





# Phenology of the black bean aphid, *Aphis fabae*, on organic crops and effect of parasitoid communities

Mohannad Ismail<sup>1</sup>  | Bertanne Visser<sup>2</sup>  | Agathe Dupuis<sup>1</sup> | Thierry Hance<sup>1</sup>  | Loulou Albittar<sup>1</sup> 

<sup>1</sup>Earth and Life Institute, Biodiversity Research Centre, Université catholique de Louvain, Louvain-la-Neuve, Belgium

<sup>2</sup>Evolution and Ecophysiology Group, Department of Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Liège, Belgium

## Correspondence

Loulou Albittar, Earth and Life Institute, Biodiversity Research Centre, Université catholique de Louvain, 4-5 Place Croix-du-Sud, B-1348 Louvain-la-Neuve, Belgium.  
Email: [louloual@yahoo.fr](mailto:louloual@yahoo.fr)

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## Abstract

1. Parasitoids represent the third trophic level in plant-insect food webs. They develop in or on herbivorous insects at the second trophic level, which in turn feed on plants that constitute the first trophic level. This food web system might be affected by agricultural landscape and practices.
2. We studied the phenology and population density of *Aphis fabae* (Hemiptera: Aphididae) and determined the impact of parasitoids in organic fields of beans and beet plants.
3. Twenty leaves per field were collected weekly. For each leaf, the total number of aphids, apterous and alate morphs, the number of mummies, and mortality of aphids were recorded.
4. Our results showed that the phenology of *A. fabae* varied throughout the season with maximal numbers observed at the beginning of summer. In addition, the production of alate morphs and the proportion of parasitism were positively correlated to an increase in aphid densities.
5. Six parasitoid species were recorded attacking *A. fabae* in the field, with *Lysiphlebus fabarum* and *Binodoxys angelicae* (Hymenoptera: Aphidiinae) being the most abundant parasitoids.
6. Field characteristics and availability of host plants in the surroundings affected *A. fabae* density. *Lysiphlebus fabarum* and *B. angelicae* appeared to be good biocontrol candidates against *A. fabae*.

## Résumé

1. Dans le système de chaîne alimentaire d'agriculture, les parasitoïdes représentent le troisième niveau trophique qui pondent dans les pucerons (deuxième niveau trophique) nourrissant sur des cultures agricoles. Cette chaîne pourrait être influencer par les conditions de terrain et l'application agriculture.
2. Dans cet étude, on s'est focalisé sur la phénologie et la densité d'*Aphis fabae* (Hemiptera: Aphididae) et a déterminé l'impact de parasitoïdes présents dans les champs organiques de féveroles et de betteraves.

Thierry Hance and Loulou Albittar co last authors.

3. Chaque semaine, 20 feuilles issues d'une vingtaine de plantes infestées (par site), ont été collectées pendant la saison. Pour chaque feuille, le nombre de pucerons et leurs stades, ainsi que le nombre de momies présentes ont été enregistrés.
4. Nos résultats ont montré que la phénologie d'*A. fabae* a varié selon la saison. Le nombre de pucerons était plus élevé en printemps et au début de l'été. En outre, la production des individus ailés et le parasitisme étaient positivement liés à l'augmentation de la densité de pucerons.
5. Six espèces de parasitoïdes ont été enregistrées, avec *Lysiphlebus fabarum* et *Binodoxys angelicae* (Hymenoptera: Aphidiinae) étant les plus abondantes.
6. Les caractéristiques du terrain y compris le site et la disponibilité des plantes hôtes dans les alentours des cultures agricoles pourraient influencer la densité de pucerons. L'abondance des *L. fabarum* et *B. angelicae* fait de ces deux espèces des excellents candidats pour le développement d'un programme de lutte biologique contre *A. fabae*.

#### KEYWORDS

agriculture, diapause, entomopathogenic fungi, interaction, landscape, quiescence, top-down

## INTRODUCTION

Food webs are constituted by interactions among different levels of trophic relations in an ecosystem. Each trophic level is influenced by lower and/or higher trophic interactions for the survival and reproduction of their constituent individuals. As a consequence, any disturbance in a food web might be pivotal for organisms, with stronger consequences for specialists (Tylianakis & Binzer, 2014). For instance, the agricultural system most often involves plant species, phytophagous insects, and natural enemies (Askew & Shaw, 1986; Dong et al., 2019; Sullivan & Völkl, 1999), which results in competition, predation and parasitism. Interactions of this kind between insect populations and the plant community are modulated by top-down and bottom-up effects. Hairston et al. (1960) in “the green world” hypothesis states that phytophagous populations are regulated by a top-down effect (downward regulation) by their predators and parasitoids on higher trophic levels. Phytophagous populations may also be regulated by bottom-up effects (upward regulation) of plants (the lower trophic level), including their nutritional quality (Hunter & Price, 1992; Chidawanyika, et al., 2020). By comparing the two effects, Vidal and Murphy (2017) found that chewing, sucking and gall-making herbivores were affected more by top-down than bottom-up effects. In addition, Hairston et al. (1960) concluded that in each of the three levels considerable interspecific competition would be found.

The food web of herbivorous insects and their natural enemies, as well as the structure of these communities, might be affected by agricultural landscape and practices (Andrade et al., 2015; Birkhofer et al., 2011; Dong et al., 2019; Harterreiten-Souza et al., 2021). Depending on the type of activities in agricultural ecosystems, an increase or decrease in habitats can be occasioned, thus affecting the phenology of herbivorous insects and consequently the abundance of their natural enemies (Kross et al., 2020; Perez-Alvarez et al., 2021).

Indeed, intensification of agriculture during the last century has resulted in an increase in agricultural areas with monocultures, with a concomitant decrease in natural habitats, insect, and crop diversity (Tscharrntke et al., 2005; Rand et al., 2012). The consequences include a negative impact on agricultural functions, such as pollination and biological control (Shi et al., 2021; Thies & Tscharrntke, 1999). In addition, to ensure high crop yields, agricultural producers have tended to use the entire land area, removing prairie strips and hedges, as well as using more synthetic fertilizers. This has resulted in a significant increase in agricultural pests, driving more extensive use of chemical pesticides to avoid or reduce the risk of infestations (Meehan, 2011). However, the use of these chemicals, in addition to threatening human health, destroys that of the environment, decreases populations of natural enemies, increases the risk of insecticide resistance and causes secondary pest outbreaks (Cloyd, 2012; Nicolopoulou-Stamati et al., 2016). As a result, new innovative approaches have been adopted in agroecology, such as conversion of 10% of a crop field to prairie strips that leads to remarkable improvements in biodiversity and ecosystem services (Schulte et al., 2017; Wezel et al., 2009). The current and future orientation of the world is to encourage organic farming in agricultural production and limit pesticide usage. In addition to the impact of agricultural practices, weather conditions may influence the phenology of insects and their natural enemies, by affecting their survival and dispersion (Santos et al., 2020).

In Belgium, sugar beet is the third most important economic crop after cereals and potatoes. Broad bean is mainly grown for the freezing and canning industries (Wesemael & Moens, 2012). Due to severe damage caused by many aphid species, including the black bean aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae), sugar beet fields have been systematically treated with the insecticide imidacloprid, as seed dressing. *Aphis fabae* is a serious, widespread pest on a range of important plants, including tobacco, broad bean, potato, as well as

various varieties of beet (Fernandez-Quintanilla et al., 2002). Both adults and nymphs feed gregariously on leaves, directly affecting sugar beet growth and the storage of sugar, by sucking on plant sap. This may promote chlorosis, as well as wilting and loss of flowers (Blackman & Eastop, 2000). Moreover, *A. fabae* produces honeydew, which encourages the growth of fungi, such as sooty mould, which hampers photosynthesis and weakens the plants (Hurej & Vanderwerf, 1993; Leroy et al. 2009). In addition, *A. fabae* is one of the main vectors of the plant virus *Beet yellows virus* (BYV; closteroviridae) that affects plant growth and reduces sugar storage in roots (Clover et al., 1999; Simpson et al., 2012). The virus infection could result in as much as 50% loss of sugar beet yield (Smith & Hallsworth, 1990).

Biological control has played an important role in controlling agricultural and forest pests with positive consequences for human health and the environment (Van Driesche et al., 2010). Thus, BC seems an attractive strategy to combat aphid infestation on sugar beet and broad bean, and its role could be enhanced by integrating good agricultural practices. In addition, it has been demonstrated that the role of parasitoids is more important in non-conventional and organic fields than in conventionally farmed fields (Ismail & Albittar, 2016; Rusch et al., 2016). Organic crops present opportunities to analyse the development of aphids and the presence of their natural enemies. Thus, in this study, we focus on the effect of characteristics of the agricultural fields of organic sugar beet and broad bean on the phenology of the aphid *A. fabae*, and on the diversity and abundance of parasitoids communities. We evaluated the impact of parasitoids on the *A. fabae* population in organic fields. We also recorded the mortality of aphids due to entomopathogenic fungi in the fields. In addition, we investigated the correlation between *A. fabae* population density and parasitoid presence on the prevalence of alate *A. fabae* (winged individuals). We further studied the impact of weather conditions on the phenology of parasitoids and their natural enemies. Finally, we also evaluated proportions of quiescent and diapausing parasitoids.

## MATERIALS AND METHODS

### Field locations and types

The work was carried out in three organic fields containing broad beans (host of *A. fabae* during winter) and plots of sugar beet (host of *A. fabae* during summer) in the Walloon Brabant region in Belgium (Appendix 1). Organic sites were very limited in the region and varied in size and agricultural landscaping. The first site was situated in Chaumont-Gistoux with a size of approximately 1 hectare, and was surrounded by hedges, grasses and buildings. This site was separated from other conventional fields of maize and sugar beet by service roads. Samples were taken from plots containing beans (0.4 hectare) and beet (0.6 hectare). The second site, in Gottechain, had a size of approximately 2 hectares (0.5 hectare broad bean and 1.5 hectare sugar beet) and was divided into two separate fields by agricultural road. There were no hedges around the field and it was completely surrounded by conventional fields of sugar beet, wheat, maize

and chicory. The last site, in Walhain, covered approximately 6 hectares of organic crops including broad bean (about 1 hectare), sugar beet (2 hectares), parsley, strawberries, lettuce and corn. It was relatively open, surrounded by grass strips. Samples were collected from beans early in the season in June, while samples were collected from beets later in the season from July till September. According to the producers, parasitoids have not previously been released in the fields. The records of weather conditions (temperature, relative humidity, rainfall and wind speed) during the study were obtained from the Royal Meteorological Institute, using the average values of each condition in a time lag of 1 week before each sampling (Appendix 2). The weather station is located in Louvain la neuve (50°40'5.092 N, 4°36'42.597 E) in the middle of the three fields (Around 10 km).

### Sampling protocol

Aphid sampling was started in May, but the first collection of aphids was recorded in June on broad bean (the top five leaves were collected from each plant and were considered as one leaf), and then on sugar beet plants (one leaf from each plant) (Appendix 3). At each site, 20 leaves from 20 plants per field (in total  $20 \times 3 = 60$  leaves) were randomly collected weekly from June to September and transferred to the laboratory. The sampling date in broad bean plants was around 30 min and in sugar beet was around 90 min in each field. For each plant leaf, the total number of aphids (alate, apterous and nymphs) was determined. The proportion of apterous morphs was calculated as the number of apterous morphs divided by the total number of aphids, and likewise for the proportion of alate morphs (expressed as percentages in figures).

### Parasitoid complex on *A. fabae* populations

We recorded the number of mummies (parasitoid-infested aphids) as well as aphid mortality. To determine the of parasitism proportion from the collected aphids, surviving individuals on each of the leaves were counted then transferred using a fine brush to individual broad bean plants enclosed in mesh cages (60 plants in total every week). These plants with aphids were then maintained in a growth chamber at  $21 \pm 1^\circ\text{C}$ , under a photoperiod 16 L: 8D, and a relative humidity  $65 \pm 10\%$  until mummification of aphids occurred. All collected mummies were placed into plastic Petri-dishes in a further growth chamber under the aforementioned conditions, to determine the species and emergence proportion of the parasitoids. Parasitism proportion was expressed as the number of mummies obtained / (number of aphids still alive + number of mummies) and expressed as a percentage in figure. For identification, collected parasitoids were preserved in 70% alcohol-containing glycerine and stored in small Eppendorf microcentrifuge tubes. Unhatched mummies were kept for 15 days at the same conditions, and then desiccated to establish the mortality of parasitoids and determine the presence of diapausing parasitoid larvae and expressed as a percentage in results. The diapause was determined as thick mustard/golden yellow larvae (Zhang et al., 2018).

Aphid mortality proportion due to entomopathogenic fungi, was determined by observing the dead aphids in the field, and calculated as the number of dead aphids recorded divided by the total number of aphids and expressed as a percentage in figure.

## Statistical analysis

Statistical analyses were performed using the statistical software R version 4.0.5 (R. Core Team, 2021). The density of total number of aphids on each plant leaf (over-dispersed data) was analysed using zero-inflated generalized linear mixed-effects models (glmmTMB), with a negative binomial error distribution, using the package *glmmTMB* and function *glmmTMB* (Brooks et al., 2017) with site, plant species and sampling date as factors. Sampling date was considered as both a linear and quadratic effect (using the function  $l(\text{date}^2)$ ). Plant leaf was included in the model as a random effect to account for repeated measures, and was nested inside the site. Binary interaction among (plant, site, date and  $l(\text{date}^2)$ ) were also included in the model. The proportion of alate morphs (data was square root transformed) and the proportion of apterous morphs (normally distributed) in respect of the total aphid number, were analysed using ANOVA with plant and site as fixed factors, and sampling date was considered as both a linear and quadratic effect. Binary interactions were included in ANOVA test. To test the correlation between the total number of aphids and weather conditions data of three sites were pooled in the same model and linear regression analysis was used. We set out 15 linear regression models based on model weights derived from information criteria, and the best model was defined according to the lowest Akaike information (dos Santos et al., 2021; Johnson & Omland, 2004), using the package “MuMin” and function *model.sel* (Bartoń, 2020).

Total parasitism proportion data was not normally distributed and was square root-transformed, then was analysed using two-way ANOVA with site and plant as fixed factors. The difference in parasitism according to parasitoid species was analysed only among the most abundant species (three species) using Generalized Linear models GLM (*glm* function with proportion data with quasibinomial distribution and logit link) with site and species as fixed factors. Mortality due to entomopathogenic fungi was analysed using zero-inflated generalized linear mixed-effects models (a negative binomial error distribution) with site and plant species as factors. Significant results of ANOVA tests at  $p < 0.05$  were followed by multiple comparison using the *multcomp* package and *glht* function (Hothorn et al., 2008). To test for a relationship between aphid density, alate morphs and mummies, we used linear regression and data were square root-transformed.

## RESULTS

### Phenology of aphids

Aphid phenology, including the peak of abundance, varied greatly from one location to the other (Figure 1). The maximal number of aphids in

the field appeared early in summer on the broad bean followed by a gradual decrease until August (Figure 1). The total number of aphid individuals varied significantly during the sampling date (Table 1). It varied significantly among sites (Table 1), with higher numbers observed at the Walhain site (Figure 2A). It also varied significantly between the two crop species with a higher number on sugar beet compared to broad bean (Table 1). Both site and plant were significantly dependent on sampling date on their effects on the total number of aphid (Table 1).

The proportions of apterous and alate morphs in respect to the total number of aphids (Figure 2B,C), did not vary among sites, nor between the two plants species (Table 2). The proportions of apterous and alate morphs did not vary during sampling date (Table 2). A significant interaction was found between plant and site on the proportion of apterous morphs (Table 2.1, Figure 2B), but not for the proportion of alate morphs (Table 2.2, Figure 2C).

During the season, particularly in June and July when aphids were collected simultaneously from both plants, the total number of aphids varied significantly between the two plants (broad bean =  $1884 \pm 416.94$  vs. beet =  $525.75 \pm 252.90$  aphid individuals,  $t = 3.47$ ,  $df = 4$ ,  $p = 0.03$ ) (Appendix 3).

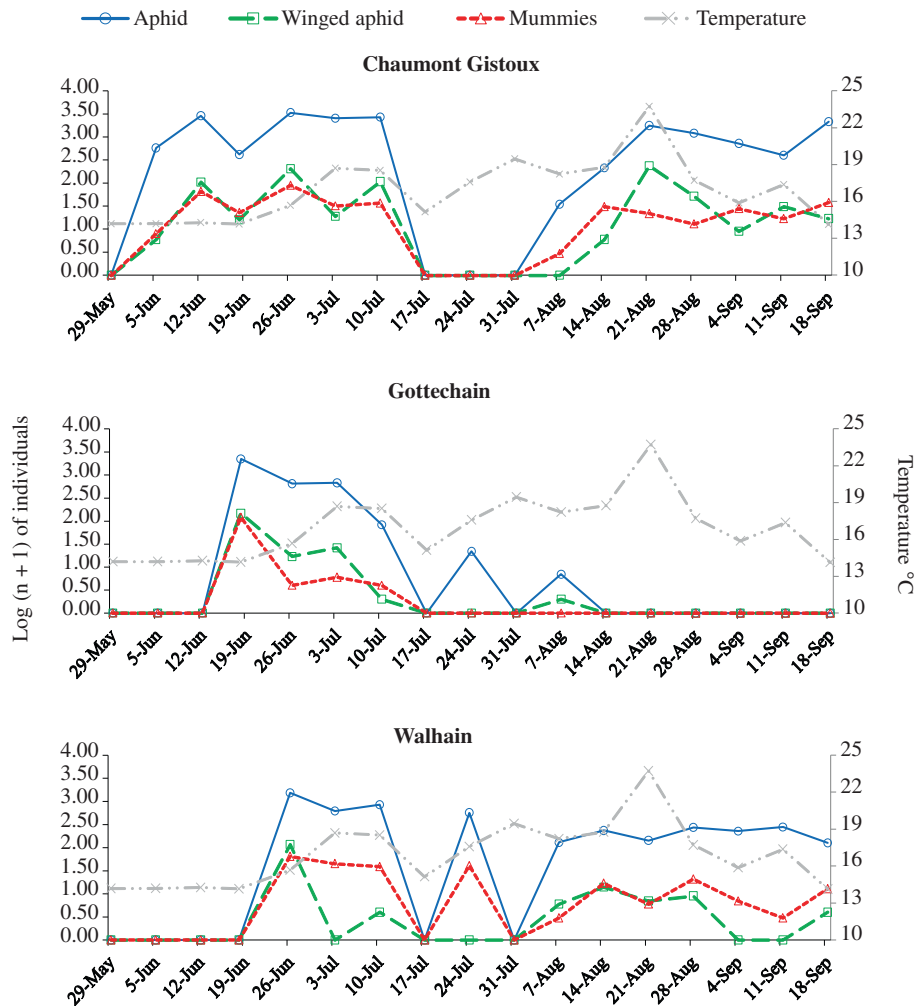
### Aphid density and weather conditions

Temperatures during the season ranged between 14.20°C and 23.73°C and humidity between 68.40% and 89.07%. Aphid density was not influenced by the majority of weather conditions: temperature ( $F_{1,14} = 0.15$ ,  $R^2 = 0.01$ ,  $p = 0.71$ ), relative humidity ( $F_{1,14} = 0.28$ ,  $R^2 = 0.02$ ,  $p = 0.60$ ) and rainfall ( $F_{1,14} = 0.12$ ,  $R^2 = 0.01$ ,  $p = 0.73$ ). Wind was the only condition that had a positive significant effect on the aphid density ( $F_{1,14} = 6.26$ ,  $R^2 = 0.31$ ,  $p = 0.02$ ), and also presented the best model (AICc = 145.30 and delta AICc = 0.00 and weight = 0.53) (Appendix 4).

### Parasitoid identification, parasitism and mortality proportions

A total of 163, 109 and 240 parasitoids emerged from mummies of collected *A. fabae* from the Chaumont-Gistoux, Gottechain, and Walhain sites, respectively. Six species of aphid parasitoids were identified, as well as two non-identified hyper-parasitoids (Table 3). The most abundant species were *Lysiphlebus fabarum*, *Binodoxys angelicae* and *B. aculephae* (Hymenoptera: Aphidiinae). Other species that were recovered included *Aphidius colemani*, *A. matricariae* and *A. avenae* (Hymenoptera: Aphidiinae) with very low abundances (Table 3). The total parasitism proportion varied significantly among sites ( $F_{2,28} = 3.76$ ,  $p = 0.03$ ) with higher parasitism observed in Chaumont-Gistoux (4.99%), followed by Walhain (3.52%) and Gottechain (1.70%) (Figure 3A). The total parasitism did not vary between the two plants ( $F_{2,28} = 0.66$ ,  $p = 0.42$ ). No interaction was found between plant and site ( $F_{2,28} = 1.80$ ,  $p = 0.18$ ).

Regarding the three most abundant species, parasitism varied among these species ( $F_{2,69} = 28.66$ ,  $p < 0.001$ ) with the highest



**FIGURE 1** Phenology of aphid number, alate morphs of *A. fabae* and mummies at the three locations. Data are presented as log ( $n + 1$ ) of individuals in the Y axis, weekly date in the X axis and temperature values in the Z axis

**TABLE 1** Zero-inflated generalized linear mixed-effects models (glmmTMB) for the effects of plant species, site, sampling date (linear and quadratic effect) and their binary interactions on the total number of aphids. Significant results are in bold ( $p < 0.05$ )

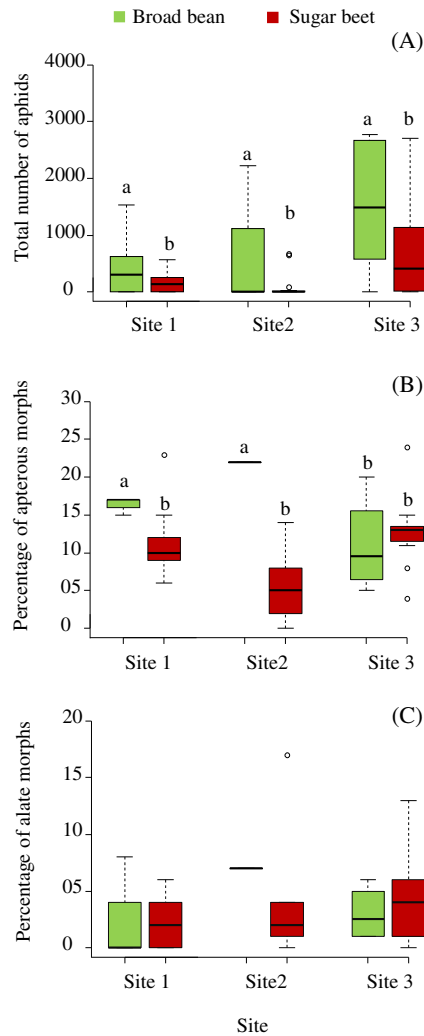
Factor	$\chi^2$	d.f.	$p$ value
Plant	4.83	1	<b>0.02*</b>
Site	45.61	2	<b>&lt; 0.001***</b>
Date	25.29	1	<b>&lt; 0.001***</b>
I(date <sup>2</sup> )	15.68	1	<b>&lt; 0.001***</b>
Plant: site	26.00	2	<b>&lt; 0.001***</b>
Plant: date	47.11	2	<b>&lt; 0.001***</b>
Plant: I(date <sup>2</sup> )	12.83	1	<b>&lt; 0.001***</b>
Site: date	16.62	2	<b>&lt; 0.001***</b>
Site: I(date <sup>2</sup> )	49.62	2	<b>&lt; 0.001***</b>

proportions observed for *L. fabarum* and *B. angelicae* and the lowest proportion for *B. aculephae*. Parasitism of the three species varied significantly among sites ( $F_{2,67} = 8.85$ ,  $p < 0.001$ ) (Table 3). A significant

interaction was found between site and parasitoid species ( $F_{4,63} = 39.29$ ,  $p < 0.001$ ), where *L. fabarum* was most abundant in Gottechain and Walhain but absent in Chaumont-Gistoux (Table 3). *Binodoxys angelicae*, however, was most abundant in Chaumont-Gistoux compared to Gottechain and Walhain (Table 3). *Binodoxys aculephae* had lower proportions in Chaumont-Gistoux and Walhain.

Among aphids coming from both broad bean and beet plants simultaneously, the parasitism percentage was almost the same on broad bean ( $2.24 \pm 0.10$ ) and on sugar beet plants ( $1.85 \pm 1.14$ ) ( $t = 0.34$ ,  $df = 4$ ,  $p = 0.76$ ).

Summer diapause was very low. On June 26th, 156 mummies were collected from the three sites: the percentage of emergence was 72.44%, the percentage of mortality inside the mummies was 19.87%, and the percentage of summer diapause was 7.39%. On September 11th, 18 mummies were collected and the percentages were as follows: emergence = 27.78%, mortality = 55.56%, and winter diapause = 16.67%. On September 18th we collected 49 mummies and the percentages were: emergence = 14.29%, mortality = 46.94% and winter diapause = 38.79%.



**FIGURE 2** Box plot of (A) total number of aphids, (B) percentage of apterous morphs and (C) percentage of alate morphs on two plants: Broad bean and sugar beet, in three sites: Chaumont-gistoux (site 1), Gottechain (site 2) and Walhain (site 3). Small letters represent significant differences between the plants in (A) and significant interaction between plant and site in (B). The boxplot summarized: Minimum (first quartile  $-1.5 \times$  interquartile range of the data), maximum (third quartile  $+1.5 \times$  interquartile range of the data), boxes: 25th and 75th percentiles, median (thick line), whiskers (continuous dash line) and outliers (data point in open circle)

The mortality proportion due to the entomopathogenic fungi did not vary among the different sites ( $\chi^2 = 1.08$ ,  $df = 2$ ,  $p = 0.58$ ) not between the two plants ( $\chi^2 = 0.32$ ,  $df = 1$ ,  $p = 0.57$ ) (Figure 3B). No interaction was found between plant and site ( $\chi^2 = 0.03$ ,  $df = 2$ ,  $p = 0.96$ ).

### Impact of aphid density on the production of alate morphs and parasitism

The total number of aphids had a significant effect on the production of alate morphs at all three sites (Chaumont-Gistoux:  $F_{1,14} = 9.23$ ,  $R^2 = 0.40$ ,  $p = 0.008$ ; Gottechain  $F_{1,14} = 269.70$ ,  $R^2 = 0.95$ ,

**TABLE 2** Effects of plant, site, sampling date and their binary interactions (two-way ANOVA) on the proportions of apterous (1) and alate (2) morphs. Significant result is in bold ( $p < 0.05$ )

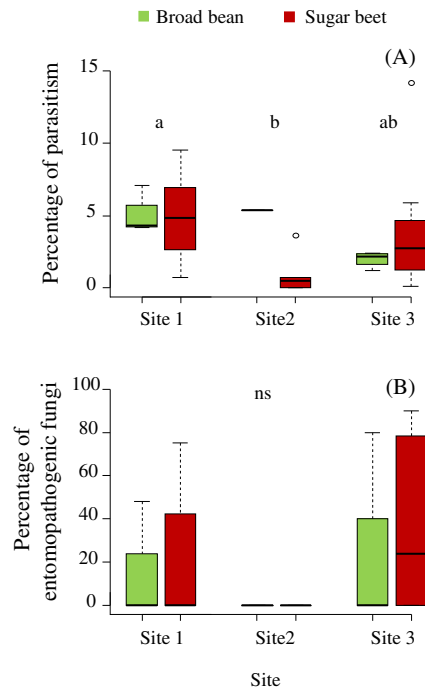
Trait	Factor	F	d.f.	p value
1- The proportion of apterous morphs	Plant	2.45	1	0.13
	Site	1.52	2	0.24
	Date	3.17	1	0.09
	I(date <sup>2</sup> )	0.17	1	0.65
	Plant: site	4.19	2	<b>0.03*</b>
	Plant: date	1.10	1	0.30
	Plant: I(date <sup>2</sup> )	0.03	1	0.87
	Site: date	1.17	2	0.33
	Site: I(date <sup>2</sup> )	0.18	2	0.83
	Residuals			20
2- The proportion of alate morphs	Plant	0.001	1	0.97
	Site	2.04	2	0.15
	Date	0.04	1	0.84
	I(date <sup>2</sup> )	0.07	1	0.78
	Plant: site	0.36	2	0.69
	Plant: date	4.83	1	0.03
	Plant: I(date <sup>2</sup> )	1.95	1	0.17
	Site: date	1.94	2	0.16
	Site: I(date <sup>2</sup> )	1.18	2	0.32
	Residuals			20

**TABLE 3** The proportion  $\pm$  (SE) of each parasitoid species among the total number of parasitoids recorded from the black bean aphid *Aphis fabae* at three sites in Belgium

Parasitoids	Site		
	Walhain	Gottechain	Chaumont-Gistoux
<i>Lysiphlebus fabarum</i>	0.71 $\pm$ 0.03 a	0.87 $\pm$ 0.03 a	0 d
<i>Binodoxys angelica</i>	0.17 $\pm$ 0.02 b	0.11 $\pm$ 0.03 c	0.75 $\pm$ 0.03 a
<i>Binodoxys acalaphae</i>	0.10 $\pm$ 0.02 c	0 d	0.18 $\pm$ 0.03 b
<i>Aphidius colemani</i>	0.00	0	0.02 $\pm$ 0.01
<i>Aphidius matricariae</i>	0.00	0.02 $\pm$ 0.1	0.01 $\pm$ 0.01
<i>Aphidius avenae</i>	0.00	0	0.01 $\pm$ 0.01
<i>Hyperparasitoid</i>	0.01 $\pm$ 0.01	0	0.03 $\pm$ 0.03

Note: Small letters represent significant interaction between site and parasitoid species (*Lysiphlebus fabarum*, *Binodoxys angelicae* and *Binodoxys acalaphae*).

$p < 0.001$ ; Walhain  $F_{1,14} = 25.97$ ,  $R^2 = 0.65$ ,  $p < 0.001$ ) (Figure 4A). Increasing aphid number also had a significant impact on parasitism at the three sites (Chaumont-Gistoux:  $F_{1,14} = 116.30$ ,  $R^2 = 0.89$ ,  $p < 0.001$ ; Gottechain  $F_{1,14} = 65.95$ ,  $R^2 = 0.82$ ,  $p < 0.001$ ; Walhain  $F_{1,14} = 49.60$ ,  $R^2 = 0.78$ ,  $p < 0.001$ ) (Figure 4B). The number of alate morphs was significantly associated with increasing parasitism at the three sites: Chaumont-Gistoux ( $F_{1,14} = 6.73$ ,  $R^2 = 0.33$ ,  $p = 0.02$ ), Gottechain ( $F_{1,14} = 182.4$ ,  $R^2 = 0.93$ ,  $p < 0.001$ ) and Walhain ( $F_{1,14} = 15.53$ ,  $R^2 = 0.53$ ,  $p = 0.002$ ) (Figure 5).



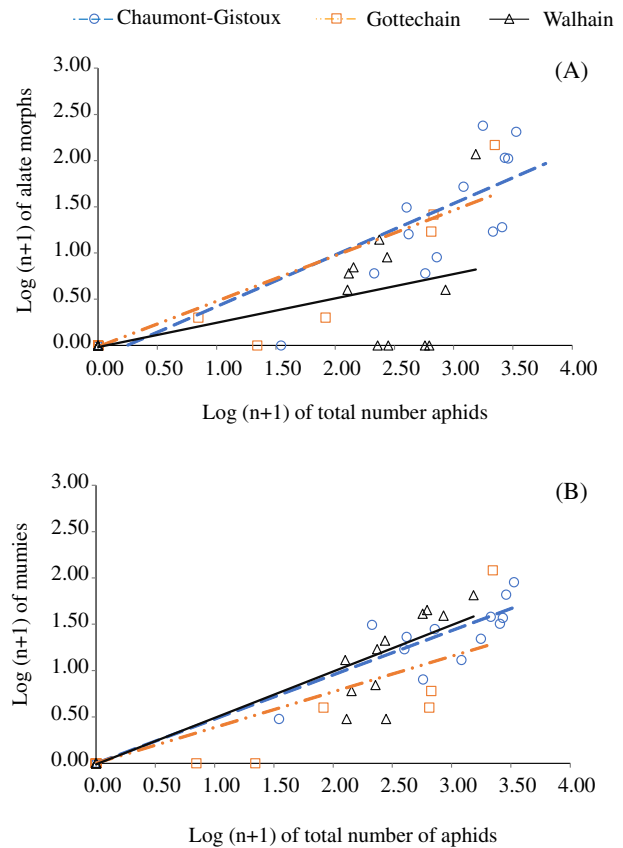
**FIGURE 3** Box plot of (A) percentage of parasitism of aphids and (B) percentage of entomopathogenic mortality on two plants: Broad bean and sugar beet, in three sites: Chaumont-gistoux (site 1), Gottechain (site 2) and Walhain (site 3). Small letters represent significant differences between sites in (a)

## DISCUSSION

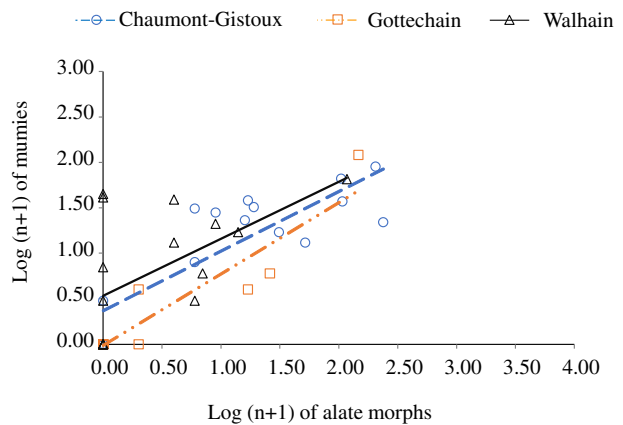
In this study, we report on the phenology of *A. fabae* and the diversity of parasitoids in organic crops of sugar beet and broad bean. Overall, the phenology of *A. fabae* varied between sugar beet and broad bean in the three sites studied. We found a low parasitism on aphids, and a significant impact of entomopathogenic fungi on mortality of aphid population.

Aphid density varied throughout the growing season with maximal numbers observed at the beginning of summer (June), followed by a reduction in August. A similar phenology with a peak of development in June and then a marked decrease in August was also observed for aphids feeding on maize, with decreasing numbers being associated with increased parasitism (Hance, 1995). The highest density of *A. fabae* was found in Walhain (Figure 2). The field was relatively open with large areas of organic crops. Such an environment might seem to favour the development of aphids, particularly without the impact of parasitoids.

At all three sites, broad bean and sugar beet were the main crops. *Aphis fabae* first appeared on broad bean, being cultivated first, then migrated to sugar beet once these plants started growing in the fields (Figure 1). The alternation of suitable crops favours a constant presence of the *A. fabae* population, but it also allows the establishment of parasitoids early in the season and may thus increase parasitoid populations for the protection of the subsequent beet crops. During the few weeks



**FIGURE 4** (A) Linear regression of alate morphs on the total number of aphids (log transformed; Chaumont-Gistoux:  $y = 0.26$  aphid density  $- 0.02$ ; Gottechain:  $y = 0.49$  aphid density  $- 0.06$ ; Walhain:  $y = 0.5574$  aphid density  $- 0.19$ ). (B) Regression of mummies (log transformed) on aphid density (log transformed; Chaumont-Gistoux:  $y = 0.50$  aphid density  $- 0.06$ ; Gottechain:  $y = 0.38$  aphid density  $- 0.06$ ; Walhain:  $y = 0.45$  aphid density  $- 0.01$ )



**FIGURE 5** Linear regression of alate morphs (log transformed) on mummies (log transformed). (Chaumont-Gistoux:  $y = 0.48$  mummy  $+0.04$ ; Gottechain:  $y = 1.11$  mummy  $+0.06$ ; Walhain:  $y = 0.87$  mummy  $+0.21$ )

when sugar beet and broad bean co-occurred (particularly in Walhain), we observed that more aphid individuals settled on broad bean compared to sugar beet. Parasitism proportions were also found to be higher on broad bean. This could be because broad bean is preferred by *A. fabae* over sugar beet (Hardie, 1981). Cockbain et al. (1963) had indeed already mentioned that many *A. fabae* settled permanently on broad bean plants, with only a few individuals settling on sugar beet plants. Host plant species have been observed to have a significant impact on the parasitoid species that attack *A. fabae* (Albittar et al., 2016; Hopkinson et al., 2013). Hence, the presence of an essential host such as the broad bean might influence the dynamics of *A. fabae* and its parasitoids.

The production of alate morphs, as well as the proportion of parasitism, were positively related to the growth of the aphid population. This indicates the possibility of a numerical response of the parasitoid through time. In aphid biology, it is well known that the production of alate morphs, and their dispersal, is influenced directly by aphid density when aphid colonies grow larger on plants, as well as by the presence of natural enemies (Bonnemai, 2010; Shaw, 1973). However, the presence of alate aphids can result in increased dispersal of associated plant virus. A better understanding of their occurrence is thus needed.

Our results showed that most environmental conditions measured in this study did not have a significant impact on density of *A. fabae*. Wind was the only condition that positively affected the aphid density. It is evident that insect dispersal is affected by meteorological parameters, particularly wind, which plays a crucial role in the migration of aphids (Ghosh et al., 2019). Similarly, dos Santos et al. (2021) found temperature, photoperiod, relative humidity, and wind speed had no impact on the density of thrips *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae).

The parasitoid community of *A. fabae* was quite diverse, with six species and one hyper-parasitoid. The parasitoids *L. fabarum*, *B. angelicae* and *B. aculephae* (Braconidae: Aphidiinae) were the most abundant species in the field. *Lysiphlebus fabarum* is known as the most common and effective parasitoid of *A. fabae* (Albittar et al., 2008; Alikhani et al., 2013; Stary et al., 2014; Stary & Havelka, 2008), whereas, *B. angelicae* is more of a generalist. While *L. fabarum* was recorded at a higher rate in Gottechain and Walhain, *B. angelica* was recorded at a low rate from both sites. However, *B. angelica* was recorded at a higher rate in Chaumont-Gistoux, in the absence of *L. fabarum*. This suggests possible competition between species for the same host. The interspecific competition between parasitoids is well known in food webs (Bogran et al., 2002; Boivin & Brodeur, 2006).

Overall, we recorded a relatively low parasitism proportion in most fields, with a higher rate in Chaumont-Gistoux. This field was surrounded by hedges, which could serve as refuges for parasitoids. The lower proportion of parasitism was found in Gottechain. The site was lacking hedges and completely surrounded by conventional fields. This might affect aphid and parasitoid densities. In addition, parasitism might be affected by the presence of both predators and entomopathogenic fungi which were present in the three locations. Indeed, our results showed that the high mortality of aphid individuals due to entomopathogenic fungi might explain the low parasitism. The specific symptoms of entomopathogenic fungal infection, such as fungal

spores with moribund (dead) aphids (Papierok et al., 2016), were most likely the reason. Further studies to identify the aphid pathogenic fungi in the field would therefore be interesting. The main fungal pathogens of aphids belong to the order of Entomophthorales and Hypocreales (Papierok et al., 2016). Indeed, *A. fabae* population was regulated by the entomopathogenic fungi rather than by parasitoids. We also observed a high number of predators in the field such as: the seven-spot ladybird *Coccinella septempunctata*, the two-spot ladybird *Adalia bipunctata* (Coleoptera: Coccinellidae) as well as the hoverflies *Eupeodes luniger* and *Syrphus torvus* (Diptera: Syrphidae). It is evident that parasitized aphids are vulnerable to predators (Xue et al., 2012) and the fungus-infected hosts are unsuitable for parasitoid larval development (Askary & Brodeur, 1999), thus may drive the proportion of parasitism down.

Our results further showed that a few parasitoid larvae inside the mummies entered diapause (quiescence) in summer. This type of diapause usually occurs when unfavourable abiotic or biotic conditions happen for a short period during the season. This may be the consequence of quite low temperature for this time of the year ranging between 14 and 15°C when we observed the quiescence. It could also be linked to the fact that aphids changed plant hosts from broad bean to beet. Little information is available regarding possible diapause or quiescence in summer for parasitoids. Tougeron et al. (2017) for instance, found by conducting summer climatic experiments, that summer diapause in two aphid parasitoids, *Aphidius avenae* and *A. rhopalosiph* (Hymenoptera: Aphidiinae), increased with increasing female densities in the patch. According to our results, the proportion of parasitism was generally low in the field. Further studies including the plant host and quality (age) as well as the aphid host species would be needed to understand summer diapause. On the other hand, we found a higher rate of winter diapause in mid-September. This was accompanied by low temperatures, reduction in daylight length as well as plant quality. Indeed, leaves are lighter in colour and quality decreased dramatically in the later growth phase of sugar beet (Varga et al., 2021). The environmental factors, particularly temperature and photoperiod, as well as biotic factors such as host life cycle and host plant quality, are the principal cues in the induction of diapause (Ahmadi et al., 2018; Legrand et al., 2004; Nakamura, 2003; Polgár et al., 1995; Polgár & Hardie, 2000; Saunders, 2011; Teraoka & Numata, 2004).

In conclusion, density of aphids depended on the field type. The parasitism proportion was very low in organic crops. Two parasitoids, *L. fabarum* and *B. angelicae*, were the most abundant parasitoids in the field, though the parasitism proportion was low. The two species appeared to be good candidates for the development of inundative biological control programmes against *A. fabae*. However, based on field results we suggest not releasing them simultaneously to avoid competition. Further studies on their interaction and competition should be done to determine which species and under what conditions would be the most successful in controlling aphid infestations. Intercropping plant systems in sugar beet fields with broad bean or any other plant attracting aphids could protect sugar beet from aphid infestation and might also enhance the presence of natural enemies. The management of the immediate surroundings of the culture by



maintaining native prairie strips also needs to be explored in conservation biological control to enhance natural parasitoid populations.

## AUTHOR CONTRIBUTIONS

LA and TH conceived research. MI, LA and ADP conducted the field work. MI and LA analysed the data, prepared the figures and wrote the paper. MI, BV, TH and LA edited and approved the manuscript.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study is available in the Mendeley Data repository: <https://DOI: 10.17632/wnvt4grz9d.1>.

## ORCID

Mohannad Ismail  <https://orcid.org/0000-0002-6258-2339>

Bertanne Visser  <https://orcid.org/0000-0003-4465-6020>

Thierry Hance  <https://orcid.org/0000-0001-5569-5020>

Loulou Albittar  <https://orcid.org/0000-0002-9077-8566>

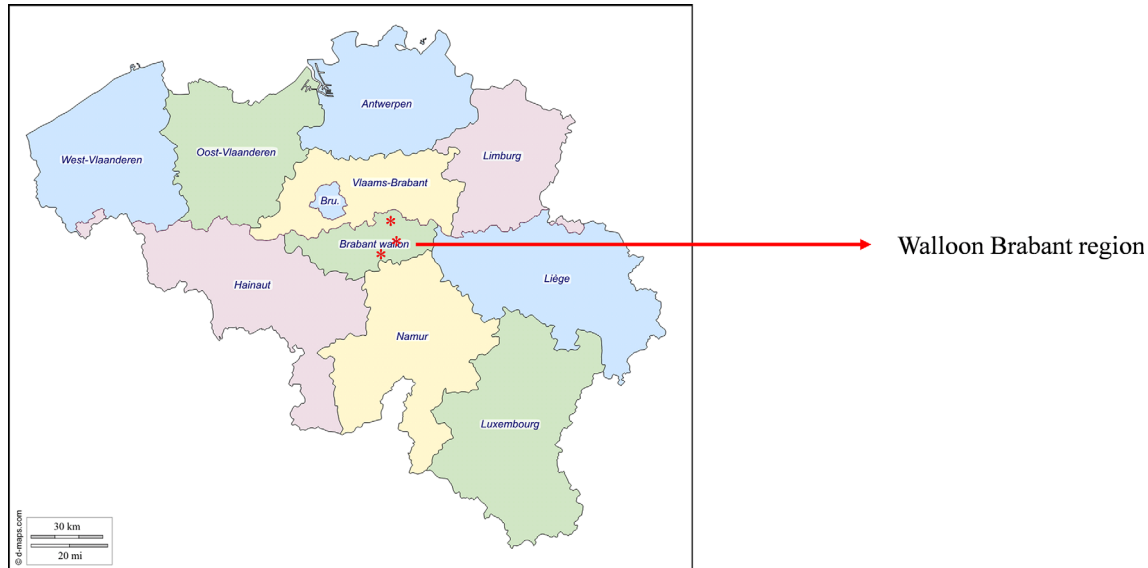
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## APPENDIX 1

 SAMPLING DISTRIBUTIONS OF ORGANIC FIELDS IN WALLOON  
BRABANT REGION IN BELGIUM


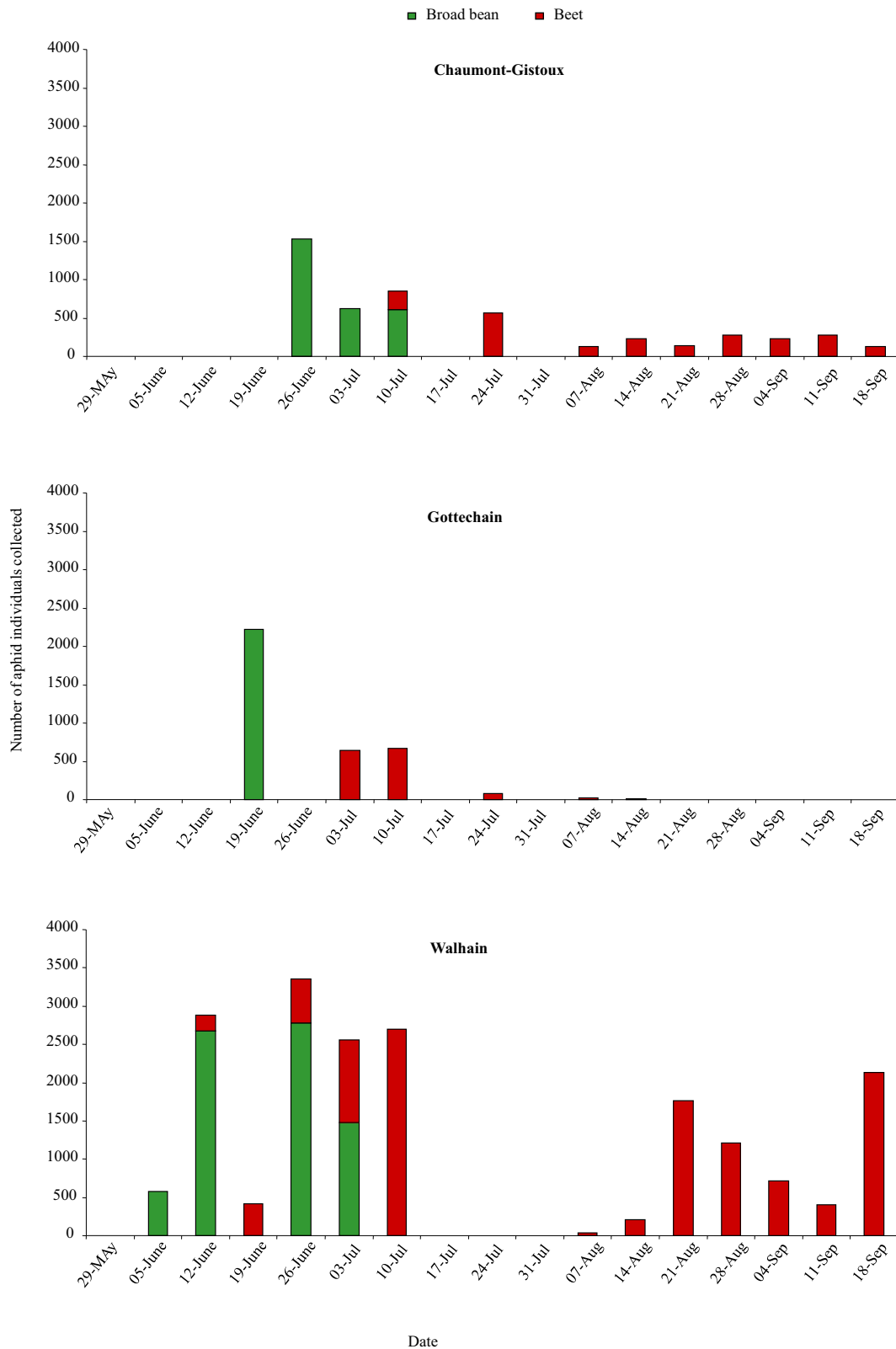
## APPENDIX 2

 THE AVERAGE VALUES OF WEATHER CONDITIONS IN A TIME  
LAG OF 1 WEEK BEFORE EACH SAMPLING (TEMPERATURE,  
RELATIVE HUMIDITY, RAINFALL AND WIND SPEED) DURING  
THE STUDY. DATA WERE COLLECTED FROM THE ROYAL  
METEOROLOGICAL INSTITUTE

Time (week)	Temperature (°C)	Rainfall (mm)	Wind (m/s)	Relative humidity %
29-mai	19.49	4.39	1.67	70.96
05-June	14.20	4.24	1.28	86.51
12-June	14.27	6.59	2.59	87.66
18-June	14.17	5.87	2.30	88.82
26-June	15.69	1.35	2.51	84.50
03-July	18.70	0.17	2.06	80.09
10-July	18.53	6.07	1.26	83.69
17-July	15.14	5.71	1.74	89.07
24-July	17.59	0.93	1.19	77.07
31-July	19.46	1.61	0.93	77.76
07-August	18.23	0.96	1.24	81.57
14-August	18.77	0.19	0.90	77.49
21-August	23.73	0.74	1.01	68.40
28-August	17.73	0.91	2.41	78.00
04-September	15.89	1.76	0.89	82.67
11-September	17.37	1.13	0.91	74.96
18-September	14.14	0.94	1.19	85.94

## APPENDIX 3

APHIDS SAMPLED FROM BROAD BEAN (GREEN) AND SUGAR BEET. ALL SAMPLES COLLECTED ON THE SAME DAY WERE SUMMED



## APPENDIX 4

FIFTEEN MODELS PREDICTING THE EFFECT OF WEATHER CONDITIONS (TEMPERATURE, WIND SPEED, RELATIVE HUMIDITY AND RAINFALL) ON THE PHENOLOGY OF *APHIS FABAE* IN ORGANIC FIELDS. THE DELTA AICC DEMONSTRATED THE PROBABILITY OF ONE MODE BEING FAVOURED OVER THE OTHER, WHERE VALUE = 0.00 PRESENTS THE BEST MODEL. AKAIKE WEIGHTS REPRESENT THE RELATIVE LIKELIHOOD OF A MODEL, AND WERE CALCULATED BY DIVIDING THE VALUE OF A MODEL BY THE SUM OF THESE VALUES ACROSS ALL MODELS

Model	Intercept	Temperature	Wind	Relative humidity	Rainfall	AICc	Delta AICc	Weight
Model 2			19.22			145.3	0.00	0.533
Model 9	52.440		21.85	-0.61490		148.5	3.17	0.109
Model 6	-18.990	1.2890	21.38			148.5	3.19	0.108
Model 10	6.800		20.85		-1.2050	148.6	3.30	0.102
Model 3	-8.897			0.54660		150.9	5.59	0.033
Model 1	50.570	-0.8716				151.1	5.74	0.030
Model 4	33.540				0.8666	151.1	5.77	0.030
Model 11	-12.960	0.9968	21.91		-0.7560	152.8	7.44	0.013
Model 14	41.990		21.94	-0.47290	-0.5101	152.8	7.50	0.013
Model 13	20.960	0.6321	21.82	-0.36070		152.8	7.51	0.012
Model 5	-51.890	0.8657		0.89250		154.5	9.18	0.005
Model 8	-11.410			0.58110	-0.1201	154.5	9.22	0.005
Model 7	45.300	-0.6370			0.5123	154.7	9.34	0.005
Model 15	-10.810	0.9656	21.92	-0.02058	-0.7398	158.1	12.77	0.001
Model 12	-67.770	1.0310		1.06400	-0.3657	158.9	13.54	0.001