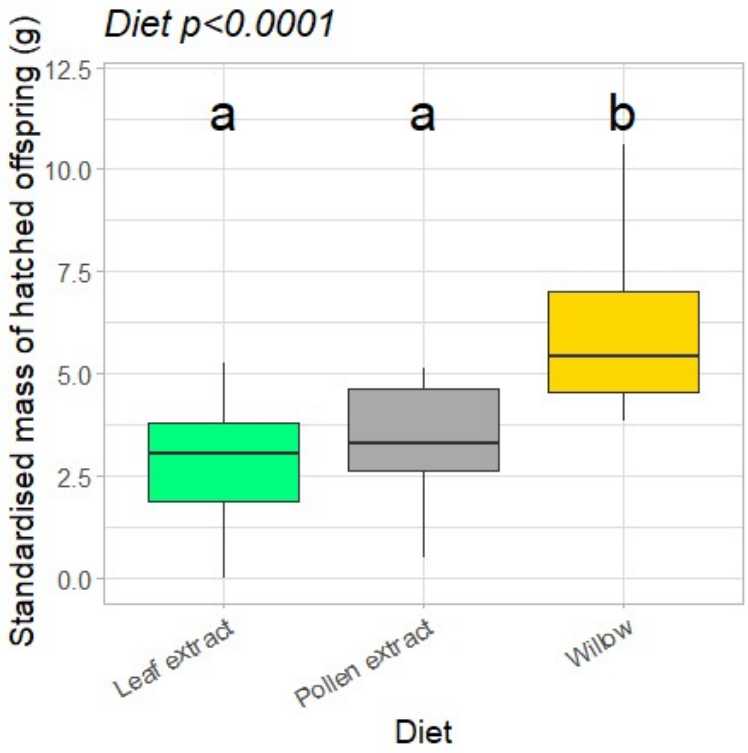


Figure 11: Influence of heather flavonoids on the total offspring mass produced. The mass of all hatched developmental stages is included. All masses were standardised by taking into account the mass of the workers of the corresponding microcolony. The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

Pollen efficacy, pollen dilution and mortality

No significant impact of the treatment on pollen efficacy was found (GLMM, $\chi2$ = 1.7115, DF = 2, *p*.value = 0.425, Appendix G Figure S4.A). Pollen dilution was however significantly affected (GLMM, $\chi2$ = 7.238, DF = 2, *p*.value = 0.0268, Figure 12) with post-hoc results showing a significantly greater pollen dilution with leaf extract supplemented willow than extract-free willow. Regarding the probability of larval ejection (GLMM, $\chi2$ = 0.0835, DF = 2, *p*.value = 0.959) and workers mortality (Cox proportional hazard model, $\chi2$ = 2.0413, DF = 2, *p*.value = 0.3604), neither parameter appeared to be affected by the presence of flavonoid extracts (Appendix G Figure S4).

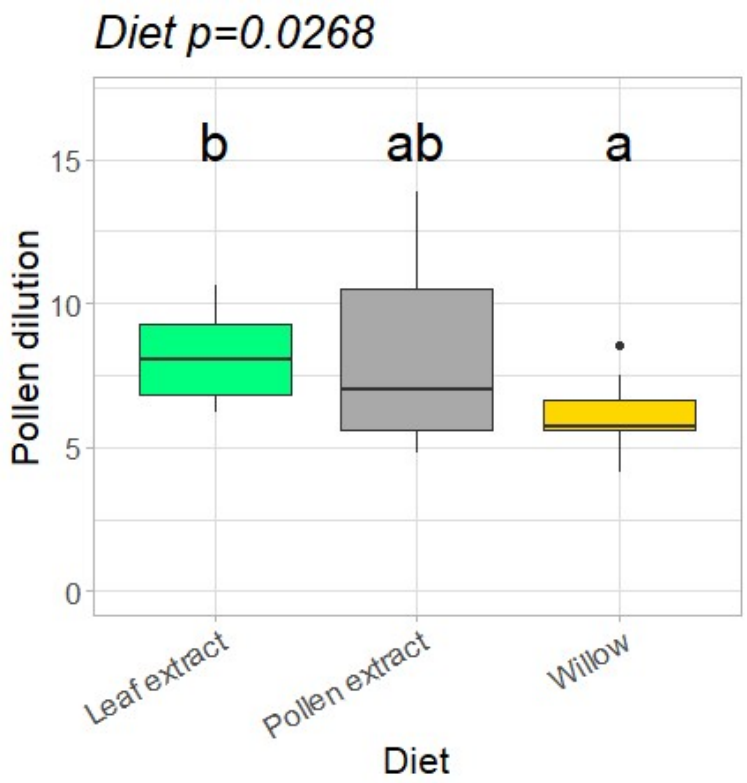


Figure 12: Influence of heather flavonoids on pollen dilution. Pollen dilution is defined as the ratio between the collection of syrup and pollen (taking evaporation into account). The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

Fat body

Two variables were studied to highlight their impact on the relative mass of the fat body. The first one, the sex of the individuals (male *vs.* female workers) was not identified as a significant factor (GLMM, $\chi2 = 1.0637$, DF = 1, *p*.value = 0.3024). The diet factor, on the other hand, significantly impacted fat bodies (GLMM, $\chi2 = 12.6369$, DF = 2, *p*.value = 0.0018, Figure 13). Post-hoc analysis revealed a higher mass of fat bodies in the presence of flavonoid extracts from leaves and pollen (*vs.* pure willow pollen). The interaction of the two parameters sex and diet was not significant (GLMM, $\chi2 = 2.4967$, DF = 2, *p*.value = 0.2869).

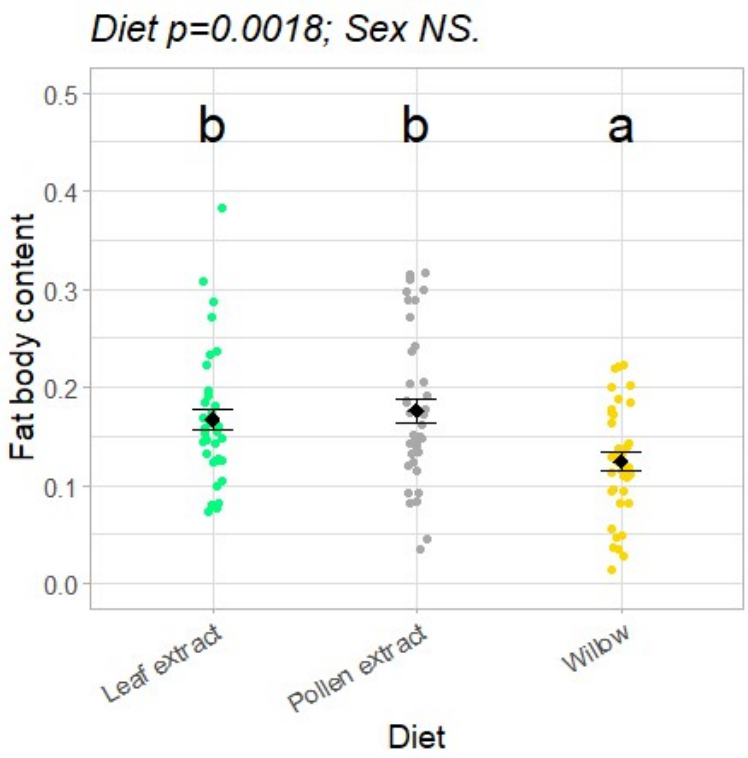


Figure 13. Influence of heather flavonoids on the fat body of bumblebees. Fat body content is calculated as the ratio of the dry mass of the fat body to the total dry mass of the abdomen. Two individuals for each sex were analysed per microcolony for a total of 40 individuals per treatment. The error bars show the standard error and the black dot in their centre indicates the mean value. The letters above the values refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. '*NS.*' refers to not significant.

IV. 3. Impacts of heather pollen and its flavonoids consumption on bumblebees

Microcolony consumption

The diet used in the different treatments had a significant effect on the collection of pollen (GLMM, $\chi2 = 12.174$, DF = 2, *p*.value = 0.0023, Figure 14.A) and syrup (GLMM, $\chi2 = 8.9972$, DF = 2, *p*.value = 0.0111, Figure 14.A). Post-hoc analyses confirmed the previous results indicating a lower consumption of pollen and syrup in the presence of pollen flavonoid extracts by comparison with pure willow pollen. However, the consumption of pollen and syrup was not significant different between the heather and willow diets. The heather diet also

supported a higher pollen collection than the flavonoid supplemented willow diet, the syrup collection was by contrast not different.

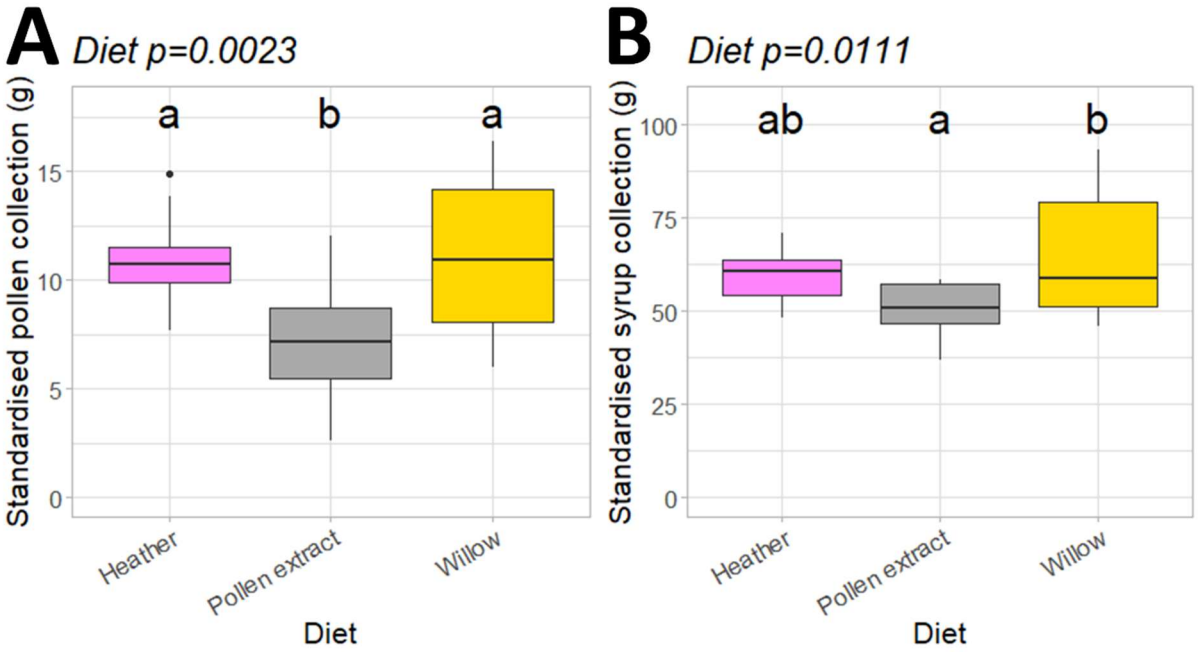


Figure 14. Effect of heather pollen and its flavonoids on resource collection. Total mass of **A.** pollen and **B.** syrup collected during the 35 days of treatment. All mass values were standardised by the mass of workers present in the microcolony and adjusted for evaporation to give the true consumption by the microcolony. The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

Offspring production

Heather pollen and its flavonoid extract had few impact on offspring production. While the treatment affected the number of post-defecating larvae (GLMM, $\chi2 = 17.035$, DF = 2, *p*.value = 0.0002, Figure 15) and emerged males (GLMM, $\chi2 = 21.444$, DF = 2, *p*.value < 0.0001, Figure 15), none of the other developmental stages was significantly affected (*p*.value > 0.05). Post-hoc analyses showed increased production of post-defecating larvae and emerged males for the willow treatment compared to the two others.

Regarding the mass of offspring produced, the treatment was found to be significant (LMM, $\chi2 = 15.304$, DF = 2, *p*.value = 0.0005, Figure 16). Post-hoc analysis indicates that the heather and willow diets do not differ significantly as well as the heather and willow diets

supplemented with pollen flavonoid extracts. However, a higher offspring total mass was again identified for the pure willow diet than for the supplemented diet.

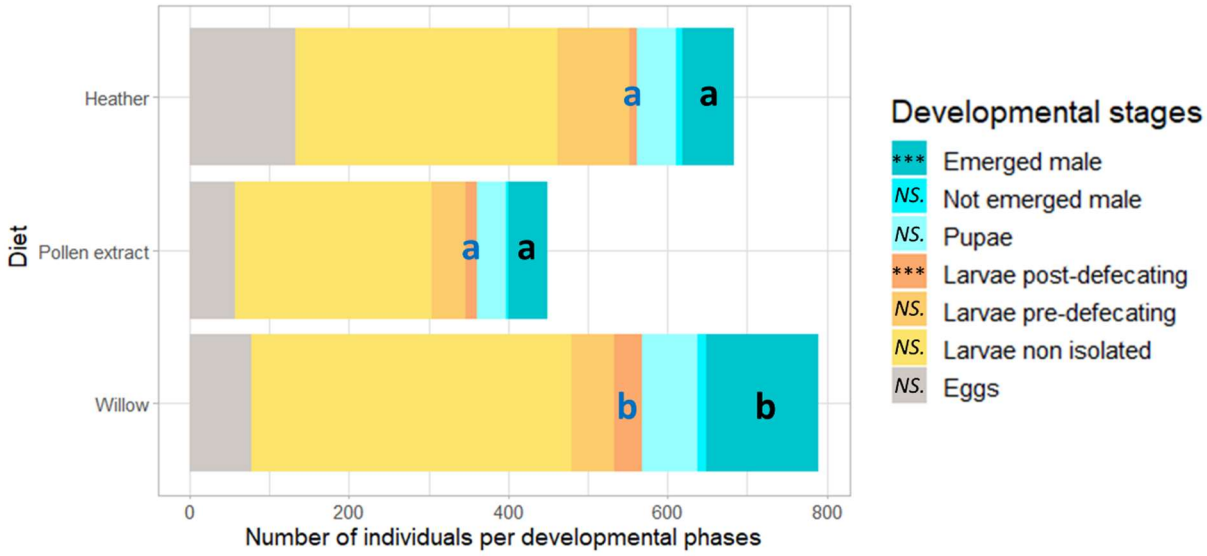


Figure 15: Influence of heather pollen on offspring production. Results of colony dissections at the end of the treatment. The total number of offspring is here divided by developmental stage. The stages significantly influenced by the diet are marked '***' in the legend, underlining a p.value < 0.001. '*NS.*' refers to not significant. The letters refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. The blue letters are associated with the post-hoc of 'Larvae post-defecating' stage, the black ones with the 'Male emerged' stage.

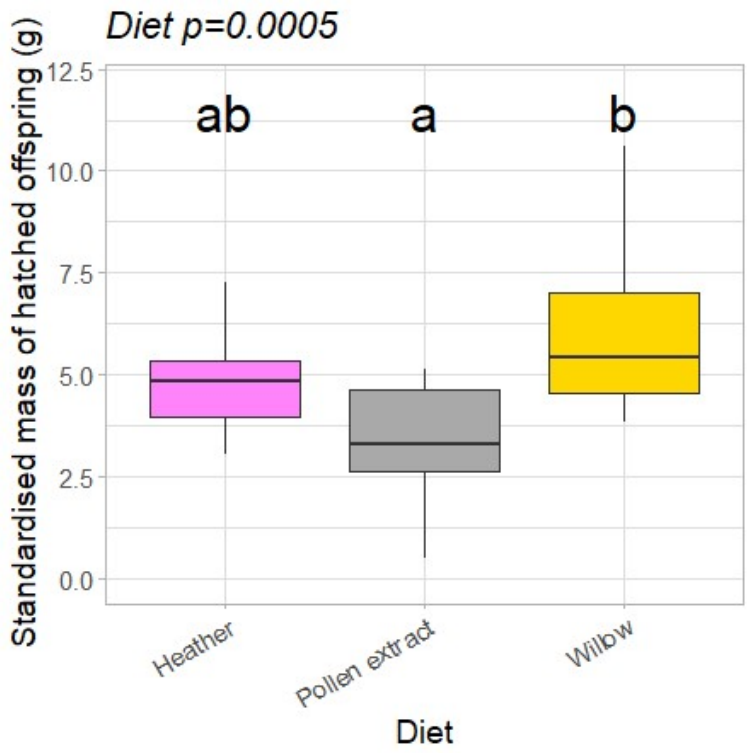


Figure 16: Influence of heather pollen on the total offspring mass produced. The mass of all developmental stages is included except for the eggs. All masses were standardised by taking into account the mass of the workers of the corresponding microcolony. The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

Pollen efficacy, pollen dilution and mortality

A significant impact of the treatment on pollen dilution was found (GLMM, $\chi2 = 15.832$, DF = 2, *p*.value = 0.0004, Figure 17.A) as well as on pollen efficacy (LMM, $\chi2 = 13.335$, DF = 2, *p*.value = 0.0013, Figure 17.B). The post-hoc analysis indicates that the pollen dilution is higher in the diet with pollen flavonoids extract than for the two pure diets of heather and willow pollen. The latter two diets were not significantly different. Regarding pollen efficacy, the willow diet had a significantly higher value than the other two diets, the latter which did not vary significantly between them. Regarding the probability of larval ejection (GLMM, $\chi2 = 0.1529$, DF = 2, *p*.value = 0.9264) and workers (Cox proportional hazard model, $\chi2 = 1.690$, DF = 2, *p*.value = 0.4296), neither parameter appeared to be affected by the treatment (Appendix G Figure S4).

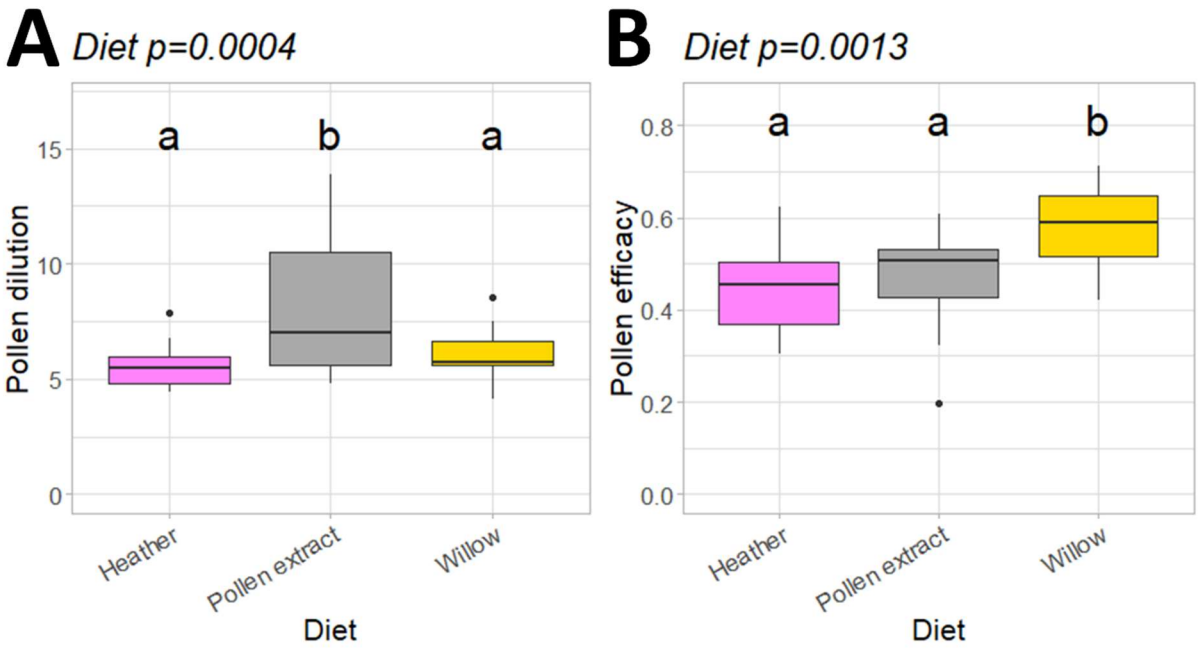


Figure 17: Influence of heather pollen and its flavonoids on pollen dilution and efficacy. A. Pollen dilution, defined as the ratio between the use of syrup and pollen and **B.** Pollen efficacy, measured as the ratio of offspring mass produced to pollen consumption. (standardised mass and taking evaporation into account). The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

Fat body

Two variables were studied to highlight their impact on the relative mass of the fat body. The first one, the sex of the individuals (male *vs.* female workers), was not identified as a significant factor (GLMM, $\chi2 = 0.5768$, DF = 1, *p*.value = 0.4476). The diet factor, on the other hand, significantly impacted the fat bodies (GLMM, $\chi2 = 10.2736$, DF = 2, *p*.value = 0.0059, Figure 18). According to the post-hoc analysis, the pure willow diet resulted in smaller fat bodies than the diet supplemented with pollen flavonoids. The other comparisons were all non-significant. The interaction of the two parameters sex and diet was not significant (GLMM, $\chi2 = 2.2932$, DF = 2, *p*.value = 0.3177).

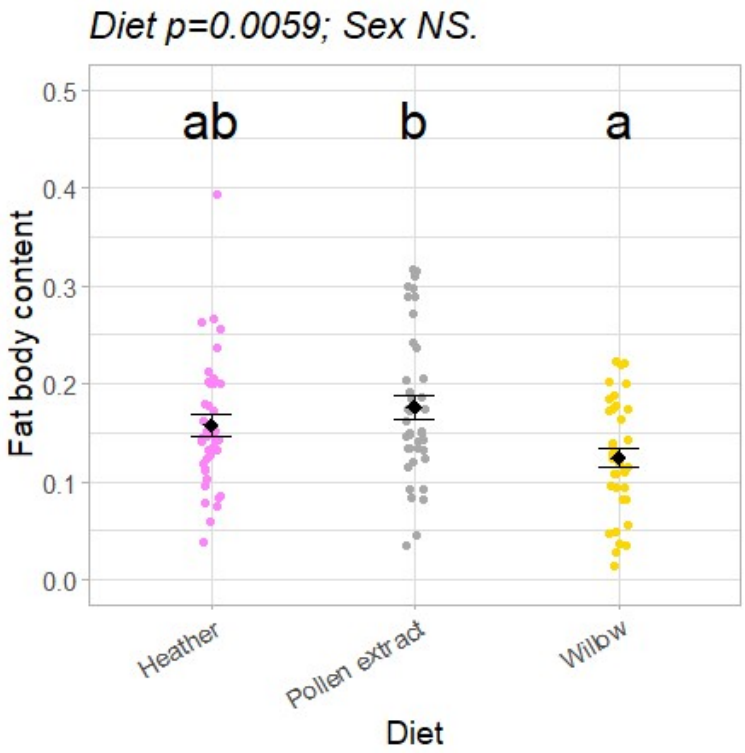


Figure 18. Influence of heather pollen on the fat body of *B. terrestris*. Fat body content is calculated as the ratio of the dry mass of the fat body to the total dry mass of the abdomen. Two individuals for each sex were analysed per microcolony for a total of 40 individuals per treatment. The error bars show the standard error and the black dot in their centre indicates the mean value. The letters above the values refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. '*NS.*' refers to not significant.

IV. 4 Impact of the floral resource on the parasitised bumblebee

Microcolony consumption

An influence of diets on the consumption of bumblebees was found. Both pollen (LMM, $\chi2 = 18.468$, DF = 3, *p*.value = 0.0004, Figure 19.A) and syrup collection (GLMM, $\chi2 = 25.563$, DF = 3, *p*.value < 0.0001, Figure 19.B) were significantly affected by the treatments. Post-hoc analyses revealed that the mass of pollen collected was higher for the treatments based on pure willow with or without the parasite than for the willow treatment supplemented with flavonoid extracts. Syrup collection was higher in the pure willow diet treatments (with and without the parasite) than in the other treatments, although the 'Willow' and 'Heather + P' treatments did not

differ significantly. The treatment 'Willow' and 'Willow + P' as well as 'Pollen extract + P' and 'Heather + P' did not differ significantly. The presence of parasites did not influence parameters as no significant difference was identified between the 'Willow' and 'Willow + P' treatments.

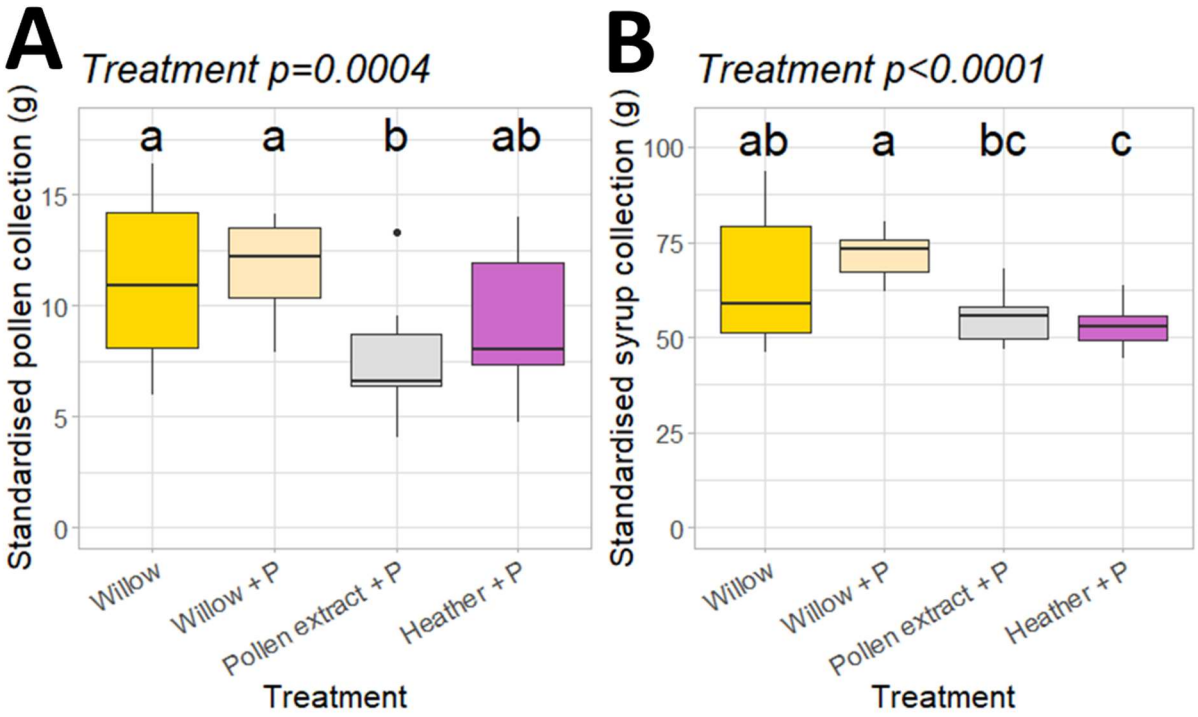


Figure 19. Effects of diet and parasite on resource consumption. Total mass of **A.** pollen and **B.** syrup consumed during the 35 days of treatment. All mass values were standardised by the mass of workers present in the microcolony and adjusted for evaporation to give the true consumption by the microcolony. The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. Treatments marked '+P' represent microcolonies with the parasite.

Offspring production

The different treatments had little impact on offspring production. While the treatment affected the number of emerged males (GLMM, $\chi2 = 43.418$, DF = 3, *p*.value < 0.0001, Figure 20), as well as pupae (GLMM, $\chi2 = 10.781$, DF = 3, *p*.value = 0.0130, but too weak differences to be supported in post-hoc analyses), none of the other developmental stages were significantly affected (*p*.value > 0.05). Post-hoc analyses showed increased production of emerged males for treatments based on a pure willow diet.

Regarding the mass of offspring produced, the treatment was found to be significant (GLMM, $\chi 2 = 22.376$, DF = 3, p.value < 0.0001, Figure 21). According to the post-hoc analysis, the treatments of pure willow with or without parasite did not differ significantly. However, they lead to a significantly higher total offspring mass than the other treatments (i.e., heather with parasite and supplemented willow with parasite).

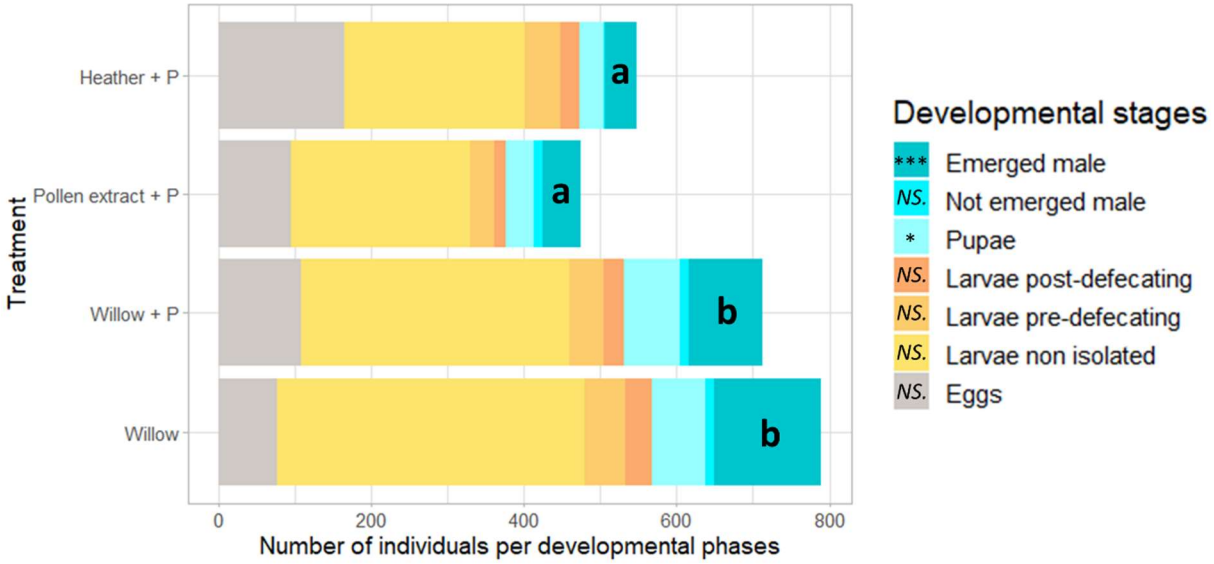


Figure 20: Effects of diet and parasite on offspring production. Results of colony dissections at the end of the treatment. The total number of offspring is here divided by developmental stage. The stage significantly influenced by the diet is marked '***' in the legend, underlining a p.value<0.001, while '*' underline a p.value between 0.01 and 0.05. '*NS.*' refers to not significant. The letters refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. Treatments marked '+P' represent microcolonies with the parasite.

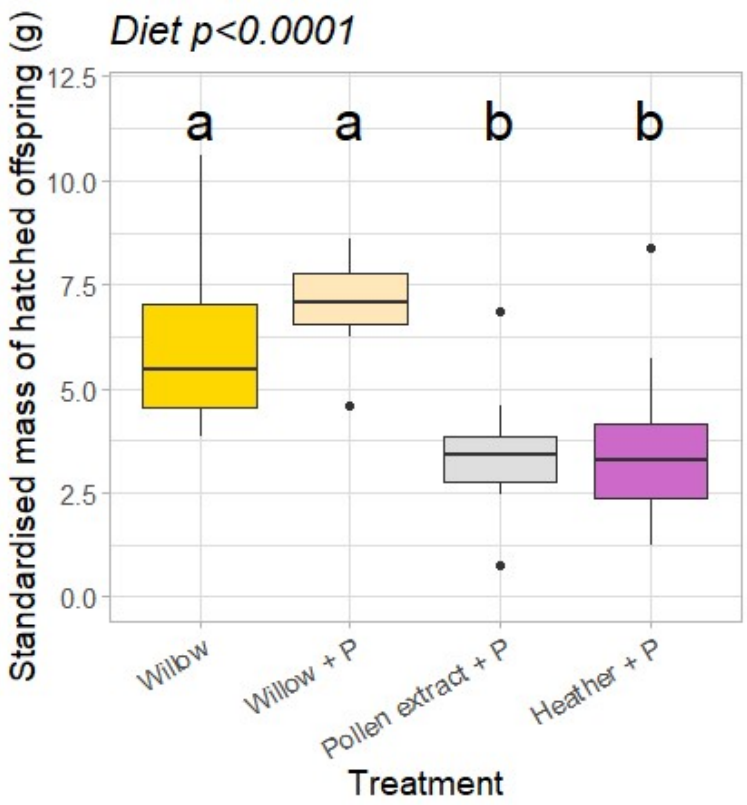


Figure 21: Effects of diet and parasite on the total offspring mass produced. The mass of all developmental stages is included except for the eggs. All masses were standardised by taking into account the mass of the workers of the corresponding microcolony. The letters above the boxplots refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. Treatments marked '+P' represent microcolonies with the parasite.

Pollen efficacy, pollen dilution and mortality

While the pollen dilution was not significantly impacted by the treatment (GLMM, $\chi2 = 9.3603$, DF = 3, *p*.value = 0.0249, but too weak differences to be supported in post-hoc analyses, Appendix G Figure S4.B), pollen efficacy was impacted (LMM, $\chi2 = 35.187$, DF = 3, *p*.value < 0.0001, Figure 22.A) with post-hoc tests showing higher pollen efficacy for pure willow treatments (with and without parasite) than for the others. Proportion of larval ejection varied not significantly (GLMM, $\chi2 = 1.2242$, DF = 3, *p*.value = 0.7472, Appendix G Figure S4.D), while the probability of worker mortality was significant between treatments (Cox proportional hazard model, $\chi2 = 11.982$, DF = 3, *p*.value = 0.0074). As illustrated on the Kaplan-Meier plot (Figure 22.B), the diet with the highest probability of survival throughout

the treatment period is the heather pollen diet. It is significantly different from all other diets except the willow diet for non-parasitised microcolonies. The latter treatment (i.e., willow) was second in survival probability and only significantly different from the flavonoid-supplemented willow treatment, which had the lowest probability of survival throughout the treatment. The latter treatment is however not significantly different from the treatment just above it, based on pure willow diet for parasitised bumblebees.

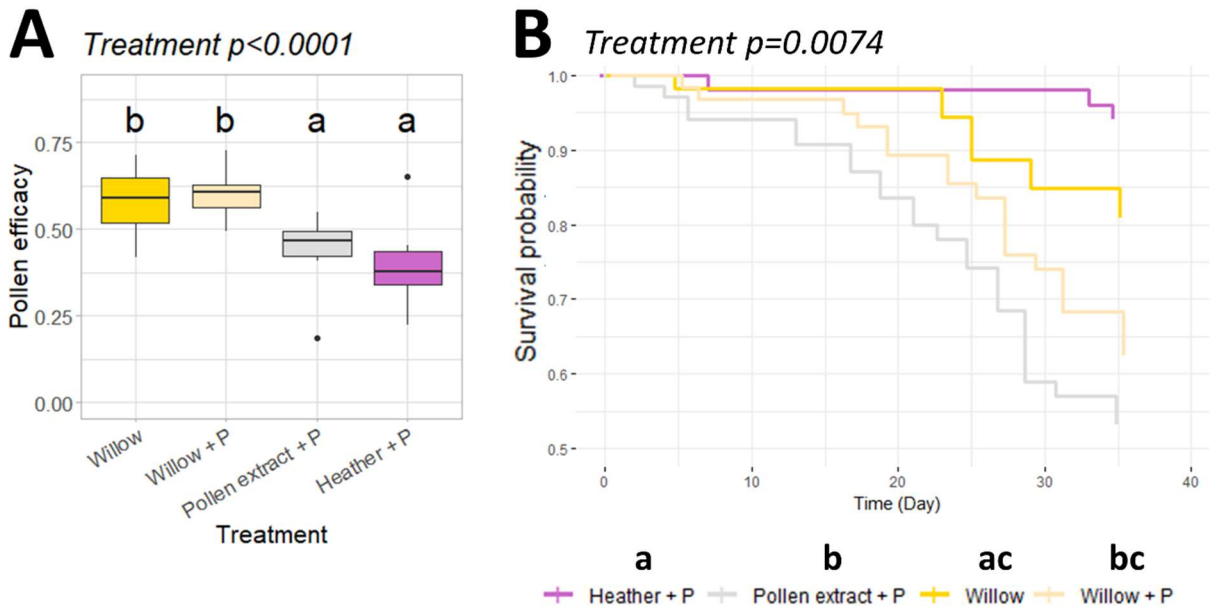


Figure 22: Effects of diet and parasite on pollen efficacy and worker survival. A. Pollen efficacy, measured as the ratio of offspring mass produced to pollen consumption (standardised mass and taking evaporation into account). **B.** Worker survival probability. Please note that for this graph, the scale has been adapted to improve readability. The letters above the boxplots (A) and on the legend (B) refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. Treatments marked '+P' represent microcolonies with the parasite.

Fat body

The sex of the individuals (male *vs.* female workers) was identified as significant factor (GLMM, $\chi2 = 8.1315$, DF = 1, *p*.value = 0.0044, Figure 23.A) with males carrying bigger fat body than workers. The treatment factor also significantly impacted fat bodies (GLMM, $\chi2 = 21.564$, DF = 3, *p*.value < 0.0001, Figure 23.B). According to the post-hoc analysis, the fat body content is higher for the parasitised diets than for the parasite-free willow diet except for the treatment based on heather pollen which did not differ significantly from any other

treatment, including willow. The interaction of the two parameters sex and diet was not significant (GLMM, $\chi 2$ = 6.4631, DF = 3, *p*.value = 0.0911).

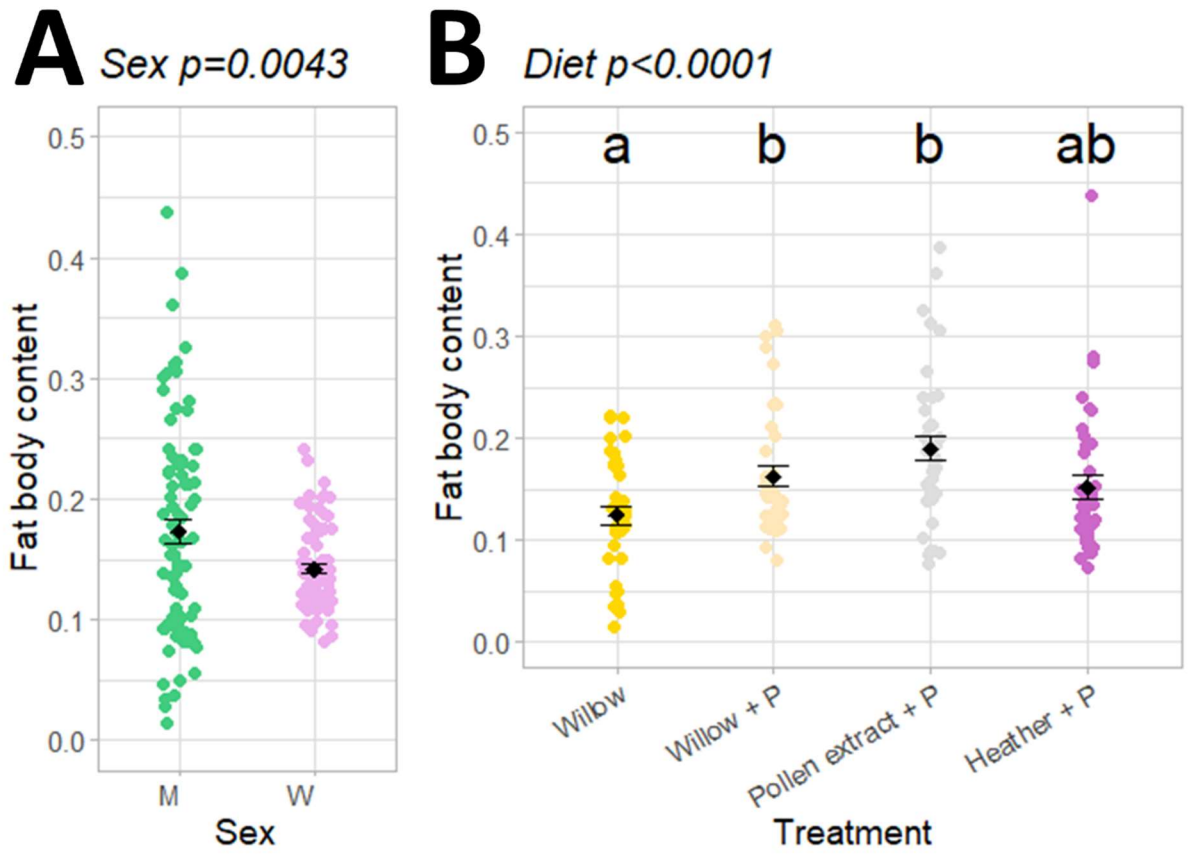


Figure 23. Effects of diet and parasite on the fat body of *B. terrestris*. A. Sex and **B.** Treatment influence on the fat body mass. Fat body content is calculated as the ratio of the dry mass of the fat body to the total dry mass of the abdomen. Two individuals for each sex were analysed per microcolony for a total of 40 individuals per treatment. The error bars show the standard error and the black dot in their centre indicates the mean value. The letters above the values refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence. '*NS.*' refers to not significant; 'M' to male bumblebees and 'W' to workers. Treatments marked '+P' represent microcolonies with the parasite.

Parasite load

For the three parasite treatments, eight successive measurements of parasite load over the 35 days of treatment were made (Figure 24). The treatment was the first factor influencing significantly the parasite load (GLMM, $\chi 2$ = 15.471, DF = 2, *p*.value = 0.0004). According to post-hoc test it is the pollen flavonoid extract based diet that significantly differs from the other

two in promoting a higher parasite load than pure heather and willow pollen. The second variable studied was the treatment day, also significantly affecting parasite load (GLMM, $\chi 2$ = 19.6385, DF = 1, *p*.value < 0.0001). The interaction of the two variables was however not significant (GLMM, $\chi 2$ = 1.2488, DF = 2, *p*.value = 0.5356).

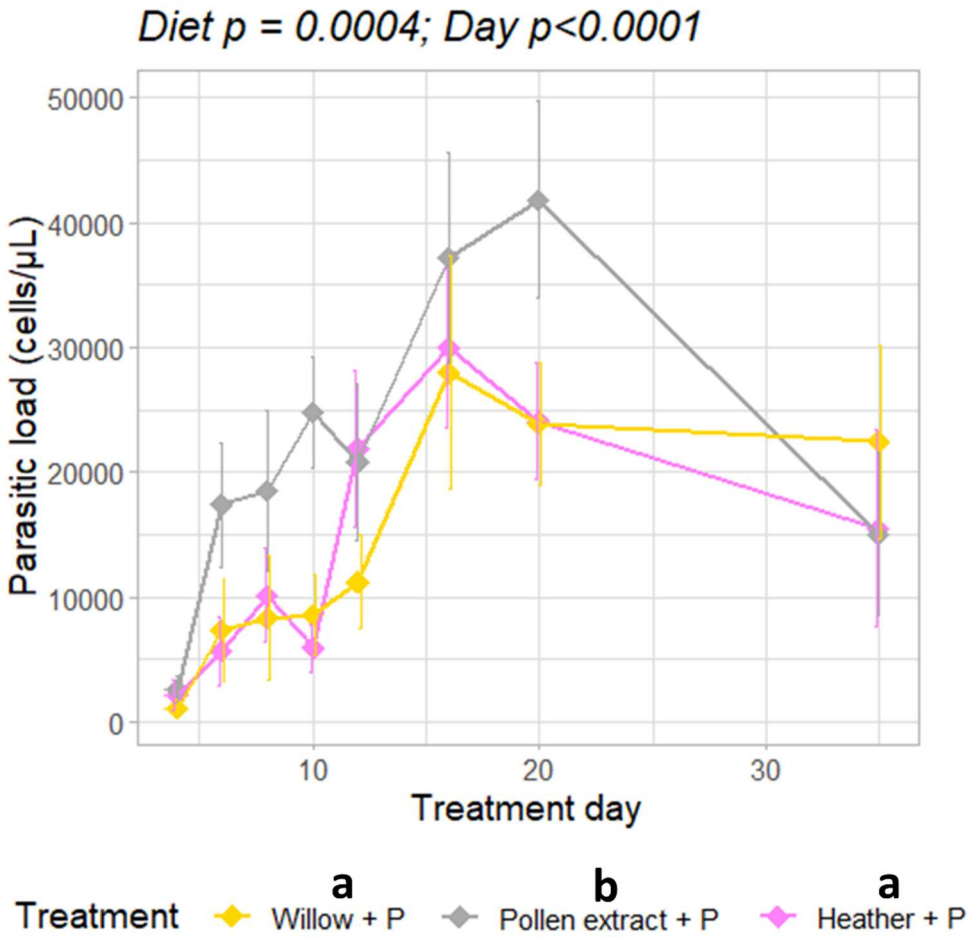


Figure 24. Effect of diet on parasite load. Evolution of the average parasite load across microcolonies per day during the 35 days of treatment under different pollen diets. Eight measurements were made, first every second day and then more spaced out until the last day of treatment. Error bars illustrate the standard error (SE). The letters on the legend refer to the results of the post-hoc analysis. Different letters for two different diets indicate a significant divergence.

V. Discussion

V. 1. Heather secondary metabolites, variable across the plant

Heather, mostly its nectar, was interesting for its content of a terpenoid, the callunene (Guyot *et al.* 1999; Dimitrova *et al.* 2006), with negative effects on *C. bombi* (Koch et al. 2019). LC-MS/MS analyses determined other secondary metabolites that were predominant abundant in heather, namely compounds from the flavonoid group. A total of 22 different flavonoids were identified in heather. The diversity of flavonoids in heather has been studied in the past and part of the 22 flavonoids identified here have already been identified or are similarly related to flavonoids already known for this species (Monschein et al. 2020 and references therein). The results however highlight how this chemical cocktail is unevenly distributed in terms of concentration and diversity among floral tissues and resources. It underlines heather ability to maintain and control its metabolite profile and associated defence through the plant. Indeed, many plants are able to modulate their secondary metabolite synthesis throughout their organs according to their interactions with their environment and more particularly according to the risk of aggression by herbivores *sensus stricto* (Zangler & Rutledge 1996; Wittstock & Gershenzon 2002). While both tissues (i.e. leaf and corolla) as well as pollen were rich in flavonoids, none of the molecules could be identified in the nectar. Conversely, the search for terpenoids was successful in the nectar with the detection of an ion that is likely to be the so-called callunene. This compound was also found in pollen but in much lower concentrations as already demonstrated (Koch et al. 2019) and still indicating a predominance of flavonoids in the pollen resource. Heather thus seems to selectively allocate its secondary metabolite profile:

(i) Nectar is a floral resource whose function is to attract and reward the pollinators. In exchange for this resource, pollinators carry pollen and improve the efficiency of pollination. Unlike in many flowering plant species (Baker 1977) and in all other types of analysed samples, heather nectar did not contain any flavonoids. This demonstrates the absence of a specific synthesis of flavonoids in the nectar as well as a lack of physiological leakage or pleiotropy from other flavonoid-rich tissues. Moreover, this resource contains a terpenoid likely to be the callunene, which is known to have a positive effect on parasited bumblebees (Koch et al. 2019). The

heather does not therefore chemically protect its nectar, at least through the production of flavonoids.

(ii) Pollen, unlike nectar, was characterised by the presence of flavonoids. Pollen is known to contain more diverse and more concentrated secondary metabolites than nectar (Cook et al. 2013; Palmer-Young et al. 2019). Pollen is crucial for the plant as it is its male reproductive component. In many species, pollen is therefore protected from different threats by a pollen-kit on its surface containing secondary metabolites that perform different functions (protection against UVs, limit pollinivory, avoid bacterial and fungal development, etc.; Pacini & Hesse 2005). Heather pollen therefore seems to display chemical defences but to a lesser extent than tested plant tissues according to its poor flavonoid diversity and, compared to leaves, its lower concentration. Heather pollen could thus be protected from excessive collection through a relatively low toxicity (Vanderplanck et al. 2020) without fully preventing pollen collection for its reproduction.

(iii) Finally, flavonoids were found to be abundant in heather leaves and corollas. These tissues, with which bees do not interact, could therefore be protected by numerous flavonoids that participate in the defence against herbivory (Bennett & Wallsgrove 1994). The diversity of these flavonoids is high and they do not overlap, as indicated by a different flavonoid profile between leaf and corolla and probably highlighting an accurate flavonoid allocation between these two tissues.

The two floral resources are therefore not or relatively less protected than the plant tissues. This specialised allocation could betray an evolutionary adaptation favouring interaction with the pollinators via the floral resources while protecting plant tissues from other herbivores (Figure 25).

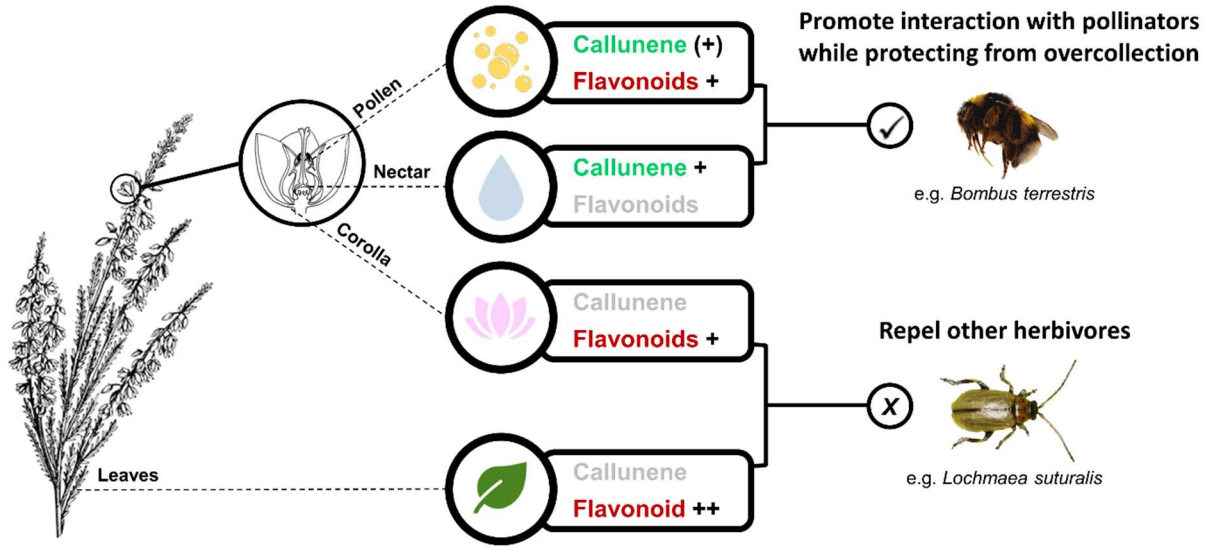


Figure 25. Summary of the interaction strategy of the heather. '(+)' trace of the molecule, '+' presence of the molecule, '++' relatively very high concentration of the molecule. Beetle picture from Rosenburgh & Marrs 2010; bumblebee picture from C. Tourbez.

V. 2. Heather leaf and pollen flavonoids negatively challenge bumblebees

The presence of flavonoids from heather in the diet of bumblebees has strongly impacted the development of their microcolonies. These flavonoid extracts induced a decrease in offspring production with a reduction in the number of individuals emerged, as well as in the total mass of offspring produced. The pollen efficacy (total mass of offspring / collected pollen) was not affected by the presence of these flavonoids and it can be concluded that this decrease in offspring production is due to a lower collection of pollen, which was significantly impacted, rather than to the lower nutritional quality of the pollen. Bumblebees seem to reduce their exposure to this toxicity by reducing their pollen consumption and by diluting the pollen more strongly with nectar (Vanderplanck et al. 2018) as shown by the associated increase in pollen dilution (collected syrup / collected pollen). Indeed, in the presence of a toxin-containing resource, social bees are able to establish a social detoxification behaviour by diluting the resource with a large amount of nectar (Berembaum & Johnson 2015). However, this flavonoid toxicity was found to only lead to sublethal effects as worker mortality and larval ejection (pre-metamorphic mortality), were not significantly affected. Finally, the ingestion of flavonoids

also had consequences at the individual level with an increase in fat body mass that may indicate an increased detoxification response which is also fat body-dependant (Li et al. 2007; Li et al. 2019). The presence of flavonoids in the diet thus seems to impact this organ used as a marker of the general health of bumblebees (Vanderplanck et al. 2021), which confirms the toxicity of flavonoids. This increase in fat body mass in the presence of toxin could be explained by an allocation of resources in this organ so that it can perform its detoxification role (Li et al. 2007; Li et al. 2019). Interestingly, in line with the literature the results confirm that a higher mass of this organ is negatively correlated with offspring production (Ellers 1996).

The screening of heather metabolites demonstrated differences in concentration and diversity of leaf and pollen flavonoids. However, flavonoid extracts from both tissues appear to have similar negative effects on bumblebees as no significant difference was identified between the leaf and pollen flavonoid supplemented diets. Only pollen dilution seems to support a higher toxicity of leaf flavonoids that differs significantly from unsupplemented pollen (willow control), whereas flavonoids extracted from pollen are not significantly different. While leaf secondary metabolites are well documented for their repellent and toxic effects on *sensus stricto* herbivores (e.g. Hoffmann-Campo et al. 2001; Chen et al. 2004; Thoison et al. 2004), few studies allow us to compare the effects triggered by pollen flavonoids. However, a parallel study carried out on the pollen of cherry tree in the Metaflore project (*Prunus avium*, Rosaceae; Apolline Michel 2022) has also identified pollen flavonoid effects and a more important toxicity of the flavonoids of leaves indicating that this difference of toxicity between the leaves and the pollen may be species dependent.

This negative influence of flavonoids on the development of bumblebees may show their use as a chemical defence by the plant for its protection against leaf herbivory (Bennett & Wallsgrove 1994) as well as against the overuse of its pollen by pollinators (Vanderplanck et al. 2020). However, in the case of pollen, secondary metabolites may have other roles. These compounds such as flavonoids could also have as a primary function of UV protection or antibacterial activity to protect pollen (Li et al. 1993; Pusztahelyi et al. 2015; Zaynab et al. 2018). The effect on pollinators could therefore be a by-product of the use of flavonoids for other purposes. While these results still illustrate very well the negative side of feeding on some pollen, it may seem surprising when one knows that flavonoids are not the most toxic molecules

for pollinators. Indeed, some plant flavonoids even have potentially positive properties such as antioxidant and antimicrobial qualities in bumblebees (Treutter 2005). Although few comparisons can be made with the literature, another work (Apolline Michel 2022) showed that flavonoids can have a negative effect on pollinators. Such effects could vary depending on the concentration, bioavailability or diversity of the compounds within the collected resources.

It is known that bees have a molecular metabolism of flavonoid detoxification based on the CYP6AS molecule, a cytochrome P450 monooxygenase (Mao et al. 2009; Feyereisen 2012). However, little is known about the toxic effect of flavonoids on bumblebees and their mode of action. These types of molecules can either directly affect the bumblebee via toxicity or interfere with nutrient uptake (Irwin et al 2014). Furthermore it can be assumed that their antimicrobial activity (Treutter 2005) negatively affects the microbiota of bumblebees. Flavonoids are thought to have two modes of action on bacteria: (i) inhibition of the nucleic acid synthesis (Wu et al. 2013) and (ii) damage to the cell membrane inducing a change in membrane fluidity, followed by the leakage of certain intracellular components (Tsuchiya & Iinuma 2000). Such effects on the microbiota have already been demonstrated in bumblebees (e.g. Billiet et al. 2016, Gekière et al. In prep.). Given the importance of this microbiota, the adverse consequences of flavonoid intake could be explained by the dysbiosis (i.e. disruption of the microbial community) they may induce. Although this hypothesis remains to be tested (and it is planned for future experiments), flavonoids could disrupt the microbial balance of the digestive system inducing difficulties to digest pollen and assimilate its nutrients (Meeus et al. 2013; Kwong & Moran 2016).

V. 3. Heather pollen and the role of pollen nutritional quality

Significant differences were observed when using heather and willow pollen. Despite the fact that both resources (pollen and syrup) were consumed in the same proportions, the heather pollen induced the production of significantly less post-defecating larvae and emerged individuals. Pollen efficacy is directly dependent on the nutrient quality of the related pollen (Pereboom et al. 2003; Hargreaves et al. 2009). This lower suitability for offspring production of heather pollen is probably related to its lower nutritional quality.

In order to elucidate how the nutrient content of heather pollen affects microcolonies, it is necessary to define its differences with willow pollen (Figure 26). The comparison of willow pollen with other pollen including heather pollen has already been done: (i) Protein content; both pollens have all essential amino acids and the same non-essential amino acid profile (Vanderplanck et al. 2014). Despite similar diversity, the relative amount of free (Huang et al. 2011) or polypeptide-bound amino acid per gram of tissue is higher in willow (Vanderplanck et al. 2014). (ii) Sterols; they are more concentrated in heather than in willow (Vanderplanck et al. 2014). Heather also has a high proportion of δ-7-avenasterol and δ-7-stigmasterol, whereas these molecules are almost absent in willow, which has large quantities of β-sitosterol, which is much less present in heather. Finally, (iii) the profile of secondary metabolites of the two pollens is free of any alkaloid or saponin (Vanderplanck et al. 2018), only heather pollen has a terpenoid, the callunene (Koch et al. 2019) and only willow has phenolamides (n = 9; Irène Semay, unpublished results). The screening also revealed that both contain flavonoids in similar concentrations (Vanderplanck et al. 2018) and that flavonoids in heather pollen (n = 3) are less diverse than those in willow (n = 10) and different (i.e., no flavonoids in common between the two pollen types). Heather pollen contained flavonoids of the kaempferol type substituted with one/two hexosides and one/two coumaroyl groups, a framework that is never found in willow where flavonols (kaempferol but also quercetin and isorhamnetin) are only linked to pento/hexosides (Irène Semay, unpublished results).

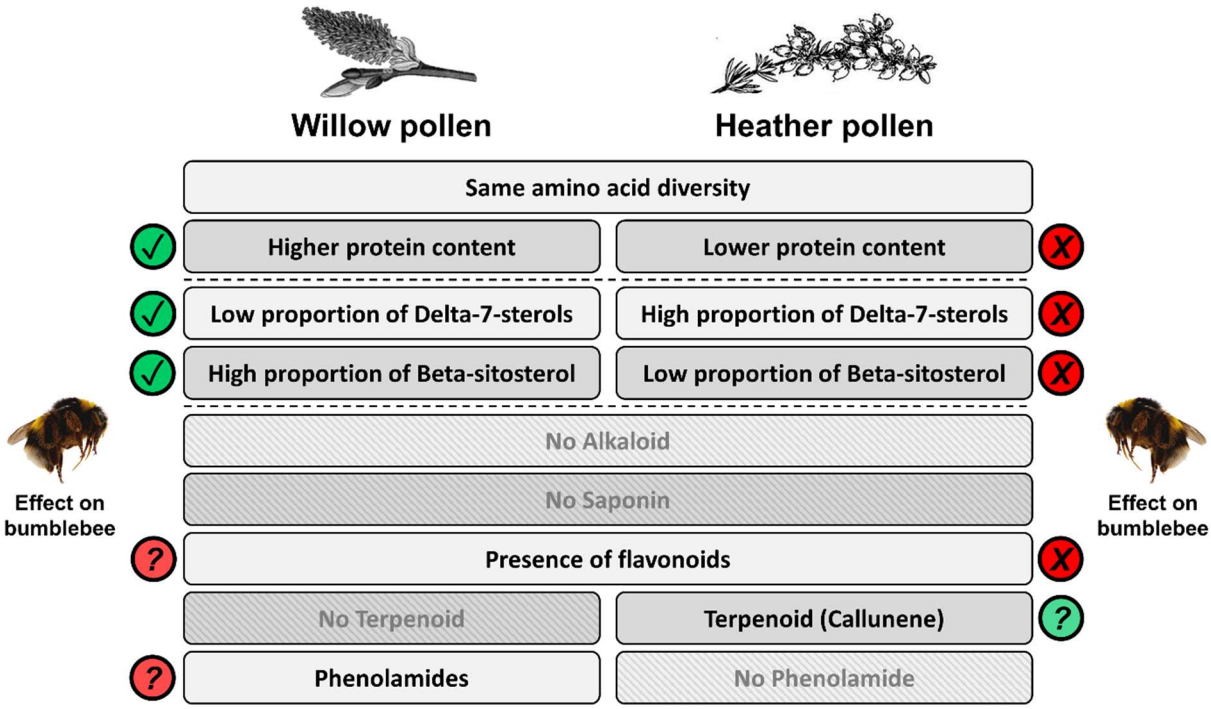
If willow pollen supports a better nutritional quality inducing a better production of offspring it is therefore first of all because of its high protein content known to be associated with higher pollen efficacy (Vanderplanck et al. 2014) thereby leading to a greater offspring development in bumblebees (Genissel et al. 2002; Tasei & Aupinel 2008b; Stabler et al. 2015) which is also true for other apoids (e.g. …; Alaux et al. 2010; Nicolson 2011). Its sterol profile is also more interesting: β-sitosterol, which it contains in greater quantities than heather, seems to favour the production of offspring (Vanderplanck et al. 2014), whereas the two δ-7-sterols of heather, on the contrary, are known to have a negative effect on insects (Behmer & Grebenol 1998; Behmer & Elias 1999), including bumblebees (Vanderplanck et al. 2018).

In terms of secondary metabolites, here presented previous results show that heather flavonoids induce negative effects on bumblebees and their microcolony. In willow pollen,

unlike heather pollen, it has flavonoids of the quercetin type that could have beneficial properties on bumblebees due to their antioxidant and antimicrobial activities (Treutter 2005). The question of the involvement of other secondary metabolites in the quality of pollens is however very complex. The presence of phenolamides in willow and their absence in heather does not support these results as these molecules have been shown to have a negative effect on microcolony development in bumblebees (Gekière et al. 2022). Regarding terpenoids (i.e., callunene) from heather, despite its ability to inhibit the *C. bombi* parasite (Koch et al. 2019), the mechanisms explaining this impact on healthy bumblebees is still unknown.

Interestingly, despite the equal concentrations of heather flavonoids in the pure heather diet and the corresponding supplemented willow diet, we observed that the supplemented pollen was less used by the workers and more diluted as evidenced by the higher pollen dilution. As the heather toxicity is identical from the point of view of secondary metabolites, it could be proposed that the bumblebees perceive the toxicity in the supplemented diet more than in the pure heather pollen and try to dilute it more as explained before (Berembaum & Johnson 2015; Vanderplanck et al. 2018). This could be explained by: (i) a decrease in the palatability of the pollen, (ii) an increase in the detection of this toxicity due to the addition of an extract directly available on the willow pollen while the flavonoids of the heather pollen are still preserved in these pollen grains or (iii) the higher concentration of flavonoids in the supplemented diet due to the presence of flavonoids already present in the willow pollen.

Figure 26. Summary of nutritional qualities and secondary metabolites and their effect on the bumblebee. Green " ✓ " refers to a variable positive effect on the bumblebee, while red " ✗ " refers to a negative effect. As secondary metabolite effects are complex and may be context-dependant we could only assessed heather flavonoid effects on bumblebees as their effects were tested in the context of this work. Other one could not be tested and are indicated " ? " while colours refer to supposed effects according to previous studies.



V. 4. Bumblebee-parasite interaction, when the heather comes into the equation

Before studying how the diet influences the bumblebee-parasite interaction and its consequences for the development of the microcolony, the impact of the parasite itself was defined. Comparison of parasitised and unparasitised bumblebee microcolonies fed all with willow pollen demonstrated the already known weak effect of the parasite (Schmid-Hempel 2001; Brown et al. 2003a; Deshwal & Mallon 2014; Goulson et al. 2018). Focusing at the microcolony level, the presence of the parasite did not significantly influence resource use, as well as the number of offspring per stage and the total mass of offspring produced. Worker mortality was slightly increased in the presence of the parasite but this trend was not significant, nor was larval ejection.

After demonstrating (i) the negative effects of heather flavonoid uptake on healthy bumblebees and (ii) the higher nutritional quality of willow pollen than heather pollen, the potential positive effect of heather pollen and its flavonoids on parasitised bumblebees was analysed. Still focusing at the microcolony level, parasitised bumblebees following a diet of heather or willow pollen supplemented with flavonoid extract were negatively affected on

several levels compared to the pure willow diet. As already seen for the non-parasitised bumblebees, we saw that for parasitised bumblebees the pollen with the best pollen efficacy is pure willow and the pollen collection is lower for the supplemented diet than for pure willow pollen but is similar between the two pure diets (i.e. heather and willow). The two diets associated with heather produced fewer offspring per developmental stage and lower total mass. Apart from the probability of worker survival which was greater under pure heather pollen than under willow pollen for the parasitised individuals, it seems that at the microcolony level, results do not allow us to demonstrate a positive effect of heather and its flavonoids on parasitised bumblebees. The microcolony-level parameters seems to be more strongly influenced by the chemical profile of the diet than by the parasite. For wild bumblebees, the presence or absence of *Crithidia bombi* is likely less influential than the nature of their diets.

The analysis of the parameters at the individual level did reveal some interesting results. The standardised fat body mass was significantly greater in the presence of the parasite than in the absence of the parasite for all diets except for the diet based on pure heather pollen. In the latter, the fat body was not significantly greater than in unparasitised individuals fed a willow diet. The protein and sterol properties of heather pollen are less interesting than those of willow pollen (Vanderplanck et al. 2014; Vanderplanck et al. 2018). If one looks at the evolution of the parasite load under the three diets it can conclude that the flavonoids of the heather could not be the metabolites responsible for this parasite-effect reduction since it: (i) stimulates a more precocious peak of the parasite and (ii) supports a higher parasite load than the other treatments until at least day 20. It is known that the influence of *C. bombi* infestation is small but may have greater consequence in the presence of another stress (Schmid-Hempel 2001; Brown et al. 2003a; Brown et al. 2003b; Deshwal & Mallon 2014). Here the flavonoids of heather represent an additional toxic stress increasing the individual-level effect of *C. bombi*, an increase that does not however extend to the social level (i.e., microcolony) as already demonstrated. If heather flavonoids do not explain these results, another potential candidate is the recorded terpenoid, probably the callunene (Koch et al. 2019) which despite a low concentration was found in pollen and might explain this decrease in the effect of the parasite on bumblebees. This molecule is known to negatively affect the parasite under lab conditions (Koch et al. 2019). These results suggest that it could also help wild bumblebee via its uptake by pollen (but especially nectar) and reduce the effects of the parasite at the individual level. However, we do

not observe such a drastic decrease in parasite load as in Koch et al. 2019. This difference could be due to the concomitant presence of flavonoids in the given pollen, weakening the bumblebees and increasing the parasite load. The concentration of terpenoid in the pollen (not quantified) probably also plays a role in explaining the results. In contrast to the concentration in nectar, the terpenoid content of pollen may be insufficient to support such a decrease (Koch et al. 2019).

These results may suggest that although the individual incidence of the parasite exists, the implementation of the immune response (allocation of resources to the fat body), allows the parasite to be countered. The individual immune response limits the impact to the individual complexity level and the effects at the microcolony level are reduced. These results are in line with the literature stating that despite the diverse effects of the parasite (see Table 2. and references therein), it has a relatively weak impact on bumblebees (Schmid-Hempel 2001; Brown et al. 2003a; Deshwal & Mallon 2014). The mitigated impact of *C. bombi* on bumblebees is encouraging given its wide distribution in wild populations as it is capable of affecting half of the individuals (Goulson et al. 2018) or even 80% of the populations (Imhoof & Schmid-Hempel 1999; Erler et al. 2012). In contrast, other parasites such as *Apicystis bombi* and *Nosema bombi* have much more detrimental consequences for bumblebees but with a much lower prevalence in the population (<1% and <6% respectively according to Goulson et al. 2018). The results support that *C. bombi* is a weak threat in bumblebees. However many potentially influencing parameters were not and should be tested in future studies: (i) at the queen level, neither queen production nor her colony founding traits have been tested here but they can be affected by the parasite (Erler et al. 2012; Goulson et al. 2018). These parameters are important as they delimit the longer-term success of the species. (ii) Regarding *in natura* foraging ability, different parameters such as pollen collection efficiency, floral manipulation and the choice of the most appropriate flowers (Hanley et al. 2008) can all be impacted by the parasite (Schmid-Hempel and Schmid-Hempel 1991; Schmid-Hempel 1998b; Schmid-Hempel & StauVer 1998) and have not been tested with this protocol. While results show here that the individual immune response limits the effect of the parasite within the microcolony, it remains to be tested how it effects the reproductive capacity of the colony through its queens as well as natural resource collection.

VI. Perspectives

VI. 1. Bumblebee immune response markers

The fat body is an organ associated with the immune response in insect as it is the main tissue responsible for the synthesis of immune proteins present in the hemolymph and other antimicrobial peptides (Hetru 1998; Arrese & Soulages 2010; Rosales 2017). As highlighted in this study, the mass of this organ varies according to the health status of the bumblebee and this organ can be used as a marker of the immune response in the bumblebee (Vanderplanck et al. 2018; Vanderplanck et al. 2021). While the suitability of this marker seems to be widely accepted, the comparison of this study with three other broadly similar studies of the Metaflore project (Begou 2021; Gekière 2021; Michel 2022) seems to highlight the lack of understanding its variation. In the here presented result and the one of Michel (2022), an increase in the mass of the fat body as a result of toxic and parasitic stress was identified. This increase was understood as an allocation of resources to the fat body, a storage organ, whose mass increases to ensure its function of immunoprotein synthesis (Michel 2022). In the results of Begou 2021 and Gekière 2021, the opposite results were observed, the same parasite, studied via a similar protocol influences the fat body differently. According to the results of this study, the toxic and parasitic stress decreases the mass of the fat body. These results are the opposite of the one reflected by this work and were explained by a decrease in the fat reserves present in the fat body and its associated mass, subsequent to the massive synthesis of immunoproteins (Gekière 2021). This problem of inconsistency of results between highly similar experiments underlines that the mechanism of variation of fat bodies is still unclear and should be taken into consideration when using this parameter. Pilot experiments should be carried out to describe the influence of different factors (e.g. *ad libitum* syrup consumption) on the evolution of the mass of this organ. Furthermore, results also highlight a high variability of fat body mass between individuals subjected to the same treatment (as well as in Gekière 2021 and Michel 2022) and in particular an importance of sex with heavier but also more variable fat body mass in males. The evolution of fat body mass should also be studied over time in order to demonstrate a potential dynamic mechanism. It is indeed possible that the mechanism of action of the fat body goes through several phases, *e.g.* a first phase of fat storage (increase in size)

and then a phase of massive defence peptide synthesis (decrease in size). While the fat body is an organ frequently used for its ease of measurement, we warn for the currently lack of understanding of its response and advise the use of other immune system markers to confirm the results indicated by its variation (reviewed in Moreno-Garcia et al. 2013). The measurement of haemocyte cell count (e.g. Cotter et al. 2004), immunoprotein in hemolymph or phenoloxidase activity (e.g. Goldsworthy et al. 2003) could provide a complementary answer that we think could be much more accurate and stable than the measurement of fat body.

VI. 2. Enhancing the parasite choice

Bombus terrestris is parasitised by several types of intracorporeal parasites such as: (i) nematodes (e.g. *Sphaerularia bombi*; Poinar & Van der Laan 1972); (ii) microsporidia (e.g. *Nosema bombi*; Otti & Schmid-Hempel 2007 & 2008); (iii) trypanosomes (e.g. *Crithidia bombi*) or (iv) Apicomplexae (e.g. *Apicystis bombi*; Plischuk & Lange 2009). The choice of *C. bombi* as the most interesting species given its prevalence in wild bumblebee populations (Goulson et al. 2018) could be questioned. Indeed, results indicate that the parasite has little impacts comparing diet impacts (Gekière 2021; Michel 2022) and the search for a metabolite that could counteract the effects of a parasite with very little impact is problematic. Similarly for experiments dealing with the recent topic of bee self-medication (de Roode & Hunter 2019; Spivak et al. 2019), it would be more interesting to ensure a significant effect of the parasite and to avoid species with weak effects such as *C. bombi*. We believe that it would be very interesting to perform similar tests with more harmful parasites such as *Apicystis bombi* or *Nosema bombi* in order to have a higher effect of the parasite. However, the use of these parasites is a dilemma as the negative effects may be so severe that we may not have sufficient microcolony development or even complete colony death raising another problem. However, this has not yet been tested and we think it would be interesting to do so. Another very interesting experiment would be to test the simultaneous effect of several parasites. In nature, bumblebees are sometimes parasitised by more than one intracorporeal parasite. Furthermore, it has been shown that the presence of *C. bombi* and its impact on the bumblebee decreases the protection provided by its immune system favouring the contamination by other parasites (Goulson et al. 2018). For example, *Nosema bombi* infections are more frequent in bumblebees already parasitised by *C. bombi* (Goulson et al. 2018). If we extend the study to other parasites

it might therefore also be interesting to test the effect of diets on bumblebees that are bi-parasitised and probably more impacted by their parasites.

VI. 3. Environmental consideration of the parasite effect

The main aim of this study was to find a metabolite group that counteracts the effect of pollinator parasites. However, experiments show that the nutritional quality of the pollen used has a more powerful impact on the development of the bumblebee colony than the parasite (Begou 2021; Gekière 2021; Michel 2022). In the end, the consequences of this parasitic interaction are mainly limited to the individual level thanks to the immune system barrier. However, for social organisms such as bumblebees, it is the situation of the colony as a whole entity that is important for the survival of the species and not that of the individual (i.e., 'superorganism'). The results suggest that in the wild, pollinators are more affected by the chemical quality of the pollen they have access to than by the presence of some parasites. Although pathogenosphere activity and effects can be influenced by human activities (Meeus et al. 2018; Brown 2022), parasites such as *C. bombi* are population regulators and just other natural selection mechanism and are not responsible for extinction-threatening damage to pollinator populations such as some human activities. While these results remain of great interest, their deepen understanding suggest that science aiming at protecting these pollinators should also focus on identifying more quality nutrient resources for these pollinators. Identifying the nutrient richness of many plant species may help to provide this pollinator with more robust health to deal with a greater number of threats rather than focusing on one of them acting as another piece in the evolution puzzle since forever.

VII. Conclusion

This study deciphered the role of secondary metabolites in the complex interaction between a flowering plant, a pollinator and a pollinator parasite (Figure 27). By first questioning the chemical defence strategy of heather (*Calluna vulgaris*), the variations of its metabolite profile across tissues and floral resources have been revealed. The heather tissues (leaves and corolla) contained diverse flavonoids which could have a role as an herbivore repellent. Regarding pollen, it also contained flavonoids which may protected it from excessive sampling by pollinators, although these compounds were in lower concentrations than in leaves and lower in molecular diversity. Interaction with the pollinator seems to be favoured since finally its nectar does not contain any flavonoids but a terpenoid, probably the callunene, also present in small quantities in the pollen and favourable for the pollinator. By performing bioassays in microcolonies of the buff-tailed bumblebee (*Bombus terrestris*), the influence of a heather-based diet on the pollinator and its parasite was studied. Despite their different concentration and profile of toxins, flavonoid extracts from leaf and pollen induced similar toxic effects in bumblebees. Then, the nutritional quality of heather pollen was found to be lower than that of control pollen (willow) probably also due to its protein poverty and unfavourable sterol profile. In order to test the impact of heather pollen and its flavonoids on *Crithidia bombi*-parasitised bumblebees, they were subjected to heather pollen and heather pollen flavonoid extract supplemented willow pollen diets. These diet influence to counteract parasite effects was then studied. While the parasite showed little influence at the microcolony level, it was found to influence the fat body, as did the flavonoids, inducing an increase in its mass in relation to an increased immune response. Although the influence of the diet was by far larger than that of the parasite on the microcolony parameters, a small effect of heather pollen to counteract the parasite could be identified at individual level. Flavonoids were not responsible as they increased the parasite load and it has been hypothesised that the low concentration of callunene present in pollen might be the candidate explaining the lower effect of the parasite at the individual level. All results shed light on the complex plant-pollinator interaction and the role of secondary metabolites in this interaction as well as on the bumblebee-parasite relationship.

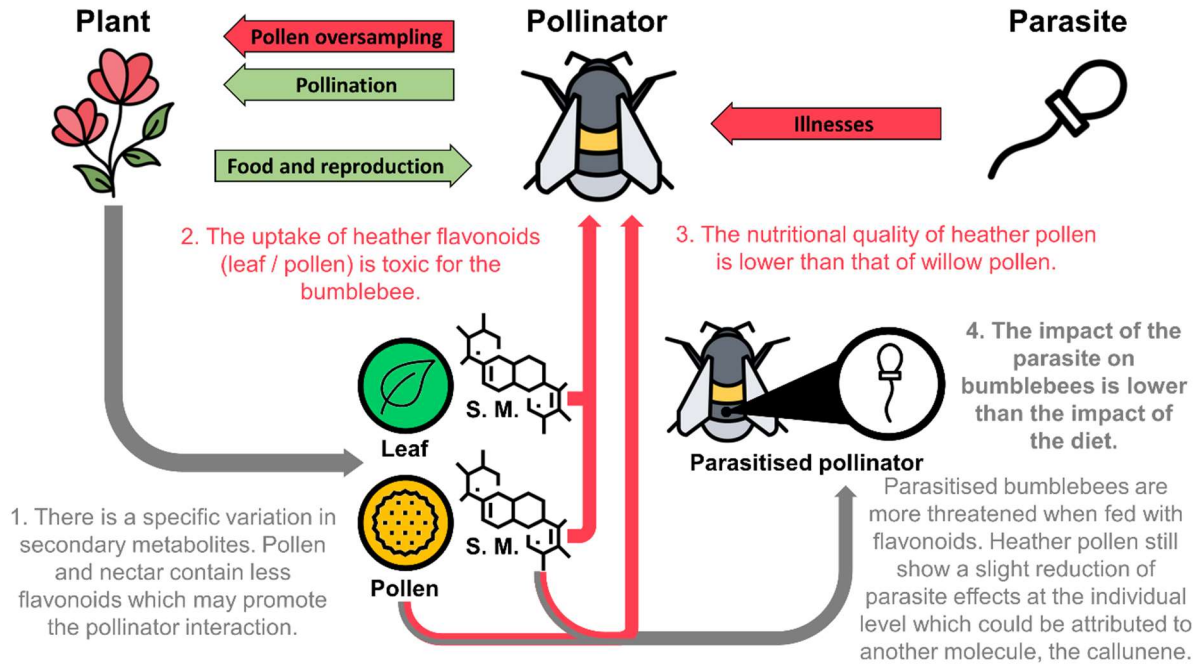


Figure 27. Summary of findings in relation to the biological questions asked. Red arrows indicate a negative impact, green arrows a positive impact of one organism on the other. S. M. refers to secondary metabolites.

VIII. References

Abbott, J. (2014). Self-medication in insects: Current evidence and future perspectives. *Ecological Entomology*, *39*(3), 273-280.

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., ... & D'Alessandro, U. (2011). Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria journal*, *10*(1), 1-12.

Ackerman, J. D. (2000). Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Pollen and Pollination*, 167-185.

Adler, L. S. (2000). The ecological significance of toxic nectar. *Oikos*, *91*(3), 409-42.

Adler, L. S., Seifert, M. G., Wink, M., & Morse, G. E. (2012). Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecology letters*, *15*(10), 1140-1148.

Aizenberg-Gershtein, Y., Izhaki, I., Santhanam, R., Kumar, P., Baldwin, I. T., & Halpern, M. (2015). Pyridine-type alkaloid composition affects bacterial community composition of floral nectar. *Scientific Reports*, *5*(1), 1-11.

Akrout, A., El Jani, H., Zammouri, T., Mighri, H., & Neffati, M. (2010). Phytochemical screening and mineral contents of annual plants growing wild in the southern of Tunisia. *Journal of Phytology*, *2*(1), 034-040.

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology letters*, *6*(4), 562-565.

Aljbory, Z., & Chen, M. S. (2018). Indirect plant defense against insect herbivores: a review. *Insect Science*, *25*(1), 2-23.

Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annual review of entomology*, *55*, 207-225.

Atsalakis, E., Chinou, I., Makropoulou, M., Karabournioti, S., & Graikou, K. (2017). Evaluation of phenolic compounds in Cistus creticus bee pollen from Greece. Antioxidant and antimicrobial properties. *Natural Product Communications*, *12*(11), 1934578X1701201141.

Augner, M. (1998). Plant defence signals and Batesian mimicry. *Evolutionary Ecology*, *12*(6), 667-679.

Aupinel, P., Genissel, A., Tasei, J. N., Poncet, J., & Gomond, S. (2000, July). Collection of spring pollens by Bombus terrestris queens. Assessment of attractiveness and nutritive value of pollen diets. In *VIII International Symposium on Pollination-Pollination: Integrator of Crops and Native Plant Systems 561* (pp. 101-105).

Arathi, H. S., Burns, I., & Spivak, M. (2000). Ethology of hygienic behaviour in the honey bee Apis mellifera L.(Hymenoptera: Apidae): behavioural repertoire of hygienic bees. *Ethology*, *106*(4), 365-379.

de Assis Junior, E. M., dos Santos Fernandes, I. M., Santos, C. S., de Mesquita, L. X., Pereira, R. A., Maracaja, P. B., & Soto-Blanco, B. (2011). Toxicity of castor bean (Ricinus communis) pollen to honeybees. *Agriculture, ecosystems & environment*, *141*(1-2), 221-223.

Bacchetta, R. 2014. Impacts de la taille et de la densité florale des populations de Calluna vulgaris (L.) Hull sur la diversité, l'abondance et la diète de sa guilde de pollinisateurs au plateau des Tailles (Ardenne, Belgique). M.S. Thesis, Université catholique de Louvain, Louvain-la-Neuve, Belgique

Baer, B., & Schmid-Hempel, P. (2001). Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, Bombus terrestris. *Evolution*, *55*(8), 1639-1643.

Baker, H. G. (1977). Non-sugar chemical constituents of nectar. *Apidologie*, *8*(4), 349-356.

Baracchi, D., Brown, M. J., & Chittka, L. (2015). Behavioural evidence for self-medication in bumblebees?. *F1000Research*, *4*.

Barlow, S. E., Wright, G. A., Ma, C., Barberis, M., Farrell, I. W., Marr, E. C., ... & Stevenson, P. C. (2017). Distasteful nectar deters floral robbery. *Current Biology*, *27*(16), 2552-2558.

Baron, G. L., Raine, N. E., & Brown, M. J. (2014). Impact of chronic exposure to a pyrethroid pesticide on bumblebees and interactions with a trypanosome parasite. *Journal of Applied Ecology*, *51*(2), 460-469.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:*1406.5823.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2021) lme4: Linear Mixed-Effects Models using "Eigen" and S4.

Bégou M. 2021. L'aubépine, une pharmacie naturelle ? Etude de la toxicité et du potentiel médicatif de Crataegus monogyna (Jacq.) sur l'abeille sauvage sociale Bombus terrestris (L.). Mémoire de Master 2 en Sciences biologiques, Université de Mons, Mons, 82 pp.

Behmer, S.T. and Grebenok, R.J. (1998) Impact of sterols on life-history traits of a caterpillar. Physiological Entomology, 23, 165–175.

Behmer, S. T., & Elias, D. O. (1999). The nutritional significance of sterol metabolic constraints in the generalist grasshopper Schistocerca americana. *Journal of Insect Physiology*, *45*(4), 339-348.

Beijerinck, W. (1940). Calluna. A monograph on the Scotch heather. *Verhandelingen Akad. Wet., Amst.*, *38*, 1-180.

Bell, R. R., Thornber, E. J., Seet, J. L., Groves, M. T., Ho, N. P., & Bell, D. T. (1983). Composition and protein quality of honeybee-collected pollen of Eucalyptus marginata and Eucalyptus calophylla. *The Journal of nutrition*, *113*(12), 2479-2484.

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New phytologist*, *127*(4), 617-633.

Berenbaum, M. R., & Johnson, R. M. (2015). Xenobiotic detoxification pathways in honey bees. *Current opinion in insect science*, *10*, 51-58.

Bernardello, G., Galetto, L., & Anderson, G. J. (2000). Floral nectary structure and nectar chemical composition of some species from Robinson Crusoe Island (Chile). *Canadian Journal of Botany*, *78*(7), 862-871.

Berrington de González, A. & Cox, D.R. (2007) Interpretation of interaction: A review. *The Annals of Applied Statistics*, **1**, 371–385.

Bilbrough, C.J. and Richards, J.H. (1993) Ecology 74,481-492

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.

Blüthgen, N., & Klein, A. M. (2011). Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic and Applied Ecology*, *12*(4), 282-291.

Boyd, J. (2012). A bit touchy: Plants' insect defenses activated by touch. *Rice University*.

Bradburn, M.J., Clark, T.G., Love, S.B. & Altman, D.G. (2003) Survival Analysis Part II: Multivariate data analysis- An introduction to concepts and methods. *British Journal of Cancer*, **89**, 431–436.

Brandt, A., Gorenflo, A., Siede, R., Meixner, M., & Büchler, R. (2016). The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (Apis mellifera L.). *Journal of insect physiology*, *86*, 40-47.

Brito, R. M., & Oldroyd, B. P. (2010). A scientific note on a simple method for karyotyping honey bee (Apis mellifera) eggs. *Apidologie*, *41*(2), 178-180.

Brochu, K. K., van Dyke, M. T., Milano, N. J., Petersen, J. D., McArt, S. H., Nault, B. A., ... & Danforth, B. N. (2020). Pollen defenses negatively impact foraging and fitness in a generalist bee (Bombus impatiens: Apidae). *Scientific reports*, *10*(1), 1-12.

Brodeur-Campbell, S. E., Vucetich, J. A., Richter, D. L., Waite, T. A., Rosemier, J. N., & Tsai, C. J. (2006). Insect herbivory on low-lignin transgenic aspen. *Environmental Entomology*, *35*(6), 1696-1701.

Brooker, N., Windorski, J., & Bluml, E. (2008). Halogenated coumarin derivatives as novel seed protectants. *Communications in agricultural and applied biological sciences*, *73*(2), 81-89.

Brooks, M., Kristensen, K., van Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Mächler, M. & Bolker, B. (2017) Modeling zero-inflated count data with glmmTMB. *bioRxiv*, 132753.

Brown, M. J. F., Loosli, R., & Schmid-Hempel, P. (2000). Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos*, *91*(3), 421-427.

Brown, M. J. F., Moret, Y., & Schmid-Hempel, P. (2003a). Activation of host constitutive immune defence by an intestinal trypanosome parasite of bumble bees. *Parasitology*, *126*(3), 253-260.

Brown, M. J., Schmid-Hempel, R., & Schmid-Hempel, P. (2003b). Strong context-dependent virulence in a host–parasite system: reconciling genetic evidence with theory. *Journal of Animal Ecology*, *72*(6), 994-1002.

Brown, M. J. (2022). Complex networks of parasites and pollinators: moving towards a healthy balance. *Philosophical Transactions of the Royal Society B*, *377*(1853), 20210161.

Bruce, M., Legg, C. J., Lazaro, A., & Kraus, D. (2010). Fire use practices for habitat and wildlife management in Scotland and the UK with a case study from Glen Tanar Estate. *Best practices of fire use. Prescribed burning and supression fire programmes in selected case studies regions. European Forest Institute, Finland*.

Brunner, F. S., Schmid-Hempel, P., & Barribeau, S. M. (2014). Protein-poor diet reduces host-specific immune gene expression in Bombus terrestris. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1786), 20140128.

Buchmann, S. L., Jones, C. E., & Little, R. J. (1983). Buzz pollination in angiosperms. Handbook of experimental pollination biology. *Handbook of experimental pollination biology*, 73-113.

Bumble Bees of Wisconsin 2021, University Ave Madison, accessed 28 September 2021, <https://wisconsinbumblebees.entomology.wisc.edu>

Burak, M., & Imen, Y. (1999). Flavonoids and their antioxidant properties. *Turkiye Klin Tip Bil Derg*, *19*, 296-304.

Calley, M., Braithwaite, R. W., & Ladd, P. G. (1993). Reproductive biology of Ravenala madagascariensis Gmel. as an alien species. *Biotropica*, 61-72.

Calliste, C. A., Trouillas, P., Allais, D. P., Simon, A., & Duroux, J. L. (2001). Free radical scavenging activities measured by electron spin resonance spectroscopy and B16 cell antiproliferative behaviors of seven plants. *Journal of Agricultural and Food Chemistry*, *49*(7), 3321-3327.

Campbell, P. J. (2012). *Pyrethroids: from Chrysanthemum to modern industrial insecticide* (Vol. 314). Springer Science & Business Media.

Campos, M., Markham, K. R., Mitchell, K. A., & da Cunha, A. P. (1997). An approach to the characterization of bee pollens via their flavonoid/phenolic profiles. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *8*(4), 181-185.

Campos, M. G., Bogdanov, S., de Almeida-Muradian, L. B., Szczesna, T., Mancebo, Y., Frigerio, C., & Ferreira, F. (2008). Pollen composition and standardisation of analytical methods. *Journal of Apicultural Research*, *47*(2), 154-161.

Cane, J. H. (2021). A brief review of monolecty in bees and benefits of a broadened definition. *Apidologie*, *52*(1), 17-22.

Cardoso, J., & Soares, M. J. (2010). In vitro effects of citral on Trypanosoma cruzi metacyclogenesis. *Memorias do Instituto Oswaldo Cruz*, *105*(8), 1026-1032.

Cariveau, D. P., Powell, J. E., Koch, H., Winfree, R., & Moran, N. A. (2014). Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (Bombus). *The ISME journal*, *8*(12), 2369-2379.

Casagrande, F. (2000). *Mode d'action des flavonoides dans les cellules de melanome humain: regulation des kinases cdks impliquees en phase g1 ou g2 du cycle cellulaire* (Doctoral dissertation, Toulouse 3).

Castelli, L., Branchiccela, B., Garrido, M., Invernizzi, C., Porrini, M., Romero, H., ... & Antúnez, K. (2020). Impact of nutritional stress on honeybee gut microbiota, immunity, and Nosema ceranae infection. *Microbial Ecology*, *80*(4), 908-919.

Chapin 111, F.S. and McNaughton, S. (1989) Oecologia 79,551-557

Chen, K., Ohmura, W., Doi, S., & Aoyama, M. (2004). Termite feeding deterrent from Japanese larch wood. *Bioresource Technology*, *95*(2), 129-134.

Cilia, G., Zavatta, L., Ranalli, R., Nanetti, A., & Bortolotti, L. (2021). Replicative Deformed Wing Virus found in the head of adults from symptomatic commercial bumblebee (Bombus terrestris) colonies. *Veterinary Sciences*, *8*(7), 117.

Çoban, İ., Toplan, G. G., Özbek, B., Gürer, Ç. U., & Sarıyar, G. (2017). Variation of alkaloid contents and antimicrobial activities of Papaver rhoeas L. growing in Turkey and northern Cyprus. *Pharmaceutical biology*, *55*(1), 1894-1898.

Cook, D., Manson, J. S., Gardner, D. R., Welch, K. D., & Irwin, R. E. (2013). Norditerpene alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on pollinators. *Biochemical Systematics and Ecology*, *48*, 123-131.

Cooper, S. M., & Owen-Smith, N. (1986). Effects of plant spinescence on large mammalian herbivores. *Oecologia*, *68*(3), 446-455.

Cordes, N., Huang, W. F., Strange, J. P., Cameron, S. A., Griswold, T. L., Lozier, J. D., & Solter, L. F. (2012). Interspecific geographic distribution and variation of the pathogens Nosema bombi and Crithidia species in United States bumble bee populations. *Journal of invertebrate pathology*, *109*(2), 209-216.

Cotter, S. C., Hails, R. S., Cory, J. S., & WIlson, K. (2004). Density-dependent prophylaxis and condition-dependent immune function in Lepidopteran larvae: a multivariate approach. *Journal of Animal Ecology*, *73*(2), 283-293.

Coughenour, M.B. (1985)Ann. MO. Bat. Card. 72,852-863

Cox, P. A. (1991). Abiotic pollination: an evolutionary escape for animal-pollinated angiosperms. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *333*(1267), 217-224.

Cremer, S., Armitage, S. A., & Schmid-Hempel, P. (2007). Social immunity. *Current biology*, *17*(16), R693-R702.

DANGLES, O., & DELUZARCHE, M. Flavonoïdes et anthocyanes.

Dellicour, S., Michez, D., & Mardulyn, P. (2015). Comparative phylogeography of five bumblebees: impact of range fragmentation, range size and diet specialization. *Biological Journal of the Linnean Society*, *116*(4), 926-939.

Denisow, B., & Denisow-Pietrzyk, M. (2016). Biological and therapeutic properties of bee pollen: a review. *Journal of the Science of Food and Agriculture*, *96*(13), 4303-4309.

De Roode, J. C., Lefèvre, T., & Hunter, M. D. (2013). Self-medication in animals. *Science*, *340*(6129), 150-151.

Descamps, C., Moquet, L., Migon, M., & Jacquemart, A. L. (2015). Diversity of the insect visitors on Calluna vulgaris (Ericaceae) in Southern France heathlands. *Journal of Insect Science*, *15*(1), 130.

Deshwal, S., & Mallon, E. B. (2014). Antimicrobial peptides play a functional role in bumblebee anti-trypanosome defense. *Developmental & Comparative Immunology*, *42*(2), 240-243.

Despres, L., David, J. P., & Gallet, C. (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in ecology & evolution*, *22*(6), 298-307.

Diemont, W. H., Heijman, W. J., Siepel, H., & Webb, N. R. (Eds.). (2013). *Economy and ecology of heathlands: heathland ecology and management*. BRILL.

Dimitrova, B., Gevrenova, R., & Anklam, E. (2007). Analysis of phenolic acids in honeys of different floral origin by solid-pase extraction and high-performance liquid chromatography. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *18*(1), 24-32.

Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F., ... & Pennacchio, F. (2013). Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proceedings of the National Academy of Sciences*, *110*(46), 18466-18471.

Dogan, G., & BAGCI, E. (2014). Essential Oil Composition of Papaver rhoeas L. Corn poppy Papaveraceae from Turkey. *Hacettepe Journal of Biology and Chemistry*, *42*(4), 545-549.

Dormann, C. F. (2011). How to be a specialist? Quantifying specialisation in pollination networks. *Network Biology*, *1*(1), 1-20.

Duchateau, M. J., & Velthuis, H. H. W. (1988). Development and reproductive strategies in Bombus terrestris colonies. Behavior 107, 186-207.

Duez, P., Gerbaux, P. & Michez, D. (2018) *METAFLORE - Recherche d'une flore optimale par la caractérisation des métabolites secondaires de plantes médicinales et mellifères*.

Duke, S. O., Paul Jr, R. N., & Lee, S. M. (1988). Terpenoids from the genus Artemisia as potential pesticides.

Durrer, S., & Schmid-Hempel, P. (1994). Shared use of flowers leads to horizontal pathogen transmission. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *258*(1353), 299-302.

Drees, B. M., Miller, R. W., Vinson, B. S., & Georgis, R. (1992). Susceptibility and behavioral response of red imported fire ant (Hymenoptera: Formicidae) to selected entomogenous nematodes (Rhabditida: Steinernematidae & Heterorhabditidae). *Journal of Economic Entomology*, *85*(2), 365-370.

Ellers, J. (1996). Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. *Netherlands Journal of Zoology*, *46*, 227-235.

El-Masry, S., El-Ghazooly, M. G., Omar, A. A., Khafagy, S. M., & Phillipson, J. D. (1981). Alkaloids from Egyptian Papaver rhoeas. *Planta medica*, *41*(01), 61-64.

Engström, Y. (1999). Induction and regulation of antimicrobial peptides in Drosophila. *Developmental & Comparative Immunology*, *23*(4-5), 345-358.

Erler, S., & Lattorff, H. M. G. (2010). The degree of parasitism of the bumblebee (Bombus terrestris) by cuckoo bumblebees (Bombus (Psithyrus) vestalis). *Insectes sociaux*, *57*(4), 371-377.

Erler, S., Popp, M., Wolf, S., & Lattorff, H. M. G. (2012). Sex, horizontal transmission, and multiple hosts prevent local adaptation of Crithidia bombi, a parasite of bumblebees (Bombus spp.). *Ecology and Evolution*, *2*(5), 930-940.

Fernandes, G. W. (1994). Plant mechanical defenses against insect herbivory. *Revista Brasileira de Entomologia*, *38*(2), 421-433.

Feyereisen, R. (2012). Insect CYP genes and P450 enzymes. In *Insect molecular biology and biochemistry* (pp. 236-316). Academic Press.

Figueroa, L. L., Grincavitch, C., & McArt, S. H. (2021). Crithidia bombi can infect two solitary bee species while host survivorship depends on diet. *Parasitology*, *148*(4), 435-442.

Finley, D. S. (1999). Patterns of calcium oxalate crystals in young tropical leaves: a possible role as an anti-herbivory defense. *Revista de biología tropical*, *47*(1-2), 27-31.

Fleming, T. H., Geiselman, C., & Kress, W. J. (2009). The evolution of bat pollination: a phylogenetic perspective. *Annals of botany*, *104*(6), 1017-1043.

Fowler, A. E., Stone, E. C., Irwin, R. E., & Adler, L. S. (2020). Sunflower pollen reduces a gut pathogen in worker and queen but not male bumble bees. *Ecological Entomology*, *45*(6), 1318-1326.

Fox, J., and Weisberg, S. (2019). *An R companion to applied regression*. Sage publications.

Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British journal of Nutrition*, *88*(6), 587-605.

Gebreziher, H. G. (2018). The role of herbivore-induced plant volatiles (HIPVs) as indirect plant defense mechanism in a diverse plant and herbivore species; a review. *International Journal of Agriculture Environment and Food Sciences*, *2*(4), 139-147.

Gegear, R. J., Otterstatter, M. C., & Thomson, J. D. (2006). Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1590), 1073-1078.

Gekière, A. (2021). From poison to potion: Effects of sunflower phenolamides on bumblebee microcolonies and individuals. *Mémoire de Master 2 en Sciences biologiques, Université de Mons, Mons, 134 pp*.

Gekière, A., Semay, I., Gérard, M., Michez, D., Gerbaux, P., & Vanderplanck, M. (2022). Poison or Potion: Effects of Sunflower Phenolamides on Bumble Bees and Their Gut Parasite. *Biology*, *11*(4), 545.

Génissel, A., Aupinel, P., Bressac, C., Tasei, J. N., & Chevrier, C. (2002). Influence of pollen origin on performance of Bombus terrestris micro-colonies. *Entomologia Experimentalis et Applicata*, *104*(2-3), 329-336.

Gerard, M., Michez, D., Debat, V., Fullgrabe, L., Meeus, I., Piot, N., ... & Vanderplanck, M. (2018). Stressful conditions reveal decrease in size, modification of shape but relatively stable asymmetry in bumblebee wings. *Scientific reports*, *8*(1), 1-10.

Ghedira, K. (2005). Les flavonoïdes: structure, propriétés biologiques, rôle prophylactique et emplois en thérapeutique. *Phytothérapie*, *3*(4), 162-169.

Gherman, B. I., Denner, A., Bobiş, O., Dezmirean, D. S., Mărghitaş, L. A., Schluens, H., ... & Erler, S. (2014). Pathogen-associated self-medication behavior in the honeybee Apis mellifera. *Behavioral Ecology and Sociobiology*, *68*(11), 1777-1784.

Giacomini, J. J., Leslie, J., Tarpy, D. R., Palmer-Young, E. C., Irwin, R. E., & Adler, L. S. (2018). Medicinal value of sunflower pollen against bee pathogens. *Scientific reports*, *8*(1), 1-10.

Gimingham, C. H. (1960). Calluna-Vulgaris (L) Hull. *Journal of Ecology*, *48*(2), 455.

Gimingham, C. H. (1972). Ecology of heathlands.

Goldsworthy, G., Mullen, L., Opoku-Ware, K., & Chandrakant, S. (2003). Interactions between the endocrine and immune systems in locusts. *Physiological Entomology*, *28*(1), 54-61.

Gosselin, M., Michez, D., Vanderplanck, M., Roelants, D., Glauser, G., & Rasmont, P. (2013). Does Aconitum septentrionale chemically protect floral rewards to the advantage of specialist bumblebees?. *Ecological Entomology*, *38*(4), 400-407.

Goulson, D. (2010). *Bumblebees: behaviour, ecology, and conservation*. Oxford University Press on Demand.

Goulson, D., O'CONNOR, S. T. E. P. H., & Park, K. J. (2018). The impacts of predators and parasites on wild bumblebee colonies. *Ecological Entomology*, *43*(2), 168-181.

Graham, L. E., & Cook, M. E. (2007). Approaches to understanding the origin and early evolution of plant body symmetry and gravity responses. *Gravitational and Space Research*, *17*(2).

Grauso, L., Emrick, S., Bonanomi, G., & Lanzotti, V. (2019). Metabolomics of the alimurgic plants Taraxacum officinale, Papaver rhoeas and Urtica dioica by combined NMR and GC–MS analysis. *Phytochemical Analysis*, *30*(5), 535-546.

Grauso, L., de Falco, B., Motti, R., & Lanzotti, V. (2021). Corn poppy, Papaver rhoeas L.: a critical review of its botany, phytochemistry and pharmacology. *Phytochemistry Reviews*, *20*, 227-248.

Guyot, C., Bouseta, A., Scheirman, V., & Collin, S. (1998). Floral origin markers of chestnut and lime tree honeys. *Journal of Agricultural and Food Chemistry*, *46*(2), 625-633.

Guyot, C., Scheirman, V., & Collin, S. (1999). Floral origin markers of heather honeys: Calluna vulgaris and Erica arborea. *Food chemistry*, *64*(1), 3-11.

Hanley, M. E., Franco, M., Pichon, S., Darvill, B., & Goulson, D. (2008). Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Functional Ecology*, 592-598.

Hao, Z., Kasumba, I., Lehane, M. J., Gibson, W. C., Kwon, J., & Aksoy, S. (2001). Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce trypanosomiasis. *Proceedings of the National Academy of Sciences*, *98*(22), 12648-12653.

Hargreaves, A. L., Harder, L. D., & Johnson, S. D. (2009). Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biological Reviews*, *84*(2), 259-276.

Harrison, X.A. (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, 2, e616.

Hartig, F. (2021) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.

Hasselrot, T. B. (1960). Studies on Swedish bumblebees (genus Bombus Latr): their domestication and biology. *Opusc. Ent.(suppl.)*.

Heilmeier, H., Schulze, E. and Whale, D.M. (1986) Oecologia 70,466-474

Heinrich, B. (1975). Thermoregulation in bumblebees. *Journal of Comparative Physiology*, *96*(2), 155-166.

Hetru, C. (1998). Antimicrobial peptides from insects. *Molecular mechanisms of immune responses in insects*.

Hillenbrand, M., Zapp, J., & Becker, H. (2004). Depsides from the petals of Papaver rhoeas. *Planta medica*, *70*(04), 380-382.

Hochuli, D. F. (2001). Insect herbivory and ontogeny: how do growth and development influence feeding behaviour, morphology and host use?. *Austral Ecology*, *26*(5), 563-570.

Hoffmann, J. A., Reichhart, J. M., & Hetru, C. (1996). Innate immunity in higher insects. *Current opinion in immunology*, *8*(1), 8-13.

Hoffmann-Campo, C. B., Harborne, J. B., & McCaffery, A. R. (2001). Pre-ingestive and post-ingestive effects of soya bean extracts and rutin on Trichoplusia ni growth. *Entomologia Experimentalis et Applicata*, *98*(2), 181-194.

Hostettmann, K., & Marston (theophylline, theobromine and caffeine) binding with DNA. *PLoS One*, *7*(12), e50019.

Howe, H. F., & Westley, L. C. (1988). *Ecological relationships of plants and animals*. Oxford University Press.

Hu, S., Dilcher, D. L., Jarzen, D. M., & Taylor, D. W. (2008). Early steps of angiosperm–pollinator coevolution. *Proceedings of the National Academy of Sciences*, *105*(1), 240-245.

Huang, T., Jander, G., & de Vos, M. (2011). Non-protein amino acids in plant defense against insect herbivores: representative cases and opportunities for further functional analysis. *Phytochemistry*, *72*(13), 1531-1537.

Husson, F., Josse, J., Le, S., Mazet, J., & Husson, M. F. (2016). Package 'factominer'. *An R package*, *96*, 698.

Imhoof, B., & Schmid-Hempel, P. (1999). Colony success of the bumble bee, Bombus terrestris, in relation to infections by two protozoan parasites, Crithidia bombi and Nosema bombi. *Insectes Sociaux*, *46*(3), 233-238.

Irwin, R. E., Cook, D., Richardson, L. L., Manson, J. S., & Gardner, D. R. (2014). Secondary compounds in floral rewards of toxic rangeland plants: impacts on pollinators. *Journal of Agricultural and Food Chemistry*, *62*(30), 7335-7344.

Jeppsson, T. (2004). *Natural selection on floral traits in Aconitum lycoctonum (Ranunculaceae) in different regions of its distribution, with special regard to the presence/absence of Bombus consobrinus (Hymenoptera)* (Doctoral dissertation, Master thesis, Department of Studies in Biology and Environmental Sciences, Umeå University, Sweden).

Jones, R. L. (1973). Gibberellins: their physiological role. *Annual review of plant physiology*, *24*(1), 571-598.

Kameyama, Y., & Kudo, G. (2009). Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, Phyllodoce aleutica: effects of pollinators and self-incompatibility. *Annals of Botany*, *103*(9), 1385-1394.

Karban, R., Agrawal, A. A., & Mangel, M. (1997). The benefits of induced defenses against herbivores. *Ecology*, *78*(5), 1351-1355.

Kaslow, D. C., & Welburn, S. (1996). Insect-transmitted pathogens in the insect midgut. In *Biology of the insect midgut* (pp. 432-462). Springer, Dordrecht.

Kassambara, A., Kosinski, M., Biecek, P., & Fabian, S. (2021). Package 'survminer'-drawing survival curves using 'ggplot2'. *R Cran*.

Kearns, C. A., Inouye, D. W., & Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual review of ecology and systematics*, *29*(1), 83-112.

Kessler, A., & Halitschke, R. (2009). Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. *Functional Ecology*, *23*(5), 901-912.

Kessler, A., & Kalske, A. (2018). Plant secondary metabolite diversity and species interactions. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 115-138.

Kevan, P. G., & Imperatriz-Fonseca, V. L. (Eds.). (2002). *Pollinating bees: the conservation link between agriculture and nature* (p. 313). Brasilia: Ministry of Environment.

Kevan, P. G., & Viana, B. F. (2003). The global decline of pollination services. *Biodiversity*, *4*(4), 3-8.

Khan, M. T. H., & Ather, A. (2006). *Lead molecules from natural products: discovery and new trends*. Elsevier.

Kleijn, D., & Raemakers, I. (2008). A retrospective analysis of pollen host plant use by stable and declining bumble bee species. *Ecology*, *89*(7), 1811-1823.

Kleisner, K., & Markoš, A. (2005). Semetic rings: towards the new concept of mimetic resemblances. *Theory in Biosciences*, *123*(3), 209-222.

Koch, H., & Schmid-Hempel, P. (2011). Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences*, *108*(48), 19288-19292.

Koch, H., Woodward, J., Langat, M. K., Brown, M. J., & Stevenson, P. C. (2019). Flagellum removal by a nectar metabolite inhibits infectivity of a bumblebee parasite. *Current Biology*, *29*(20), 3494-3500.

Kostic, D. A., Mitic, S. S., Mitic, M. N., & Velickovic, J. M. (2010). Phenolic contents, antioxidant and antimicrobial activity of Papaver rhoeas L. extracts from Southeast Serbia. *Journal of Medicinal Plants Research*, *4*(17), 1727-1732.

Koulianos, S., & Schwarz, H. H. (1999). Reproduction, development and diet of Parasitellus fucorum (Mesostigmata: Parasitidae), a mite associated with bumblebees (Hymenoptera: Apidae). *Journal of Zoology*, *248*(2), 267-269.

Krauss, S. L., Phillips, R. D., Karron, J. D., Johnson, S. D., Roberts, D. G., & Hopper, S. D. (2017). Novel consequences of bird pollination for plant mating. *Trends in Plant Science*, *22*(5), 395-410.

Kraus L, Gehrmann B, Seitz R (2007). Calluna. In: Blaschek W, Ebel S, Hackenthal E, Holzgrabe U, Keller K, Reichling J, Schulz V (Eds.), Hagers Enzyklopaedie der Arzneistoffe und Drogen. 6. Auflage. Wissenschafltiche Verlagsgesellschaft mbH, Stuttgart, pp. 586-592.

Kudo, G., Hirao, A. S., & Kawai, Y. (2011). Pollination efficiency of bumblebee queens and workers in the alpine shrub Rhododendron aureum. *International Journal of Plant Sciences*, *172*(1), 70-77.

Kumarasamy, Y., Cox, P. J., Jaspars, M., Nahar, L., & Sarker, S. D. (2002). Screening seeds of Scottish plants for antibacterial activity. *Journal of ethnopharmacology*, *83*(1-2), 73-77.

Kuromori, T., Seo, M., & Shinozaki, K. (2018). ABA transport and plant water stress responses. *Trends in plant science*, *23*(6), 513-522.

Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. *Nature reviews microbiology*, *14*(6), 374-384.

Lee, Y. K., Yuk, D. Y., Lee, J. W., Lee, S. Y., Ha, T. Y., Oh, K. W., ... & Hong, J. T. (2009). (−)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. *Brain research*, *1250*, 164-174.

Lee, S. B., Park, K. H., Choi, Y. C., Kim, W. T., Kang, S. W., Ihm, Y. B., ... & Park, I. G. (2012). Comparison of the Pollinating Activities of Bombus terrestris Hive Produced by Domestic Bumblebee Rearing Companies at the Beefsteak-tomato Houses in Korea. *Korean Journal of Apiculture*.

Lenth, R. (2018) lsmeans: Least-Squares Means.

Lev-Yadun, S., & Inbar, M. (2002). Defensive ant, aphid and caterpillar mimicry in plants?. *Biological Journal of the Linnean Society*, *77*(3), 393-398.

Li, J., Ou-Lee, T. M., Raba, R., Amundson, R. G., & Last, R. L. (1993). Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *The Plant Cell*, *5*(2), 171-179.

Li, X., Schuler, M. A., & Berenbaum, M. R. (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annu. Rev. Entomol.*, *52*, 231-253.

Li, S., Yu, X., & Feng, Q. (2019). Fat body biology in the last decade. *Annual Review of Entomology*, *64*, 315-333.

Liener, I. E. (2003). Plant antinutritional factors| detoxification.

Lipa, J. J., & Triggiani, O. (1988). Crithidia bombi sp. n. a flagellated parasite of a bumble-bee Bombus terrestris L.(Hymenoptera, Apidae). *Acta protozoologica*, *27*(3-4), 287-290.

Logan, A., Ruiz-González, M. X., & Brown, M. J. F. (2005). The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. *Parasitology*, *130*(6), 637-642.

Loken, A. (1973). Studies on scandinavian bumble bees (Hymenoptera, Apidae).

Lozano, G. A. (1998). Parasitic stress and self-medication in wild animals. *Advances in the Study of Behaviour*, *27*, 291-318.

Ludwiczuk, A., Skalicka-Woźniak, K., & Georgiev, M. I. (2017). Terpenoids. In *Pharmacognosy* (pp. 233-266). Academic Press.

Lunau, K., Piorek, V., Krohn, O., & Pacini, E. (2015). Just spines—mechanical defense of malvaceous pollen against collection by corbiculate bees. *Apidologie*, *46*(2), 144-149.

Lüttge, U. (1977). Nectar composition and membrane transport of sugars and amino acids: a review on the present state of nectar research. *Apidologie*, *8*(4), 305-319.

Macrae, R., Robinson, R. K., & Sadler, M. J. (1993). Encyclopaedia of food science, food technology and nutrition.

Madsen, H. B., & Calabuig, I. (2012). Kommenteret checkliste over danmarks bier–Del 5: Apidae (hymenoptera, apoidea). *Entomologiske Meddelelser*, *80*(1), 7-52.

Mahy, G., Sloover, J. D., & Jacquemart, A. L. (1998). The generalist pollination system and reproductive success of Calluna vulgaris in the Upper Ardenne. *Canadian Journal of Botany*, *76*(11), 1843-1851.

Mallon, E. B., Loosli, R., & SCHMID-HEMPEL, P. A. U. L. (2003). Specific versus nonspecific immune defense in the bumblebee, Bombus terrestris L. *Evolution*, *57*(6), 1444-1447.

Manson, J. S., Otterstatter, M. C., & Thomson, J. D. (2010). Consumption of a nectar alkaloid reduces pathogen load in bumble bees. *Oecologia*, *162*(1), 81-89.

Mao, W., Rupasinghe, S. G., Johnson, R. M., Zangerl, A. R., Schuler, M. A., & Berenbaum, M. R. (2009). Quercetin-metabolizing CYP6AS enzymes of the pollinator Apis mellifera (Hymenoptera: Apidae). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *154*(4), 427-434.

Martin, C. D., Fountain, M. T., & Brown, M. J. (2018). Bumblebee olfactory learning affected by task allocation but not by a trypanosome parasite. *Scientific reports*, *8*(1), 1-8.

Martinez, A. P. (2020). pairwiseAdonis: pairwise multilevel comparison using adonis. R package version 0.3.

Maurizi, A., De Michele, A., Ranfa, A., Ricci, A., Roscini, V., Coli, R., ... & Burini, G. (2015). Bioactive compounds and antioxidant characterization of three edible wild plants traditionally consumed in the Umbria Region (Central Italy): Bunias erucago L.(corn rocket), Lactuca perennis L.(mountain lettuce) and Papaver rhoeas L.(poppy). *Journal of Applied Botany and Food Quality*, *88*(1).

Mayfield, M. M., Waser, N. M., & Price, M. V. (2001). Exploring the 'most effective pollinator principle'with complex flowers: bumblebees and Ipomopsis aggregata. *Annals of Botany*, *88*(4), 591-596.

McNaught, A. D. (1997). *Compendium of chemical terminology* (Vol. 1669). Oxford: Blackwell Science.

Meeus, I., Mommaerts, V., Billiet, A., Mosallanejad, H., Van de Wiele, T., Wäckers, F., & Smagghe, G. (2013). Assessment of mutualism between Bombus terrestris and its microbiota by use of microcolonies. *Apidologie*, *44*(6), 708-719.

Meeus, I., Pisman, M., Smagghe, G., & Piot, N. (2018). Interaction effects of different drivers of wild bee decline and their influence on host–pathogen dynamics. *Current opinion in insect science*, *26*, 136-141.

Merschjohann, K., Sporer, F., Steverding, D., & Wink, M. (2001). In vitro effect of alkaloids on bloodstream forms of Trypanosoma brucei and T. congolense. *Planta medica*, *67*(07), 623-627.

Michel A. 2022. Les vergers de merisiers représentent-ils une pharmacie naturelle pour les bourdons parasités ? Mémoire de Master 2 en Sciences biologiques, Université de Mons, Mons, 82 pp.

Michener, C. D. (2000). *The bees of the world* (Vol. 1). JHU press.

Michez, D., Patiny, S., Rasmont, P., Timmermann, K., & Vereecken, N. J. (2008). Phylogeny and host-plant evolution in Melittidae sl (Hymenoptera: Apoidea). *Apidologie*, *39*(1), 146-162.

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annual review of plant biology*, *63*, 431-450.

Monschein, M., Neira, J. I., Kunert, O., & Bucar, F. (2010). Phytochemistry of heather (Calluna vulgaris (L.) Hull) and its altitudinal alteration. *Phytochemistry Reviews*, *9*(2), 205-215.

Moure-Oliveira, D., Hirotsu, C. M., Serrano, J. C., & Garófalo, C. A. (2019). Host-parasitoid interactions between the solitary bee Centris analis (Apidae: Centridini) and conopid flies (Diptera: Conopidae). *The Science of Nature*, *106*(7), 1-8.

Morales, P., Ferreira, I. C., Carvalho, A. M., Sánchez-Mata, M. C., Cámara, M., Fernández-Ruiz, V., ... & Tardío, J. (2014). Mediterranean non-cultivated vegetables as dietary sources of compounds with antioxidant and biological activity. *LWT-Food Science and Technology*, *55*(1), 389-396.

Moreno-García, M., Córdoba-Aguilar, A., Condé, R., & Lanz-Mendoza, H. (2013). Current immunity markers in insect ecological immunology: assumed trade-offs and methodological issues. *Bulletin of Entomological Research*, *103*(2), 127-139.

Morris, J. L., Puttick, M. N., Clark, J. W., Edwards, D., Kenrick, P., Pressel, S., ... & Donoghue, P. C. (2018). The timescale of early land plant evolution. *Proceedings of the National Academy of Sciences*, *115*(10), E2274-E2283.

Müller, C. B. (1994). Parasitoid induced digging behaviour in bumblebee workers. *Animal Behaviour*, *48*(4), 961-966.

Müller, A., Diener, S., Schnyder, S., Stutz, K., Sedivy, C., & Dorn, S. (2006). Quantitative pollen requirements of solitary bees: implications for bee conservation and the evolution of bee–flower relationships. *Biological conservation*, *130*(4), 604-615.

Nabhan, G. P., & Buchmann, S. L. (1997). Services provided by pollinators. *Nature's Services: societal dependence on natural ecosystems*, 133-150

Näpflin, K., & Schmid-Hempel, P. (2018). High gut microbiota diversity provides lower resistance against infection by an intestinal parasite in bumblebees. *The American Naturalist*, *192*(2), 131-141.

Ngor, L., Palmer-Young, E. C., Nevarez, R. B., Russell, K. A., Leger, L., Giacomini, S. J., ... & McFrederick, Q. S. (2020). Cross-infectivity of honey and bumble bee-associated parasites across three bee families. *Parasitology*, *147*(12), 1290-1304.

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.

Nigam, Y., Maudlin, I., Welburn, S., & Ratcliffe, N. A. (1997). Detection of Phenoloxidase Activity in the Hemolymph of Tsetse Flies, Refractory and Susceptible to Infection withTrypanosoma brucei rhodesiense. *Journal of invertebrate pathology*, *69*(3), 279-281.

Niklas, K. J. (1997). *The evolutionary biology of plants*. University of Chicago Press.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... & Wagner, H. (2020). Vegan: community ecology package (version 2.5-7). *The Comprehensive R Archive Network*.

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals?. *Oikos*, *120*(3), 321-326.

Olsen, O. W. (1986). *Animal parasites: their life cycles and ecology*. Courier Corporation.

Osborne, J.W. (2010) Improving your data transformations: Applying the Box-Cox transformation. *Practical Assessment, Research and Evaluation*, **15**, 1–9.

Otterstatter, M. C., Gegear, R. J., Colla, S. R., & Thomson, J. D. (2005). Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behavioral Ecology and Sociobiology*, *58*(4), 383-389.

Otterstatter, M. C., & Thomson, J. D. (2007). Contact networks and transmission of an intestinal pathogen in bumble bee (Bombus impatiens) colonies. *Oecologia*, *154*(2), 411-421.

Otti, O., & Schmid-Hempel, P. (2007). Nosema bombi: a pollinator parasite with detrimental fitness effects. *Journal of invertebrate pathology*, *96*(2), 118-124.

Otti, O., & SCHMID-HEMPEL, P. A. U. L. (2008). A field experiment on the effect of Nosema bombi in colonies of the bumblebee Bombus terrestris. *Ecological Entomology*, *33*(5), 577-582.

Pacini, E., & Hesse, M. (2005). Pollenkitt–its composition, forms and functions. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *200*(5), 399-415.

Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., & Bansal, Y. K. (2015). Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy*, *9*(3), 293-304.

Palmer-Young, E. C., Farrell, I. W., Adler, L. S., Milano, N. J., Egan, P. A., Irwin, R. E., & Stevenson, P. C. (2019). Secondary metabolites from nectar and pollen: a resource for ecological and evolutionary studies. *Ecology*, *100*(4).

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*.

Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., & Estevinho, L. M. (2014). Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. *Food and Chemical Toxicology*, *63*, 233-239.

Pelletier, S. W. (Ed.). (1983). Alkaloids: chemical and biological perspectives.

Pereboom, J. J. M. (2000). The composition of larval food and the significance of exocrine secretions in the bumblebee Bombus terrestris. *Insectes Sociaux*, *47*(1), 11-20.

Pereboom, J. J. M., Velthuis, H. H. W., & Duchateau, M. J. (2003). The organisation of larval feeding in bumblebees (Hymenoptera, Apidae) and its significance to caste differentiation. *Insectes sociaux*, *50*(2), 127-133.

Pérez-Mellado, V., Riera, N., Potter, C., Piccolo, V., & Hernández-Estevéz, J. Á. (2006). A Complex Case of Interaction between Lizards and Plants. The Dead Horse Arum (" Drancunculus muscivorus") and the Balearic Lizard (" Podarcis lilfordi"). *A Complex Case of Interaction between Lizards and Plants. The Dead Horse Arum (" Drancunculus muscivorus") and the Balearic Lizard (" Podarcis lilfordi")*, 1000-1028.

Piessens, K., & Hermy, M. (2006). Does the heathland flora in north-western Belgium show an extinction debt?. *Biological Conservation*, *132*(3), 382-394.

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2020). R Core Team (2020) nlme: linear and nonlinear mixed effects models. R package version 3.1–148.

Plischuk, S., & Lange, C. E. (2009). Invasive Bombus terrestris (Hymenoptera: Apidae) parasitized by a flagellate (Euglenozoa: Kinetoplastea) and a neogregarine (Apicomplexa: Neogregarinorida). *Journal of Invertebrate Pathology*, *102*(3), 263-265.

Plischuk, S. (2010). *Protistas (Protista) entomopatógenos asociados a apoideos (Hymenoptera: Apoidea) polinizadores de la región pampeana* (Doctoral dissertation, Universidad Nacional de la Plata).

Plischuk, S., Pocco, M. E., & Lange, C. E. (2013). The tracheal mite Locustacarus buchneri in South American native bumble bees (Hymenoptera: Apidae). *Parasitology international*, *62*(6), 505-507.

Plischuk, S., Antúnez, K., Haramboure, M., Minardi, G. M., & Lange, C. E. (2017). Long-term prevalence of the protists C rithidia bombi and A picystis bombi and detection of the microsporidium N osema bombi in invasive bumble bees. *Environmental microbiology reports*, *9*(2), 169-173.

Poinar, G. O., & Van der Laan, P. A. (1972). Morphology and life history of Sphaerularia bombi. *Nematologica*, *18*(2), 239-252.

Poinar Jr, G. O., & Hess, R. (1972). Food uptake by the insect-parasitic nematode, Sphaerularia bombi (Tylenchida). *Journal of nematology*, *4*(4), 270.

Poinar, G. O., & Danforth, B. N. (2006). A fossil bee from Early Cretaceous Burmese amber. *Science*, *314*(5799), 614-614.

Ponchau, O., Iserbyt, S., Verhaeghe, J. C., & Rasmont, P. (2006, January). Is the caste-ratio of the oligolectic bumblebee Bombus gerstaeckeri Morawitz (Hymenoptera: Apidae) biased to queens?. In *Annales de la Société entomologique de France* (Vol. 42, No. 2, pp. 207-214). Taylor & Francis Group.

Pouvreau, A. (1967). Contribution a l'étude morphologique et biologique d'Aphomia sociella L.(Lepidoptera, Heteroneura, Pyralidoidea, Pyralididæ), parasite des nids de bourdons (Hymenoptera, Apoidea, Bombus Latr.). *Insectes sociaux*, *14*(1), 57-72.

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.

Praz, C. J., Müller, A., & Dorn, S. (2008). Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen. *Ecology*, *89*(3), 795-804.

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N., & Weis, A. E. (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual review of Ecology and Systematics*, *11*(1), 41-65.

Pritchard, D. J., & Vallejo-Marín, M. (2020). Buzz pollination. *Current Biology*, *30*(15), R858-R860.

Pusztahelyi, T., Holb, I. J., & Pócsi, I. (2015). Secondary metabolites in fungus-plant interactions. *Frontiers in plant science*, *6*, 573.

Pyke, G. H. (1991). What does it cost a plant to produce floral nectar?. *Nature*, *350*(6313), 58-59.

Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science*, *318*(5851), 812-814.

Rasmont, P., Leclercq, J., Jacob-Remacle, A., Pauly, A., & Gaspar, C. (1993). The faunistic drift of Apoidea in Belgium. *Bees for pollination. Commission of the EC*, 65-87.

Raven, J. A., & Edwards, D. (2014). Photosynthesis in early land plants: adapting to the terrestrial environment. In *Photosynthesis in bryophytes and early land plants* (pp. 29-58). Springer, Dordrecht.

Regali, A., & Rasmont, P. (1995). Nouvelles méthodes de test pour l'évaluation du régime alimentaire chez des colonies orphelines de Bombus terrestris (L)(Hymenoptera, Apidae). *Apidologie*, *26*(4), 273-281.

Richardson, L. L., Adler, L. S., Leonard, A. S., Andicoechea, J., Regan, K. H., Anthony, W. E., ... & Irwin, R. E. (2015). Secondary metabolites in floral nectar reduce parasite infections in bumblebees. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1803), 20142471.

Rivest, S., & Forrest, J. R. (2020). Defence compounds in pollen: why do they occur and how do they affect the ecology and evolution of bees?. *New Phytologist*, *225*(3), 1053-1064.

Roberts, D.W. (2019) labdsv: Ordination and Multivariate Analysis for Ecology. <https://cran.r-project.org/package=labdsv>.

Roger, N., Michez, D., Wattiez, R., Sheridan, C., & Vanderplanck, M. (2017). Diet effects on bumblebee health. *Journal of Insect Physiology*, *96*, 128-133.

de Roode, J. C., & Hunter, M. D. (2019). Self-medication in insects: when altered behaviors of infected insects are a defense instead of a parasite manipulation. *Current opinion in insect science*, *33*, 1-6.

Rosales, C. (2017). Cellular and molecular mechanisms of insect immunity. *Insect Physiology and Ecology. InTeach Publicaiton. CCBY*, 179-212.

Röseler, P. F. (1970). Unterschiede in der Kastendetermination zwischen den Hummelarten Bombus hypnorum und Bombus terrestris/differences in the caste determination between the bumblebee species Bombus hypnorum and Bombus terrestris. *Zeitschrift für Naturforschung B*, *25*(5), 543-548.

Rosenburgh, A., & Marrs, R. (2010). The Heather Beetle: a review. *Unpublished report to the Heather Trust (available from www. heathertrust. org. uk)*.

Rosenkranz, V., & Wink, M. (2008). Alkaloids induce programmed cell death in bloodstream forms of trypanosomes (Trypanosoma b. brucei). *Molecules*, *13*(10), 2462-2473.

Rosenthal, J. P., & Kotanen, P. M. (1994). Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution*, *9*(4), 145-148.

Rotariu, L., Lagarde, F., Jaffrezic-Renault, N., & Bala, C. (2016). Electrochemical biosensors for fast detection of food contaminants–trends and perspective. *TrAC Trends in Analytical Chemistry*, *79*, 80-87.

Royal Botanic Garden, UK (2016). State of the world's plants report-2016. *State of the world's plants report-2016.*

Ruiz-González, M. X., Bryden, J., Moret, Y., Reber-Funk, C., Schmid-Hempel, P., & Brown, M. J. (2012). Dynamic transmission, host quality, and population structure in a multihost parasite of bumblebees. *Evolution: International Journal of Organic Evolution*, *66*(10), 3053-3066.

Runckel, C., DeRisi, J., & Flenniken, M. L. (2014). A draft genome of the honey bee trypanosomatid parasite Crithidia mellificae. *PloS one*, *9*(4), e95057.

Saito, K. (2004). Sulfur assimilatory metabolism. The long and smelling road. *Plant Physiology*, *136*(1), 2443-2450.

Sakai, S., Kato, M., & Inoue, T. (1999). Three pollination guilds and variation in floral characteristics of Bornean gingers (Zingiberaceae and Costaceae). *American Journal of Botany*, *86*(5), 646-658.

Sarni-Manchado, P., & Cheynier, V. (2006). Les polyphénols en agroalimentaire,(Ed.) Lavoisier (Tec&Doc). *Sciences et techniques agro-al*, *61*(377), 2073-85.

Schmid-Hempel, P., & Schmid-Hempel, R. (1990). Endoparasitic larvae of conopid flies alter pollination behavior of bumblebees. *Naturwissenschaften*, *77*(9), 450-452.

Schmid-Hempel, R., & Schmid-Hempel, P. (1991). Endoparasitic flies, pollen-collection by bumblebees and a potential host-parasite conflict. *Oecologia*, *87*(2), 227-232.

Schmid-Hempel, P., & Schmid-Hempel, R. (1993). Transmission of a pathogen in Bombus terrestris, with a note on division of labour in social insects. *Behavioral Ecology and Sociobiology*, *33*(5), 319-327.

Schmidt, L. S., Schmidt, J. O., Rao, H., Wang, W., & Xu, L. (1995). Feeding preference and survival of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. *Journal of Economic Entomology*, *88*(6), 1591-1595.

Schmid-Hempel, R., & Schmid-Hempel, P. (1998). Colony performance and immunocompetence of a social insect, Bombus terrestris, in poor and variable environments. *Functional Ecology*, *12*(1), 22-30.

Schmid-Hempel, P. (1998). *Parasites in social insects* (Vol. 60). Princeton University Press.

SCHMID-HEMPEL, P. A. U. L., & STAUFFER, H. P. (1998). Parasites and flower choice of bumblebees. *Animal behaviour*, *55*(4), 819-825.

Schmid-Hempel, P. (2001). On the evolutionary ecology of host–parasite interactions: addressing the question with regard to bumblebees and their parasites. *Naturwissenschaften*, *88*(4), 147-158.

Schmid-Hempel, P. (2005). Natural insect host–parasite systems show immune priming and specificity: puzzles to be solved. *Bioessays*, *27*(10), 1026-1034.

Schmid-Hempel, P., Schmid-Hempel, R., Brunner, P. C., Seeman, O. D., & Allen, G. R. (2007). Invasion success of the bumblebee, Bombus terrestris, despite a drastic genetic bottleneck. *Heredity*, *99*(4), 414-422.

SCHMID-HEMPEL, R. E. G. U. L. A., & Tognazzo, M. (2010). Molecular divergence defines two distinct lineages of Crithidia bombi (Trypanosomatidae), parasites of bumblebees. *Journal of Eukaryotic Microbiology*, *57*(4), 337-345.

Schmid-Hempel, P., Aebi, M., Barribeau, S., Kitajima, T., du Plessis, L., Schmid-Hempel, R., & Zoller, S. (2018). The genomes of Crithidia bombi and C. expoeki, common parasites of bumblebees. *PloS one*, *13*(1), e0189738.

Schmitt, A., Roy, R., & Carter, C. J. (2021). Nectar antimicrobial compounds and their potential effects on pollinators. *Current Opinion in Insect Science*.

Seigler, D. S. (1998). *Plant secondary metabolism*. Springer Science & Business Media.

Servais, T., Cascales-Miñana, B., Cleal, C. J., Gerrienne, P., Harper, D. A., & Neumann, M. (2019). Revisiting the Great Ordovician Diversification of land plants: Recent data and perspectives. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *534*, 109280.

Shear, W. A. (1991). The early development of terrestrial ecosystems. *Nature*, *351*(6324), 283-289.

Shykoff, J. A., & Schmid-Hempel, P. (1991). Incidence and effects of four parasites in natural populations of bumble bees in Switzerland. *Apidologie*, *22*(2), 117-125.

Sıralı, R., Ugur, A., & Kocamanaoğlu, C. (2012). The possibilities of using bumble bees in greenhouses. *Arıcılık Araştırma Dergisi 4 (7)*, 16-20.

Söderhäll, K., & Cerenius, L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. *Current opinion in immunology*, *10*(1), 23-28.

Soulimani, R., Younos, C., Jarmouni-Idrissi, S., Bousta, D., Khalouki, F., & Laila, A. (2001). Behavioral and pharmaco-toxicological study of Papaver rhoeas L. in mice. *Journal of Ethnopharmacology*, *74*(3), 265-274.

Southwick, E. E. (1984). Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology*, *65*(6), 1775-1779.

Spivak, M., & Reuter, G. S. (2001). Resistance to American foulbrood disease by honey bee colonies Apis mellifera bred for hygienic behavior. *Apidologie*, *32*(6), 555-565.

Spivak, M., Goblirsch, M., & Simone-Finstrom, M. (2019). Social-medication in bees: the line between individual and social regulation. *Current opinion in insect science*, *33*, 49-55.

Sreevidya, V. S., Srinivasa Rao, C., Sullia, S. B., Ladha, J. K., & Reddy, P. M. (2006). Metabolic engineering of rice with soybean isoflavone synthase for promoting nodulation gene expression in rhizobia. *Journal of experimental botany*, *57*(9), 1957-1969.

Stabler, D., Paoli, P. P., Nicolson, S. W., & Wright, G. A. (2015). Nutrient balancing of the adult worker bumblebee (Bombus terrestris) depends on the dietary source of essential amino acids. *The Journal of experimental biology*, *218*(5), 793-802.

van der Steen, J. J. (2008). Infection and transmission of Nosema bombi in Bombus terrestris colonies and its effect on hibernation, mating and colony founding. *Apidologie*, *39*(2), 273-282.

Stevenson, P. C. (2020). For antagonists and mutualists: the paradox of insect toxic secondary metabolites in nectar and pollen. *Phytochemistry Reviews*, *19*(3), 603-614.

Stiles, B., & Paschke, J. D. (1980). Midgut pH in different instars of three Aedes mosquito species and the relation between pH and susceptibility of larvae to a nuclear polyhedrosis virus. *Journal of Invertebrate Pathology*, *35*(1), 58-64.

Strauss, S. Y., & Agrawal, A. A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends in ecology & evolution*, *14*(5), 179-185.

Strauss, S. Y., & Whittall, J. B. (2006). Non-pollinator agents of selection on floral traits. *Ecology and evolution of flowers*, *2006*, 120-138.

Strobl, V., Yañez, O., Straub, L., Albrecht, M., & Neumann, P. (2019). Trypanosomatid parasites infecting managed honeybees and wild solitary bees. *International journal for parasitology*, *49*(8), 605-613.

Szczesna, T. (2006). Long-chain fatty acids composition of honeybee-collected pollen. *Journal of Apicultural Science*, *50*(2), 65-79.

Tadmor-Melamed, H., Markman, S., Arieli, A., Distl, M., Wink, M., & Izhaki, I. (2004). Limited ability of Palestine sunbirds Nectarinia osea to cope with pyridine alkaloids in nectar of tree tobacco Nicotiana glauca. *Functional Ecology*, *18*(6), 844-850.

T'ai, H. R., & Cane, J. H. (2000). Pollen nutritional content and digestibility for animals. *Pollen and pollination*, 187-209.

Tasei, J. N., & Aupinel, P. (2008a). Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (Bombus terrestris, Hymenoptera: Apidae). *Apidologie*, *39*(4), 397-409.

Tasei, J. N., & Aupinel, P. (2008b). Validation of a method using queenless Bombus terrestris micro-colonies for testing the nutritive value of commercial pollen mixes by comparison with queenright colonies. *Journal of Economic Entomology*, *101*(6), 1737-1742.

Taylor, E. L., Taylor, T. N., & Krings, M. (2009). *Paleobotany: the biology and evolution of fossil plants*. Academic Press.

Team, R. C., Team, M. R. C., Suggests, M. A. S. S., & Matrix, S. (2018). Package "Stats.". *The R Stats Package*.

Team, R. C., (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL, https://www.R-project.org/.

Tehel, A., Brown, M. J., & Paxton, R. J. (2016). Impact of managed honey bee viruses on wild bees. *Current opinion in virology*, *19*, 16-22.

Therneau, T.M. (2020) coxme: Mixed Effects Cox Models.

Therneau, T.M. (2021) survival: Survival Analysis.

Thoison, O., Sévenet, T., Niemeyer, H. M., & Russell, G. B. (2004). Insect antifeedant compounds from Nothofagus dombeyi and N. pumilio. *Phytochemistry*, *65*(14), 2173-2176.

Thorp, R. W. (2000). Pollen collection by bees. *Plant Systematics and Evolution*, *222*, 211-223.

Tiwari, R., & Rana, C. S. (2015). Plant secondary metabolites: a review. *International Journal of Engineering Research and General Science*, *3*(5), 661-670.

Tomás-Lorente, F., Garcia-Grau, M. M., Nieto, J. L., & Tomás-Barberán, F. A. (1992). Flavonoids from Cistus ladanifer bee pollen. *Phytochemistry*, *31*(6), 2027-2029.

Treutter, D. (2005). Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant biology*, *7*(06), 581-591.

Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I., & Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology letters*, *8*(8), 857-874.

Tsuchiya, H., & Iinuma, M. (2000). Reduction of membrane fluidity by antibacterial sophoraflavanone G isolated from Sophora exigua. *Phytomedicine*, *7*(2), 161-165.

Tunon, H., Olavsdotter, C., & Bohlin, L. (1995). Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. *Journal of Ethnopharmacology*, *48*(2), 61-76.

Tyler, E. R., Adams, S., & Mallon, E. B. (2006). An immune response in the bumblebee, Bombus terrestris leads to increased food consumption. *BMC physiology*, *6*(1), 1-4.

Vaidya, C., Fisher, K., & Vandermeer, J. (2018). Colony development and reproductive success of bumblebees in an urban gradient. Sustainability, 10(6), 1936.

Vanderplanck, M., Moerman, R., Rasmont, P., Lognay, G., Wathelet, B., Wattiez, R., & Michez, D. (2014). How does pollen chemistry impact development and feeding behaviour of polylectic bees?. *PloS one*, *9*(1), e86209.

Vanderplanck, M., Decleves, S., Roger, N., Decroo, C., Caulier, G., Glauser, G., ... & Michez, D. (2018). Is non-host pollen suitable for generalist bumblebees?. *Insect Science*, *25*(2), 259-272.

Vanderplanck, M., Martinet, B., Carvalheiro, L. G., Rasmont, P., Barraud, A., Renaudeau, C., & Michez, D. (2019). Ensuring access to high-quality resources reduces the impacts of heat stress on bees. *Scientific reports*, *9*(1), 1-10.

Vanderplanck, M., Gilles, H., Nonclercq, D., Duez, P., & Gerbaux, P. (2020). Asteraceae paradox: Chemical and mechanical protection of Taraxacum pollen. *Insects*, *11*(5), 304.

Vanderplanck, M., Michez, D., Albrecht, M., Attridge, E., Babin, A., Bottero, I., ... & Gérard, M. (2021). Monitoring bee health in European agro-ecosystems using wing morphology and fat bodies. *One Ecosystem*, *6*, e63653.

Vardavas, C. I., Majchrzak, D., Wagner, K. H., Elmadfa, I., & Kafatos, A. (2006). Lipid concentrations of wild edible greens in Crete. *Food Chemistry*, *99*(4), 822-834.

Vaudo, A. D., Stabler, D., Patch, H. M., Tooker, J. F., Grozinger, C. M., & Wright, G. A. (2017). Correction: Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. *Journal of Experimental Biology*, *220*(3), 507-507.

Vaudo, A. D., Tooker, J. F., Patch, H. M., Biddinger, D. J., Coccia, M., Crone, M. K., ... & Grozinger, C. M. (2020). Pollen protein: Lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects*, *11*(2), 132.

Vermerris, W., & Nicholson, R. (2007). *Phenolic compound biochemistry*. Springer Science & Business Media.

Verpoorte, R., & Alfermann, A. W. (Eds.). (2000). *Metabolic engineering of plant secondary metabolism*. Springer Science & Business Media.

Vogelweith, F., Thiery, D., Moret, Y., & Moreau, J. (2013). Immunocompetence increases with larval body size in a phytophagous moth. *Physiological entomology*, *38*(3), 219-225.

Wadhawan, S., & Rao, C. K. (1993). Saponins in pollen. *Grana*, *32*(4-5), 255-259.

Wang, X. Y., Tang, J., Wu, T., Wu, D., & Huang, S. Q. (2019). Bumblebee rejection of toxic pollen facilitates pollen transfer. *Current Biology*, *29*(8), 1401-1406.

Webb, N. R. (1998). The traditional management of European heathlands. *Journal of Applied ecology*, *35*(6), 987-990.

Wells, T., Wolf, S., Nicholls, E., Groll, H., Lim, K. S., Clark, S. J., ... & Haughton, A. J. (2016). Flight performance of actively foraging honey bees is reduced by a common pathogen. *Environmental microbiology reports*, *8*(5), 728-737.

Westerkamp, C., & Classen-Bockhoff, R. (2007). Bilabiate flowers: the ultimate response to bees?. *Annals of botany*, *100*(2), 361-374.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*. springer.

Wink, M. (2010). *Annual plant reviews, functions and biotechnology of plant secondary metabolites* (Vol. 39). John Wiley & Sons.

Williams, K. S., & Gilbert, L. E. (1981). Insects as selective agents on plant vegetative morphology: egg mimicry reduces egg laying by butterflies. *Science*, *212*(4493), 467-469.

Williams, P. H. (1998). An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini). *Bulletin-Natural History Museum Entomology Series*, *67*, 79-152.

Williams, P. H., & Osborne, J. L. (2009). Bumblebee vulnerability and conservation world-wide. *Apidologie*, *40*(3), 367-387.

Willmer, P. G., Bataw, A. A. M., & Hughes, J. P. (1994). The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology*, *19*(3), 271-284.

Wilson, H. M., & Anderson, L. I. (2004). Morphology and taxonomy of paleozoic millipedes (Diplopoda: Chilognatha: Archipolypoda) from Scotland. *Journal of Paleontology*, *78*(1), 169-184.

Wilson-Rich, N., Dres, S. T., & Starks, P. T. (2008). The ontogeny of immunity: development of innate immune strength in the honey bee (Apis mellifera). *Journal of insect physiology*, *54*(10-11), 1392-1399.

Wittstock, U., & Gershenzon, J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current opinion in plant biology*, *5*(4), 300-307.

Wood, A. J. (2005). Eco-physiological adaptations to limited water environments. *Plant abiotic stress*, 1.

Wooller, R. D., & Wooller, S. J. (2003). The role of non-flying animals in the pollination of Banksia nutans. *Australian Journal of Botany*, *51*(5), 503-507.

Wuyts, N., De Waele, D., & Swennen, R. (2006). Extraction and partial characterization of polyphenol oxidase from banana (Musa acuminata Grande naine) roots. *Plant physiology and biochemistry*, *44*(5-6), 308-314.

Yoneda, M., Furuta, H., Kanbe, Y., Tsuchida, K., Okabe, K., & Goka, K. (2008). Reproduction and transmission within a colony of bumblebee tracheal mite Locustacarus buchneri (Acari: Podapolipidae) in Bombus terrestris (Hymenoptera: Apidae). *Applied entomology and zoology*, *43*(3), 391-395.

Young, T. P., & Okello, B. D. (1998). Relaxation of an induced defense after exclusion of herbivores: spines on Acacia drepanolobium. *Oecologia*, *115*(4), 508-513.

Zangerl, A. R., & Rutledge, C. E. (1996). The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *The American Naturalist*, *147*(4), 599-608.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial pathogenesis*, *124*, 198-202.

Zeng, R. S., Su, Y. J., Ye, M., Xie, L. J., Chen, M., & Song, Y. Y. (2008). Plant induced defense and biochemical mechanisms. *Journal of South China Agricultural University*, *29*(2), 1-6.

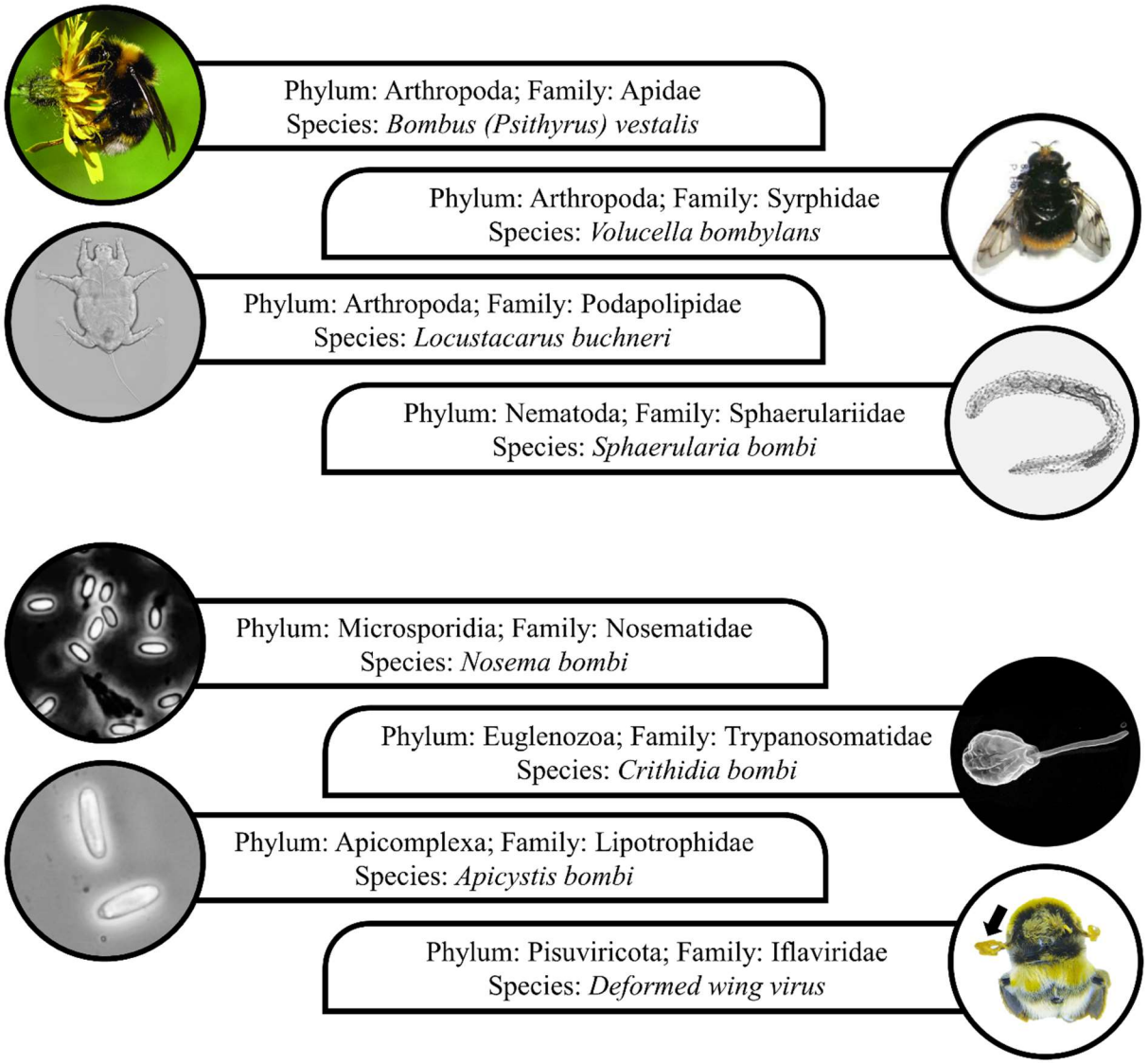
Zhou, D. C., Zittoun, R., & Marie, J. P. (1995). Homoharringtonine: an effective new natural product in cancer chemotherapy. *Bulletin du cancer*, *82*(12), 987-995.

Ziegler, J., Diaz-Chávez, M. L., Kramell, R., Ammer, C., & Kutchan, T. M. (2005). Comparative macroarray analysis of morphine containing Papaver somniferum and eight morphine free Papaver species identifies an O-methyltransferase involved in benzylisoquinoline biosynthesis. *Planta*, *222*(3), 458-471.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science and Business Media.

IX. Appendices

A. Bumblebee parasite diversity

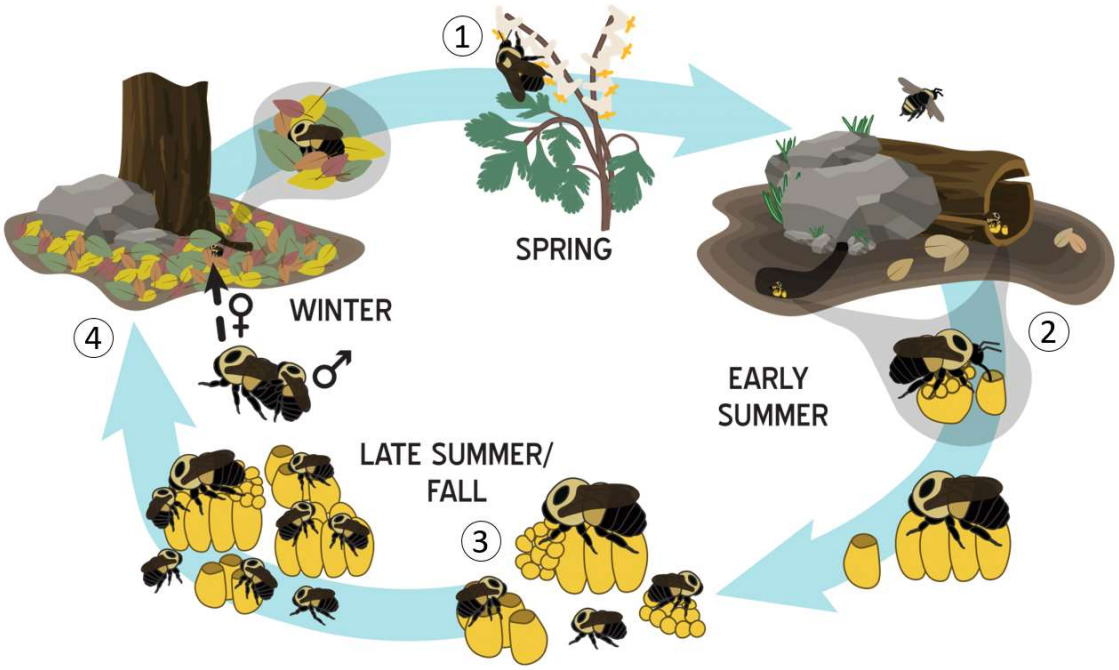


Appendix figure S1. Illustration of the diversity of parasites found in the genus *Bombus*. Bumblebees are parasitised by a wide variety of organisms at different levels of their complexity and organism. *Psithyrus* bumblebees such as *Bombus (Psithyrus) vestalis* (Photo: Madsen & Calabuig 2012) take advantage of colony resources, other insects such as the fly *Volucella bombylans* (Kleisner & Markoš 2005) lay eggs on the larvae. At the individual level, some mites act as extracorporeal parasites like *Locustacarus buchneri* (Plischuk et al. 2013). Many multi or single cell parasites affect bumblebees as intracorporeal parasites such as *Sphaerularia bombi* (Poinar & Hess 1972), *Nosema bombi* (Plischuk et al. 2017), *Crithidia bombi* (Schmid-Hempel & Tognazzo 2010) or *Apicystis bombi* (Plischuk 2010). Bumblebees are also the target of different viruses such as the *Deformed wing virus* (Cilia et al. 2021).

B. Organisation and life cycle of bumblebee colony

The eusocial colony lifestyle found in bumblebees allows for greater efficiency in resource collection, productivity of worker and reproductive individuals (Goulson 2010). Bumblebees are heterothermic, an example of the incredible abilities resulting from this social support between individuals, and have the ability to thermoregulate the whole nest (Heinrich 1975). Nests are often located underground in ancient rodent nests (Goulson 2010). Bumblebee colonies range from a few individuals to several hundred and individuals are split into three casts; workers, queens and males. The bumblebees build honeypots made of wax in which they feed and build cells in which the queen lays the eggs that develop later into adults (Goulson 2010).

According to Duchateau & Velthuis 1988, wild bumblebee cycle and colonies development evolve in several stages (Supplementary Figure 2): the colony is first born from a queen who, after emerging from hibernation, chooses a cavity to lay her eggs. She begins by laying diploid eggs which she takes care of alone and which will give rise to the first non-laying females or workers. Then, when the first workers emerge, the colony enters the eusocial phase and it is the workers who take care of the colony's tasks instead of the queen. The queen continues to lay new workers, enlarging the population of the colony, and will start to lay unfertilised (haploid) eggs which will give rise to reproductive males (switch point). Finally, when the colony has reached a sufficient size, a last phase starts with the arrival of worker bees capable of laying eggs (competition point) and which will therefore also start to lay eggs (which could only lead to males as they are not fertilised), thus competing with the queen and generating a general aggressiveness in the hive with numerous egg destructions. Among the surviving queen eggs, there are females with a fully developed reproductive system which form the queens of the next generation. These queens fly away, leave the hive and are fertilised by the males before founding their own colonies after hibernation while the males die during the reproduction period (Goulson 2010).

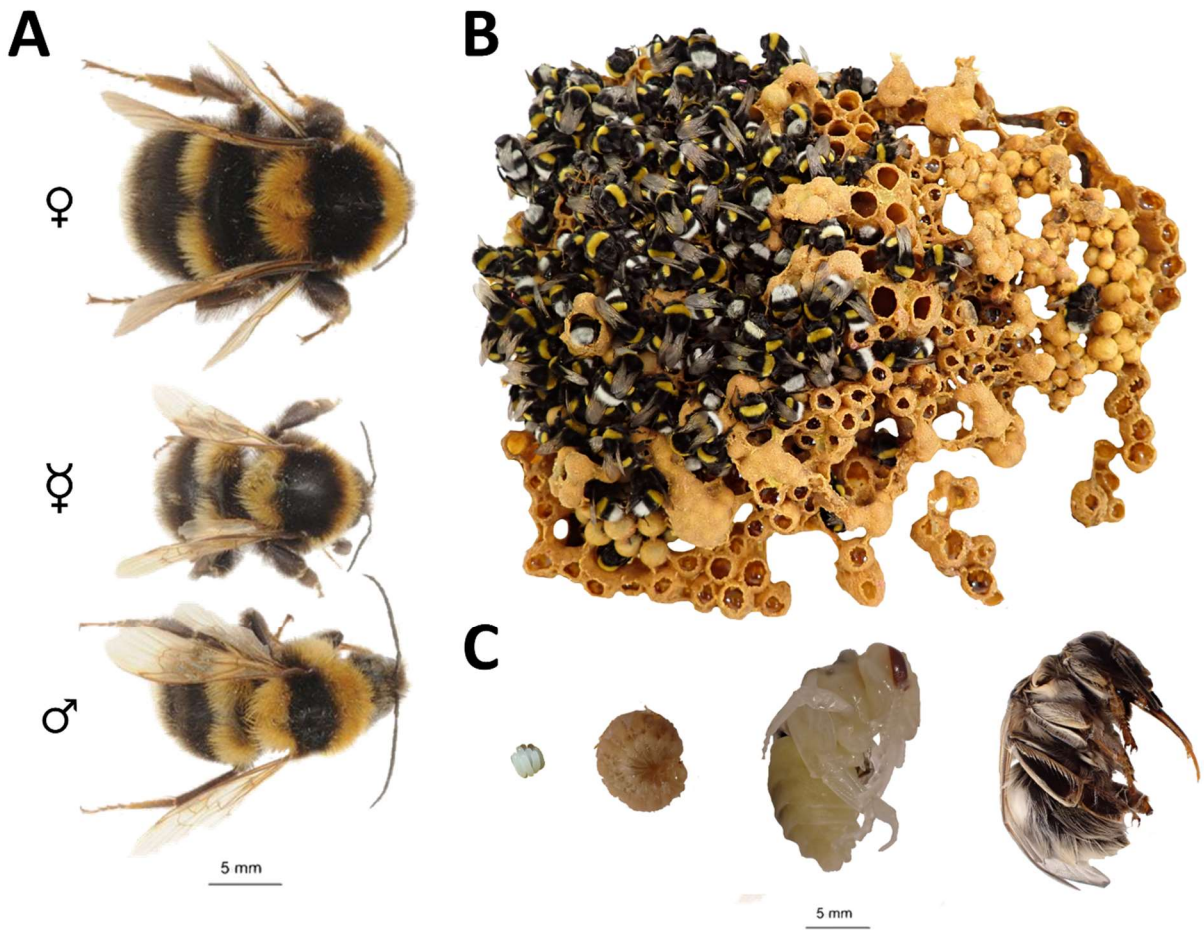


Appendix figure S2. Diagram of the general life cycle of the bumblebee. 1. Emergence of the hibernating queens at the beginning of the spring, the queens start to search for food. **2.** Nest creation, underground cavities are used and the queen builds honey pots and cells where she lays her first eggs. **3.** Emergence of the first workers, the queen continues to lay eggs but it is the workers which carry out the colony tasks. **4.** At the end of the summer, the colony produces males and new queens who mates. The queens then spend the winter hibernating before starting a colony the next season. (Source: Modified from Bumblebees of Wisconsin Website 2021).

C. Caste regulation and individual development in bumblebees

In bumblebees, the imagos can belong to three castes (Supplementary Figure 3A) namely; worker, queen (both diploid) and male (haploid), the last two being the sexual individuals ensuring the reproductive process and the next generation (Brito & Oldroyd 2010; Goulson 2010). The control of the worker ability to lay eggs is achieved by the queen and the pheromones she emits (Röseler 1970). In the absence of the queen, the workers develop their egg-laying system and start to lay eggs. The competition point (i.e. the arrival of worker bees capable of laying eggs) is the result of an increase in the hive population. The queen is no longer able to produce enough of the contraceptive hormone and to deposit it in the different structures of the colony (i.e. the wax of pollen and nectar pots, a typical colony is represented Supplementary Figure 3B). As a result, the workers develop the ability to lay eggs (Röseler 1970). During this phase, the diploid eggs (females) laid by the queen are also less subject to the hormone and are better fed than the previous ones due to the size of the colony. The amount of food does not seem to be the most important factor but rather the ability of the workers to meet the needs of the larvae (Pereboom et al. 2003). These two events combined lead to the development of the reproductive systems and these females become not workers but future queens (Röseler 1970). In contrast to the honeybee (*A. mellifera*), the quality of food is not altered and therefore would not be important for caste differentiation (Pereboom *et al.* 2000).

The individual development of bumblebees corresponds to a classical holometabolous cycle with a larval phase that continues post-metamorphosis into an adult phase (Supplementary Figure 3C; Goulson 2010). The eggs are laid in spherical structures in the colony and grow and segment into larvae. These larvae are first called pre-defaecating when they still possess metabolic waste products in their bodies which are visible in the larva. After the formation of the digestive tract, the larvae eject these metabolic residues and are then called post-defaecating (Goulson 2010). Latter, larvae enter metamorphosis as pupae and then give rise to the adult imago emerging from their developmental cell (Goulson 2010).



Appendix figure S3. Growth stage and castes of the bumblebee. A. Illustration of the three castes found in the social structure of the genus *Bombus* (here *Bombus ruderatus*). From top to bottom, queen (♀) bigger in size than the two others castes, worker (☿) and males (♂) this last caste with longer antennae (Photos: A. Pauly). **B.** Photo of a typical colony showing the cell structure containing honey or eggs to pupae bumblebees, here for *B. terrestris* (Photo: C. Tourbez). **C.** Different stages of development in bumblebees. From left to right, egg, larva, pupa and ready to emerge imago of *B. terrestris*. (Photos: C. Tourbez)

D. Preparation of diets

The massive samples of pollen and leaves having been subjected to the flavonoid extraction protocols, these extracts could be used (pollen: 209.79g; leaves: 11.75g) to supplement diets. Quantification was first done in triplicate (3 X 50g for the pollen) the flavonoids present in pollen and extracts via the HPLC-MS/MS protocol with a quercetin equivalent as already described. Following concentrations were deduced: (i) Salix pollen: 17.46 +/- 0.16 mg flavonoids/g fresh pollen; (ii) Calluna pollen: 14.73 +/- 1.69 mg/g fresh pollen; (iii) Calluna pollen extract: 40.63 +/- 0.72 mg/g extract & (iv) Calluna leaf extract: 66.53 +/- 1.50 mg/g extract. With these concentrations the amounts of each component to be added could be calculated (Supplementary Table S1) in order to have similar concentrations of ethanol 50% (16.58 - 20.5 µl/g diet) between all treatments, as well as flavonoids (11.85 - 12.8 mg/g diet) except for the diet with leaf extract (5.21 mg/g)

Appendix Table S1. Constitution of the diets given to the microcolonies. Each microcolony was fed every two days with pollen candies made up according to the proportions presented here. The values are presented here for 10g, corresponding to the quantity necessary to feed a set of 10 microcolonies (one treatment) at the beginning of the bioassays (1g/microcolony then increasing proportionally to the consumption of the microcolony up to 4g for some treatments). The proportions of each component were evaluated in order to have a similar amount of ethanol (for all treatments) and flavonoids (for treatments based on Calluna pollen and its pollen extracts) and thus avoid potential bias. '*' The amount of flavonoids only contained in the added extract without taking into account the flavonoids already present in the willow pollen being 11.67 mg/g for the diet with pollen extract and 11.99 mg/g for the diet with leaf extract.

DIET TREATMENTS				
	Control diet (Salix)	**Natural diet (Calluna)**	**Supplemented diet (Salix + pollen flavonoid extract)**	**Supplemented diet (Salix + leaf flavonoid extract)**
Pollen (g)	10 (Salix)	10 (Calluna)	10 (Salix)	10 (Salix)
65% sugar solution (numbers of drops)	5	5	0	0
Aqueous ethanol (v:v, 1:1) (ml)	0.5	0.5	0	0
Distilled water (ml)	4	1.5	0	0
Flavonoid extract (ml)	0	0	4.5	4.6
Final candy mass (g)	14.69	12.19	14.98	14.56
Ethanol in final candy (µl/g)	17.02	20.50	19.80	16.58
Pollen in final candy (g/g)	0.68	0.82	0.67	0.69
Flavonoid in final candy (mg/g)	11.89	12.08	11.85*	5.21*

E. Characterization of identified flavonoids

Appendix Table S2. List of flavonoids identified and quantified in heather tissues and resources. Identification and quantification by HPLC-MS/MS of flavonoids from the two heather tissues (i.e., corolla and leaf) and from heather pollen. Quantification was performed in three replicates of five individuals by analysis of flavonoid concentration per quercetin equivalent (QE). The concentrations (mg/g sample) represent the average obtained for the five replicate individuals. No flavonoids were identified in the nectar.

Molecular name	Molecular formula [M]	*m/z* $[M-H]^-$	Sample type	Concentration (mg QE/g sample)
Kaempferol-O-caffeoyl ester	$C_{24}H_{16}O_9$	447.2	Leaf	3.712
Quercetin-O-feruloyl ester	$C_{25}H_{18}O_{10}$	477.2	Leaf	0.628
Unknow molecule	$C_{23}H_{14}O_9$	433.15	Leaf	2.869
Kaempferol-O-caffeoylpentoside	$C_{29}H_{24}O_{13}$	579.25	Leaf	0.789
Quercetin-O-caffeoyl ester	$C_{24}H_{16}O_{10}$	463.2	Leaf	7.524
Quercetin-O-caffeoylpentoside	$C_{29}H_{24}O_{14}$	595.25	Leaf	0.630
Quercetin-O-hexoside isomer A	$C_{21}H_{20}O_{12}$	463,15	Corolla	0.136
Myricetin-O-rhamnoside	$C_{21}H_{20}O_{12}$	463,15	Corolla	0.134
Quercetin-O-hexoside isomer B	$C_{21}H_{20}O_{12}$	463,15	Corolla	0.441
Quercetin-O-hexoside isomer C	$C_{21}H_{20}O_{12}$	463,15	Corolla	0.141
Kaempferol-O-hexoside	$C_{21}H_{20}O_{11}$	447,15	Corolla	0.072
Quercetin-O-rhamnoside	$C_{21}H_{20}O_{11}$	447,15	Corolla	0.512
Flavonoid glycosyled	$C_{21}H_{22}O_{11}$	449.15	Corolla	0.253
Quercetin-O-pentoside isomer A	$C_{20}H_{18}O_{11}$	433,1	Corolla	0.066
Quercetin-O-pentoside isomer B	$C_{20}H_{18}O_{11}$	433,1	Corolla	0.183
Apigenin-O-hexuronide	$C_{21}H_{18}O_{11}$	445.1	Corolla	1.816
Kaempferol-O-rhamnoside	$C_{21}H_{20}O_{10}$	431.1	Corolla	2.364
Kaempferol-O-coumaroylhexoside isomer A	$C_{30}H_{26}O_{13}$	593,2	Corolla	0.548
			Pollen	0.608
Kaempferol-O-coumaroylhexoside isomer B	$C_{30}H_{26}O_{13}$	593,2	Corolla	0.125
Apigenin O-substitued	$C_{23}H_{20}O_{12}$	487.2	Corolla	4.737
Kaempferol-O-dicoumaroylhexoside	$C_{39}H_{32}O_{15}$	739.2	Pollen	0.273
Kaempferol-O-coumaroyldihexoside	$C_{36}H_{36}O_{18}$	755.2	Pollen	6.363

F. Summary of statistical outputs

Appendix Table S3. Compilation of statistical test results. All the parameters of each statistical test carried out in this thesis and their results are compiled in this table. The statistical tests are separated into four parts in relation to the structuring of the result chapter. Significant *p*.values ($p<0.05$) are indicated in bold underlined and '**' refers to significant value not supported by post-hoc tests. GLMM: Generalized linear mixed model. LMM: Linear mixed model. LNI: Non-isolated larvae. LI: Isolated larva.

1. DISTRIBUTION OF FLAVONOID PROFILE OF *C. VULGARIS*

Response variable (Statistical analysis)	Explanatory variable	Chi-squared value (χ^2)	Degree of freedom (DF)	*p*.value
Flavonoid profile (perMANOVA)	Tissue	160.98 (F Value)	3	**0.0001**
Flavonoid concentration (Kruskal-Wallis)	Tissue	15.067	3	**0.0018**

2. EFFECTS OF HEATHER FLAVONOIDS ON BUMBLEBEES

Response variable (Statistical analysis)	Explanatory variable	Chi-squared value (χ^2)	Degree of freedom (DF)	*p*.value
Pollen consumption (Gamma GLMM)	Diet	16.294	2	**0.0003**
Syrup consumption (Gamma GLMM)	Diet	12.064	2	**0.0024**
Egg number (Negative binomial GLMM)	Diet	4.555	2	0.1025
LNI number (Negative binomial GLMM)	Diet	2.904	2	0.2341
LI pre (Negative binomial GLMM)	Diet	0.234	2	0.8896
LI post (Negative binomial GLMM)	Diet	2.933	2	0.2308

Pupae (Negative binomial GLMM)	Diet	5.394	2	0.0674
Non emerged drone (Poisson GLMM)	Diet	5.954	2	0.0509
Emerged drone (Quasi-poisson GLMM)	Diet	37.997	2	**<0.0001**
Total offspring mass (Gaussian LMM)	Diet	20.335	2	**<0.0001**
Pollen dilution (Gamma GLMM)	Diet	7.238	2	**0.0268**
Pollen efficacy (Gamma GLMM)	Diet	1.712	2	0.425
Larval ejection (Binomial – GLMM)	Diet	0.084	2	0.9591
Worker survival (Cox analysis)	Diet	2.041	2	0.3604
Fat body content (Logit Gamma GLMM)	Diet	12.637	2	**0.0018**
	Sex	1.603	1	0.3024
	Diet * Sex	2.497	2	0.2869

3. IMPACTS OF HEATHER POLLEN AND ITS FLAVONOIDS CONSUMPTION ON BUMBLEBEES

Response variable (Statistical analysis)	**Explanatory variable**	**Chi-squared value (χ^2)**	**Degree of freedom (DF)**	***p*.value**
Pollen consumption (Gamma GLMM)	Diet	12.174	2	**0.0023**
Syrup consumption (Gamma GLMM)	Diet	8.997	2	**0.0111**
Egg number (Negative binomial GLMM)	Diet	1.865	2	0.3937
LNI number (Negative binomial GLMM)	Diet	2.111	2	0.3481

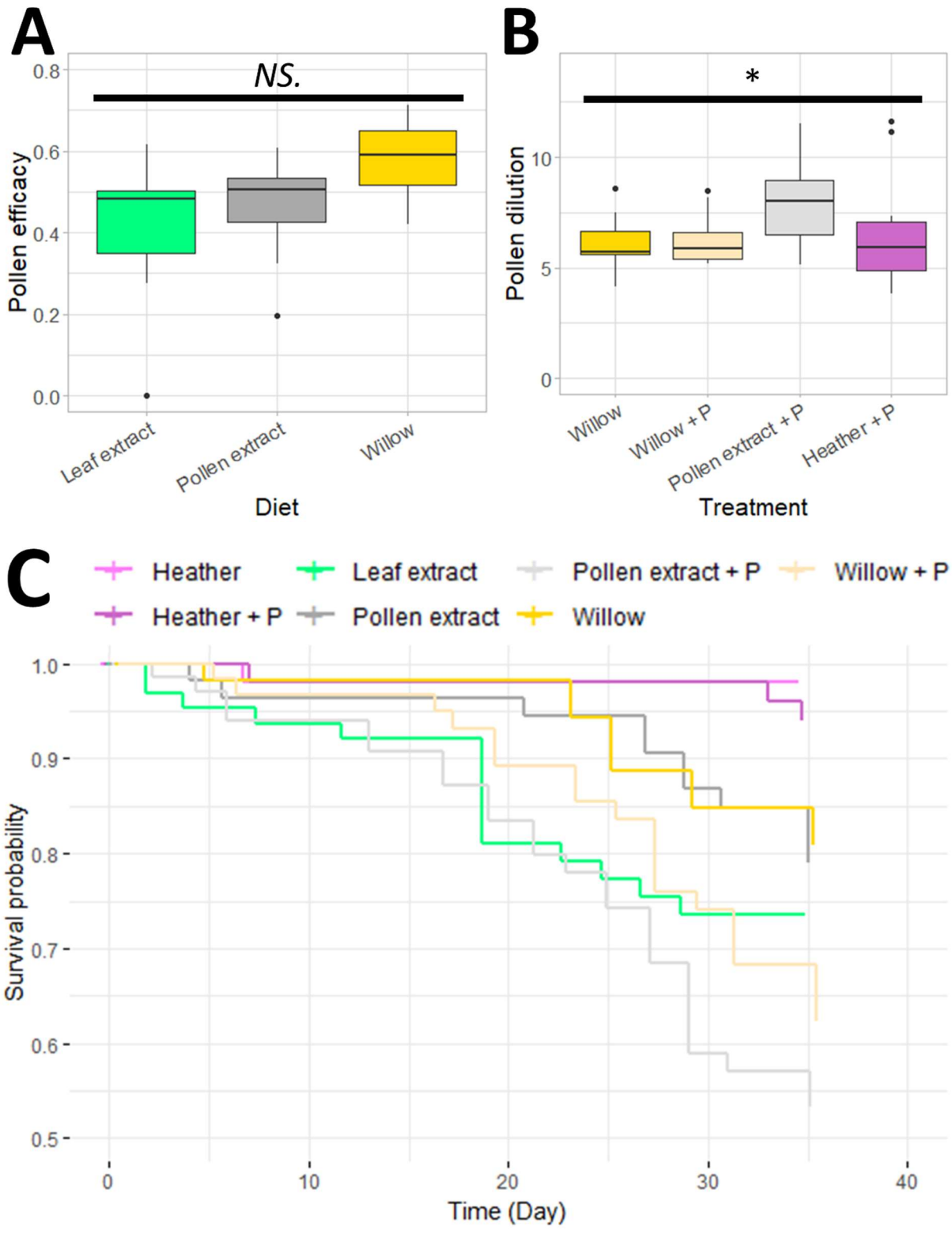
LI pre (Negative binomial GLMM)	Diet	3.776	2	0.1514
LI post (Poisson GLMM)	Diet	17.035	2	**0.0002**
Pupae (Negative binomial GLMM)	Diet	4.810	2	0.0903
Non emerged drone (Poisson GLMM)	Diet	4.698	2	0.0955
Emerged drone (Quasi-poisson GLMM)	Diet	21.444	2	**<0.0001**
Total offspring mass (Gaussian LMM)	Diet	15.304	2	**0.0005**
Pollen dilution (Gamma GLMM)	Diet	15.832	2	**0.0004**
Pollen efficacy (Gaussian LMM)	Diet	13.335	2	**0.0013**
Larval ejection (Binomial – GLMM)	Diet	0.153	2	0.9264
Worker survival (Cox analysis)	Diet	1.690	2	0.4296
Fat body content (Logit Gamma GLMM)	Diet	10.274	2	**0.0059**
	Sex	0.577	1	0.4476
	Diet * Sex	2.293	2	0.3177

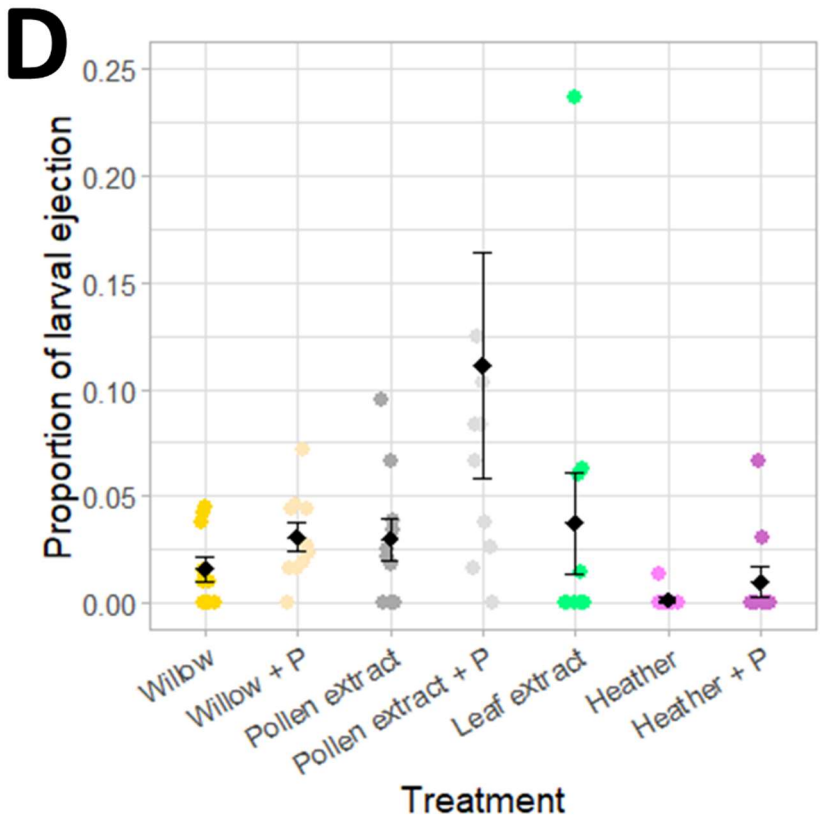
4. IMPACT OF THE FLORAL RESOURCE ON THE PARASITISED BUMBLEBEE

Response variable (Statistical analysis)	**Explanatory variable**	**Chi-squared value (χ^2)**	**Degree of freedom (DF)**	***p*.value**
Pollen consumption (Gaussian LMM)	Treatment	18.468	3	**0.0004**
Syrup consumption (Gamma GLMM)	Treatment	25.563	3	**<0.0001**

Egg number (Negative binomial GLMM)	Treatment	1.200	3	0.7531
LNI number (Negative binomial GLMM)	Treatment	5.212	3	0.1569
LI pre (Negative binomial GLMM)	Treatment	2.034	3	0.5654
LI post (Negative binomial GLMM)	Treatment	2.700	3	0.4402
Pupae (Negative binomial GLMM)	Treatment	10.781	3	0.0130**
Non emerged drone (Poisson GLMM)	Treatment	5.748	3	0.1245
Emerged drone (Quasi-poisson GLMM)	Treatment	43.418	3	**<0.0001**
Total offspring mass (Gamma GLMM)	Treatment	22.376	3	**<0.0001**
Pollen dilution (Gamma GLMM)	Treatment	9.360	3	0.0249**
Pollen efficacy (Gaussian LMM)	Treatment	35.187	3	**<0.0001**
Larval ejection (Binomial – GLMM)	Treatment	1.224	3	0.7472
Worker survival (Cox analysis)	Treatment	11.982	3	**0.0074**
Fat body content (Gamma GLMM)	Treatment	21.564	3	**<0.0001**
	Sex	8.132	1	**0.0044**
	Treatment * Sex	6.463	3	0.0911
Parasitic load (Negative binomial GLMM)	Treatment	15.471	2	**0.0004**
	Day	19.638	1	**<0.0001**
	Treatment * Day	1.248	2	0.5356

G. Supplementary graphs





Appendix figure S4. Compilation of non-significant result plots. A. Influence of heather flavonoids on pollen efficacy (ratio of offspring mass produced to pollen consumption). **B.** Influence of diet and parasite on pollen dilution (ratio between the use of syrup and pollen). **C.** Probability of survival of workers under each treatment (note that y-axis scale has been changed). **D.** Proportion of larval ejection (ratio of dead larvae to total number of offspring) under each treatment. The error bars show the standard error and the black dot in their centre indicates the mean value, '*NS.*' refers to not significant.