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How Diet Leads to Defensive Dynamism

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HOW DIET LEADS TO DEFENSIVE DYNAMISM: EFFECT OF THE DIETARY QUALITY ON AUTOGENOUS ALKALOID RECOVERY RATE IN A CHEMICALLY DEFENDED BEETLE

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HOW DIET LEADS TO DEFENSIVE DYNAMISM: EFFECT OF THE DIETARY QUALITY ON AUTOGENOUS ALKALOIDS RECOVERY RATE IN A CHEMICALLY DEFENDED BEETLE

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Abstract-The impact of different diets on chemical defense has been extensively studied in animals that sequester defensive chemicals from food. However, there are fewer studies of diet-mediated variation in autogenously produced defenses. Ladybird beetles, which use autogenously synthesized defensive alkaloids, are used as models in a wide diversity of studies of chemical defense, specifically in studies of intraspecific variation in color pattern and chemical defense. Many aphidophagous ladybirds consume a wide diversity of aphid prey, which vary in quality and thus could affect the synthesis of chemical defense. We measured alkaloid recovery rate after reflex bleeding by the ladybird *Adalia bipunctata* on two different aphid diets, the high quality *Acyrthosiphon pisum* and the lower quality *Aphis fabae*. Alkaloids reaccumulated in ladybirds more slowly when they were fed *A. fabae* than when they were fed *A. pisum* and females generally had more alkaloid than males, but reaccumulated alkaloid more slowly. Recovery times were in excess of 12 days. There appeared to be a weak positive relationship between alkaloid level and time since reflex bleeding for eggs of *A. pisum*- but not *A. fabae*-fed females. Our findings on diet and alkaloid synthesis in ladybirds suggest that chemical defense levels are very dynamic, indicating that studies conducted at a single point in time, such as those focused on ladybird color pattern, fail to consider a wide diversity of temporal variation that occurs in the field. This is likely true for many autogenously produced chemical defense systems in a diversity of other organisms.

Key Words-Coccinellidae, chemical defense, *Adalia bipunctata*, dietary generalist, adaline.

Declarations

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Authors' contributions:

ZO and JJS formulated the idea and developed the methodology. ZO conducted the experiments and statistical analysis, with advice from JJS. ZO and JJS wrote the manuscript.

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INTRODUCTION

Chemical defense is widely used as a protective mechanism in insects and other animals. Defensive chemicals are generally obtained either by sequestration from the diet or through autogenous synthesis (e.g. Zvereva and Kozlov 2016; Ruxton et al. 2018). Intraspecific variation in both quality and quantity of these toxins has often been observed (Brower et al.

1982; Jones et al. 1987; Holliday et al. 2009), being frequently quantitative rather than qualitative (Speed et al. 2012). While there are a lot of examples of variation due to diet in directly sequestered defenses (e.g. Lampert and Bowers 2010; Ramos et al. 2012; Agrawal et al. 2021), there are very few documented cases related to diet mediated variation in autogenously synthesized defensive chemicals (Brückner and Heethoff 2018).

Ladybird beetles (Coccinellidae) use endogenously synthesized taxon-specific defensive alkaloids (Pasteels et al. 1973; Daloze et al. 1995; King and Meinwald 1996; Laurent et al. 2005). These alkaloids protect the beetles against a diversity of natural enemies (e.g. Marples et al. 1989; Marples 1993; Hemptinne et al. 2000). When disturbed, adults and larvae emit droplets of alkaloid-bearing hemolymph (Happ and Eisner 1961; Kendall 1971), a defense mechanism known as reflex bleeding. Eggs and pupae also are protected by alkaloids (Daloze et al. 1995; King and Meinwald 1996). Synthesis and use of these endogenous alkaloids are energetically costly processes (Holloway et al. 1991; Grill and Moore 1998; Bayoumy et al. 2020).

Ladybirds have been used as models in a wide diversity of studies of chemical defense (e.g. de Jong et al. 1991; Marples 1993; Grill and Moore 1998; Sloggett et al. 2009; Arenas et al. 2015). One area of considerable interest has been the link between color pattern variation and defensive capability, both intra- and interspecifically (Bezzerides et al. 2007; Blount et al. 2012; Winters et al. 2014; Arenas et al. 2015; Wheeler et al. 2015). However, although intraspecific variation in chemical defense concentration is accepted as a given in many of these studies, the bases of this variability are only partially understood. Genetic variation in the defensive capability of ladybirds is known (de Jong et al. 1991) and rearing temperature, microbial infection and food quantity appear to play a role (Blount et al. 2012; Steele et al. 2020a, b), however dietary quality mediated via prey species has not been studied. Generalist aphidophagous ladybirds consume many different aphid prey species of differing dietary quality (Hodek and Evans 2012). Thus, dietary quality related to prey species is potentially a very significant factor in determining chemical defense strength.

In this paper we take a dynamic temporal approach to measuring chemical defense responses to dietary quality, by measuring alkaloid accumulation in ladybirds provided with qualitatively different aphid diets after reflex bleeding. For this we use a well-established model system that has been used to study the effect of different aphid diets for over 50 years, the two-spotted ladybird *Adalia bipunctata* (L.) and its aphid prey, the pea aphid *Acyrthosiphon pisum* (Harris) and the black bean aphid *Aphis fabae* Scopoli. While *A. pisum* is a high-quality prey, *A. fabae* has been shown to be poorer for the ladybird: deleterious effects have previously been observed on larval survival, development time, subsequent adult weight, adult pre-oviposition period, fecundity, fertility, and longevity (Blackman 1965, 1967; El-Hariri 1966a, b; Rana et al. 2002). In spite of this *A. fabae* is regularly used for feeding and breeding by *A. bipunctata* in the wild (e.g. Banks 1955). *Adalia bipunctata* synthesizes adaline (Tursch et al. 1973) as a major alkaloid and adalinine (Lognay et al. 1996) as a minor one for its defense. As in other ladybirds these alkaloids are synthesized in the fat body of the beetle from fatty acid precursors (Laurent et al. 2001; Haulotte et al. 2012). Using this model system, this study asked how diet affects the reaccumulation of the autogenously synthesized alkaloids of male and female *A. bipunctata* over time, and also its effect on the alkaloid content of the eggs that the females produce.

METHODS AND MATERIALS

Insect Culture. Adult *A. bipunctata* were acquired from Entocare Biologische Gewasbescherming Wageningen, The Netherlands. Ladybirds were maintained in a constant-climate cabinet set at 21 °C, 70% RH and a 16L:8D light regime. They were maintained in 9 cm diameter Petri dishes, the interior of which had been previously scraped with a coarse scouring pad, giving a roughened surface to facilitate easy movement. *Aphis fabae*, and *A.*

pisum (Hemiptera: Aphididae) colonies were reared separately on broad bean plants (*Vicia faba* L., cultivar Witkiem) in the lab (20±2.0 ^{o}C) under a constant light source. Ladybirds were fed aphids collected from the host plant daily, without plant material, which causes condensation to accumulate in Petri dishes (cf. Majerus et al. 1989).

Care Prior to Reflex Bleeding. The progression of the experiment is shown in Fig. 1. Ladybirds were immobilized using a flow of carbon dioxide and sexed under a dissecting microscope using the criteria of Randall et al. (1992). They were placed in single-sex Petri dishes with a density of approximately ten ladybirds per dish. Each dish was replaced daily, and the ladybirds were fed with an excess of aphids without plant material, either *A. pisum* or *A. fabae*. On the second day of feeding, females and males were paired in a 5.5 cm diameter Petri dish, roughened as described previously, under a light source for two hours with an excess of aphids. The pairs were maintained in the Petri dish after mating, and only pairs that copulated, and thus were are able to lay fertile eggs, were used in the subsequent experiment.

Reflex Bleeding. All adult *A. bipunctata* were reflex bled on the sixth day of the experiment, based on the assumption that new alkaloid would be synthesized after alkaloid loss. Reflex bleeding is also expected to minimize the starting variation in alkaloid quantity that would be found in untreated ladybirds as a consequence of natural variation and possible prior reflex bleeding. The methodology of stimulating reflex bleeding was modified from de Jong et al. (1991). Ladybirds were fixed by taping the elytra on to sample slides (Knapp et al. 2018). All the six femora and sides of the prontoum were squeezed clockwise with forceps until the secretion ceased. The reflex blood was removed using filter paper. This method maximized the excretion of reflex blood from the ladybird and ensured that all ladybirds were in a comparable baseline state at the beginning of the experiment.

Collection of Sample. Six or seven pairs of ladybirds were frozen for analysis directly after reflex bleeding and also after 1, 3, 5, 8, and 12 days. On each occasion, each individual was separately weighed inside a 5.5 cm diameter Petri dish, using a Satorius CPA225D Semi Microbalance (precision of 0.01 mg). All ladybird samples were transferred into separate 1.2 mL glass test tubes held inside a 2.0 mL Eppendorf safe-lock tube and stored at -80 ^{o}C.

The remaining dishes were inspected daily for dead ladybirds and the presence of eggs. Dishes were replaced, and the ladybirds were provided with fresh aphids. The numbers of eggs were recorded. Each egg cluster with greater than 25 eggs, was weighed using a Mettler Toledo Balance XS205 with precision of 0.01 mg. Each cluster of <25 eggs was combined with eggs from a neighboring day from the same female, because small numbers of eggs are difficult to weigh accurately. These pooled samples were allocated to the day from which the larger number of eggs came in subsequent statistical analysis. Females that laid <25 eggs over more than two days were excluded from the analysis. The collected eggs were stored in a freezer at -25 ^{o}C. The difference in the temperature from the storage of the adult samples was for space reasons, and is not expected to affect the amount of the alkaloid recovered.

Alkaloid Extraction. The extraction of alkaloids from the adult ladybird samples was conducted as follows; 200 μL of methanol was pipetted into a 1.2 mL test tube with each ladybird and 5 μL of a 10 mg/mL nicotine solution was added as an internal standard. Extraction of egg samples followed the same procedure with 100 μL of methanol and 5 μL of a 2 mg/mL nicotine solution added to a single clutch of eggs. The samples were crushed and the solution with the remains was left for 15 min to extract the maximum amount of alkaloid into the solvent. The test tube was placed inside a 2.0 mL Eppendorf Safe-lock tube and centrifuged with an Eppendorf 5424 microcentrifuge for 5 minutes at 15,000 rcf at room temperature. The supernatant was transferred into a new glass tube and the undissolved parts were discarded. The methanolic solution was dried with nitrogen gas to remove the methanol and redissolved in 100 μL of chloroform for adult ladybird samples and 50 μL for eggs. It was shaken until everything had dissolved. The solution, containing the extracted alkaloids, was subsequently transferred into GC-MS vials with a 150 µL glass low volume insert and stored at -80 ^{o}C if GC-MS was not carried out immediately.

Quantitative Analysis. The analysis of 1 µL chloroform containing the alkaloid extracts was performed using a Shimadzu GC-2010 Plus gas chromatograph with an AOC-20i autoinjector. The column injection was performed using an OPTIC-4 Multi Inlet System. The GC column was a SH-Rxi-5ms (30m length; 0.25 mm inner diameter; 0.25 µm film thickness). The GC was coupled to a 2010 Ultra Mass Spectrometer. The carrier gas helium flow was at a constant rate of 1.05 mL min^{-1}. Mass spectra were recorded with an EIMS (70 eV). The injection temperature was 50 ^{o}C rising to 200 ^{o}C at 5 ^{o}C/sec. This was found to minimize the degradation of adalinine. The GC program was held at 50 ^{o}C for 30 sec, then increased to 170 ^{o}C at 20 ^{o}C/min, then to 290 ^{o}C at 10 ^{o}C/min and a final increase to 325 ^{o}C at 20 ^{o}C/min, and the final temperature was held for 3 min.

The alkaloids and standard were identified by comparison to published mass spectra (Lognay et al. 1996; Hautier et al. 2008) or by injection of the pure compound (nicotine). Retention times were approximately 8.66 min for nicotine, 12.53 min for adaline and 13.30 min for (undegraded) adalinine. The amount of alkaloid in samples was calculated by comparison of the area of the alkaloid peaks to the nicotine peak as mg nicotine equivalents (see Supplementary Table S1 and S2). Results are given per mg wet mass. Even with precise adjustment on the GC-MS to control the injection temperature to achieve only minimal degradation of adaline, both analysis on adults and eggs showed a very small amount of adaline degradation (RT = 10.90 min) as in other studies (cf. Hautier et al. 2008). This was nonetheless an exceedingly small amount, at the limits of detection and was thus was not quantified. The proportion of degraded adalinine (RT = 14.49 min) was much higher: it significantly correlated with the undegraded adalinine (Fig. S1a, b). Thus, both degraded and undegraded adalinine were combined for the rest of the analysis. It is worth noting that our allocations of peaks to undegraded and degraded adalinine were based on mass spectra and might potentially be reversed; however this does not affect our results due to the two being combined. Between the two analyzed alkaloids, adalinine and adaline show a significant positive correlation (Fig. S1c, d). Both alkaloids were combined for the rest of the alkaloid analysis, labelled as 'total alkaloid' in the results. Data for individual alkaloids is provided in the electronic supplementary material (Fig. S2 to S4).

Data Analysis. Alkaloid concentrations (µg/mg wet mass) were calculated by dividing alkaloid calculated in each sample (see above) by the sample mass. This measure, also used by some other authors (e.g. de Jong et al. 1991; Wheeler et al. 2015) and which accounts for variation in body mass, gives the best measure of defensive capability, as alkaloid concentration in the body or reflex blood determines deterrent capability (cf. Pasteels et al. 1973). Comparative analysis was conducted differentiating diets and sexes of ladybirds. An analysis of covariance (ANCOVA) was performed to investigate the reaccumulation of alkaloid as a function of both diet and sex using time as a covariate. Similar analyses testing reaccumulation as a function of diet were done for eggs. Levene’s test was conducted to check for equality of variances. To further examine the relationship between diet/sex and alkaloid recovery rate, individual Pearson correlations were calculated for adults and eggs. In addition, a Pearson test for the correlation of the two endogenous synthesized major and minor alkaloids, adaline and adalinine was also performed. The data was analyzed using IBM SPSS Version 25.0 and R version 3.4.2 (R Core Team 2013).

Daily oviposition rate was calculated as the total number of eggs laid by each female divided by the number of days she was alive in the experiment. Both this and egg mass between diets were analyzed using a one-tailed Mann-Whitney U-test, with alternative hypotheses based on prior literature (Blackman 1967) that *A. fabae* fed ladybirds would lay fewer smaller eggs.

RESULTS

Effect of Diet on Adult Alkaloid Level. Measure of alkaloid reaccumulation in adult ladybirds on the two aphid diets (Fig. 2) showed that the total alkaloid reaccumulation for adult *A. bipunctata* was affected significantly for both the interactive term Diet*Time and Sex*Time, indicating that reaccumulation rate differed with Diet and Sex (Table 1). The rate was lower for ladybirds fed on the lower quality *A. fabae* and for female than males: correlations with time were also weaker for females and on *A. fabae* diets (Fig. 2). Females generally had a higher concentration of alkaloid than males (fixed term Sex) but the term Diet was not significant, possibly because reflex bled ladybirds in both dietary treatments started with similarly low alkaloid levels. Results for each of the individual alkaloids adaline and adalinine, were similar, except that Sex*Time was not significant for adaline (Table S3, Fig. S2 to S4).

Effect of Diet on Egg Production and Alkaloid Levels. When female two-spotted ladybird were fed with *A. fabae*, the oviposition rate per day was lower than when fed *A. pisum* (Fig. 3a; Mann-Whitney U test: $U = 402$, $P = 0.049$), consistent with earlier studies (El-Hariri 1966b; Rana et al. 2002). No effect was observed on the weight of the eggs (Fig. 3b; $t = 1.547$, $df = 40$, $P = 0.13$), although this has been observed previously (Blackman 1967).

In an ANCOVA there was no significant effect of Diet*Time for total alkaloid (Table 2), adaline or adalinine (Table S4). In individual correlations, a positive but weak relationship between alkaloid concentration and time since reflex bleeding was observed for eggs of *A. pisum* fed ladybirds for total alkaloid and adaline but not adalinine (Fig. 4, Fig. S4a). There was no correlation for eggs of *A. fabae* fed ladybirds for any alkaloid measure (Fig. S4b).

DISCUSSION

After reflex bleeding, alkaloids reaccumulated in ladybirds more slowly when they were fed *A. fabae* than when they were fed *A. pisum*. Thus, synthesis of alkaloids in *A. bipunctata* was dependent on dietary quality, both directly, via food content, and because the adult ladybirds could eat fewer of the less palatable *A. fabae* (Blackman 1967), although they clearly do eat sufficient to sustain reproduction. It is worth noting that this dietary effect exists irrespective of the reasons for alkaloid synthesis. We frame the subsequent discussion in terms of recovery, i.e. alkaloid increase, after reflex bleeding, although potentially alkaloid might have increased in the two groups over time irrespective of this. Given earlier studies (e.g., de Jong et al. 1991), however, it does not seem much more likely that the documented increase arose primarily as a result of prior alkaloid depletion.

This relationship between the quality of the fed aphids and the amount of the synthesized alkaloids can be explained through the synthesis of defensive chemicals, for which the energy from food is needed. Previous research has emphasized an integral role for fatty acids as precursors involved in the synthesis of ladybird alkaloids, including adaline (Attygalle et al. 1994; Laurent et al. 2002; Haulotte et al. 2012). Given their importance, a link is to be expected to the level of fat reserves in the beetle: alkaloid synthetic rate could decline with a reduction in *A. bipunctata* fat reserves, which are lower with an *A. fabae* diet (El-Hariri 1966a). Other nutritional factors may also contribute: for example, the amino acid glutamine is suggested to play significant role in adaline synthesis (Laurent et al. 2002).

Interestingly males accumulated alkaloid faster than the female ladybirds, and the effect was stronger, although female *A. bipunctata,* like other ladybirds, synthesize a greater volume of alkaloids than the males (de Jong et al. 1991). However, females allocate a considerable proportion of the alkaloid they synthesize to eggs, which likely explains the lower reaccumulation rate in females and lower correlations with time. No consistent effects on the alkaloid investment in eggs by females between the different diets were observed, although there was limited evidence for a weak increase in alkaloid investment over time on the higher quality diet. Possibly, because alkaloid reaccumulated in *A. pisum*-fed females faster this allowed them to allocate slightly more alkaloid to eggs in the later stages of the experiment. Absence of an effect on the allocation of alkaloids in the egg from diet and level of reaccumulation could possibly be due to allocation of the alkaloids in the eggs being kept constant by the female, ensuring that eggs receive consistent levels of protection. However, study by Kajita et al. (2010) showed the amount of alkaloids across egg clutches significantly varied, and was not constant.

Assuming that our results do represent alkaloid reaccumulation after reflex bleeding, which is supported by other studies (de Jong et al. 1991), it is notable that all alkaloid reaccumulation periods were exceedingly long with both treatments, even the high quality one: in our study they were still increasing even after 12 days. This is consistent with the synthesis of autogenously produced chemical defenses in some other animals (Rossini et al. 1997; Jared et al. 2014), although it can be faster (Heethoff 2012). Although the long recovery time can partly be explained by us having reflex bled our ladybirds to exhaustion, it still seems likely that the recovery period can run to days, given that ladybirds could produce quite large quantities of reflex fluid during reflex bleeding (Holloway et al. 1993).

Studies of chemically defended organisms often rely on single measures to quantify defense levels, usually at peak strength. This has been true for studies testing whether interspecific variation in color pattern and quantitative aspects of chemical defense are linked (Speed et al. 2012). Our study suggests that this approach is unrealistic as it fails to capture a vast amount of variation that can occur even within individuals over time. As reflex bleeding itself can lead to differing levels of chemical defense and long recovery times after alkaloid use, this mechanism only increases the differences between individuals over time. Although individuals of generalist species, such as *A. bipunctata* consume only a few species of aphids in their lives, the potential prey of such species extends to hundreds of different aphids which vary in quality (Majerus 1994; Hodek and Evans 2012) exerting a further substantial intraspecific effect on the amount of ladybirds alkaloid. For ladybirds these effects potentially undermine studies linking color pattern, which does not change over time (except with age:

Majerus 1994), to defense levels, which clearly do. Any correlation detected in laboratory studies with single measurements of defense levels may be drowned out by environmental variation in the field. Similar effects to those we observed here undoubtedly occur in other chemically defended organisms, suggesting that a more temporally dynamic view is needed to better understand the functioning of chemical defense under natural conditions (Brückner and Heethoff 2018). This needs to reflect that many defenses temporarily decline with use and may increase again slowly and heterogeneously across individuals due to factors such as diet, as has been shown here, and potentially other environmental factors such as temperature (Steele et al. 2020a).

Our findings on differences in reaccumulation of alkaloid in relation to quality of diet in the two-spotted ladybird adds to our understanding of alkaloid synthesis in ladybirds, and suggests that the level of chemical defense in ladybirds are much more dynamic than had previously been considered. In the context of this work, how diet and alkaloids are mechanistically linked and a detailed understanding of allocation of alkaloid investment between the eggs and females remain to be studied. Our broader findings on this ladybird, are without doubt applicable to many other chemically defended organisms that synthesize their own toxins, indicating that a more dynamic approach is needed across a wide diversity of studies of chemical defense.

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Figures

Fig. 1 Experimental scheme of care prior to sample collection for analysis. Adult *A. bipunctata* was obtained on the starting day and fed for two days. On the second day of feeding, females and males were paired, only copulated pairs were used in the experiment. All individuals were reflex bled on the sixth day of the experiment. Pairs of ladybirds were frozen for analysis directly after reflex bleeding and also after 1, 3, 5, 8, and 12 days.

Fig. 2 Mean ±SE of alkaloid concentration in *A. bipunctata* over 12 days after reflex bleeding for male (a) and female (b) adult ladybirds. For each sampling day and treatment 6 or 7 pairs were analyzed. Units are µg nicotine equivalent/mg wet mass ladybirds. Pearson Correlation Analysis: (a) *A. pisum* - $r^2 = 0.28$, $n = 38$, $P < 0.001$; *A. fabae* - $r^2 = 0.20$, $n = 39$, $P = 0.004$. (b) *A. pisum* - $r^2 = 0.10$, $n = 38$, $P = 0.006$; *A. fabae* - $r^2 = 0.03$, $n = 39$, $P = 0.32$. Data for each diet are offset horizontally to facilitate interpretation of error bars.

Fig. 3 Reproductive parameters of females fed on two aphid diets. (a) Mean ±SE daily oviposition rate by each female. For *A. pisum* $n = 32$, and *A. fabae* $n = 33$. *$P < 0.05$. Numbers are lower than the Figure 2 as the ladybirds were killed on day 0, could not lay any eggs. (b) Average weight of individual eggs laid by each female. For *A. pisum* $n = 20$ and *A. fabae* $n = 22$.

Fig. 4 Mean ±SE of alkaloid concentration in eggs over 12 days after reflex bleeding of females. For each day and diet the number of samples is between 25 and 66. Units are µg nicotine equivalent/mg wet mass of eggs. Pearson Correlation Analysis: *A. pisum* - $r^2 = 0.061$, $n = 72$, $P = 0.036$; *A. fabae* - $r^2 = 0.20$, $n = 59$, $P = 0.29$. Data for each diet are offset horizontally to facilitate interpretation of error bars.

Tables

Table 1 Results of an ANCOVA analysis on total alkaloid concentration in adult *A. bipunctata*, with fixed effects Diet and Sex and covariate Time since reflex bleeding

Fixed Variable	*df*	*F*	*P*
Diet	1	0.499	0.481
Sex	1	9.486	**0.002**
Diet*Sex	1	0.209	0.648
Diet*Time	1	5.015	**0.027**
Sex*Time	1	4.298	**0.040**
Diet*Sex*Time	1	1.056	0.306

Bold letters indicate a significance of ($P < 0.05$). Number of analyzed adult pairs of each aphid diet: A. pisum - $n = 38$; A. fabae - $n = 39$. $N = 154$.

Table 2 Results of an ANCOVA analysis on egg total alkaloid concentration in adult *A. bipunctata*, with fixed effect Diet and covariate Time since female reflex bleeding

Fixed Variable	*df*	*F*	*P*
Diet	1	0.583	0.446
Diet*Time	1	1.162	0.283

Number of analyzed egg samples from each female aphid diet: A. pisum - *n = 72;* A. fabae - *n = 59. N = 131.*

Figure 1:

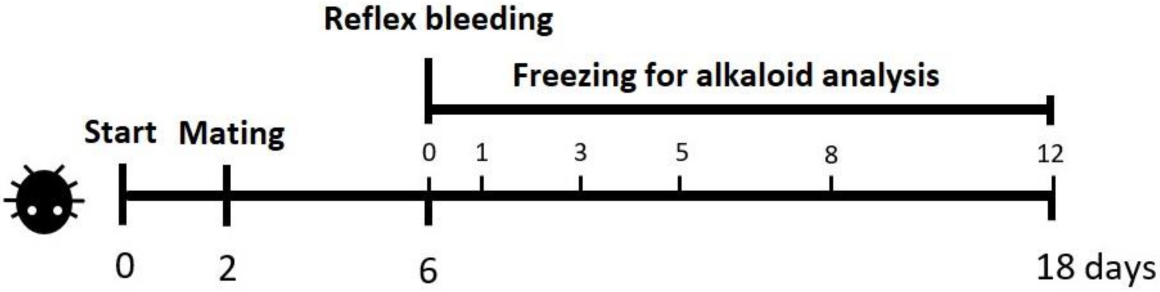

Reflex bleeding
Freezing for alkaloid analysis
Start
Mating
0
1
3
5
8
12
0
2
6
18 days

Figure 2:

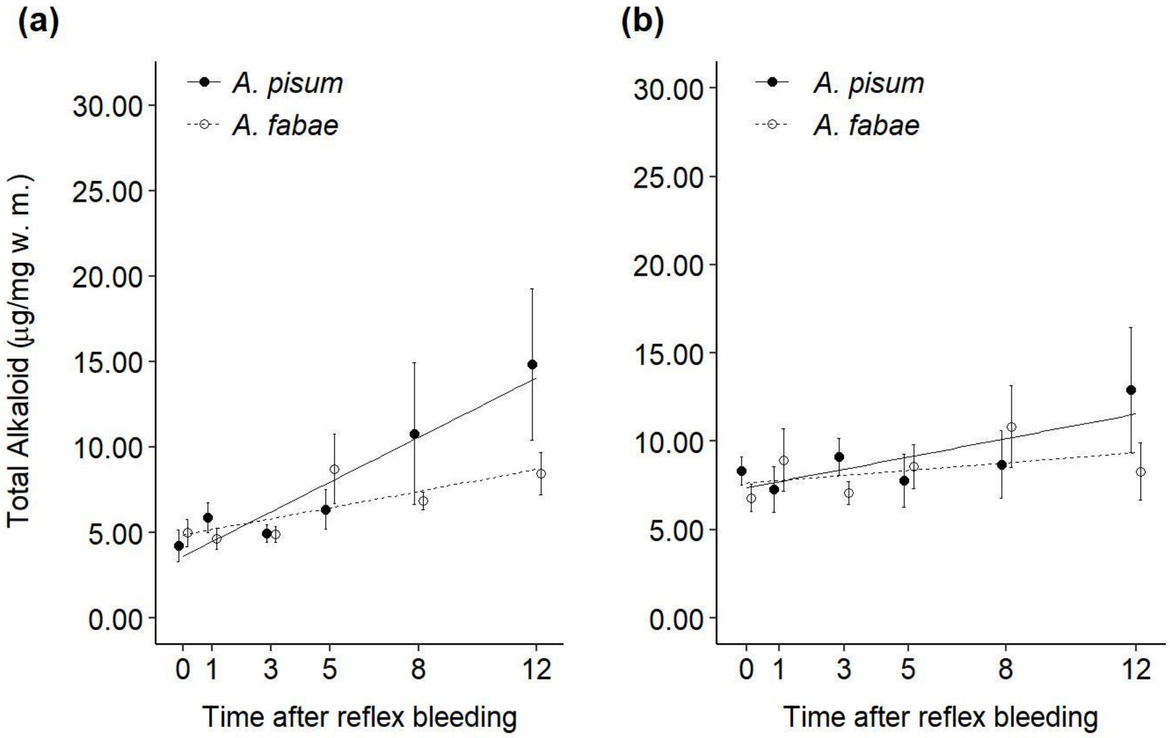

(a)
(b)
A. pisum
A. fabae
Total Alkaloid (µg/mg w. m.)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0
1
3
5
8
12
Time after reflex bleeding

Figure 3:

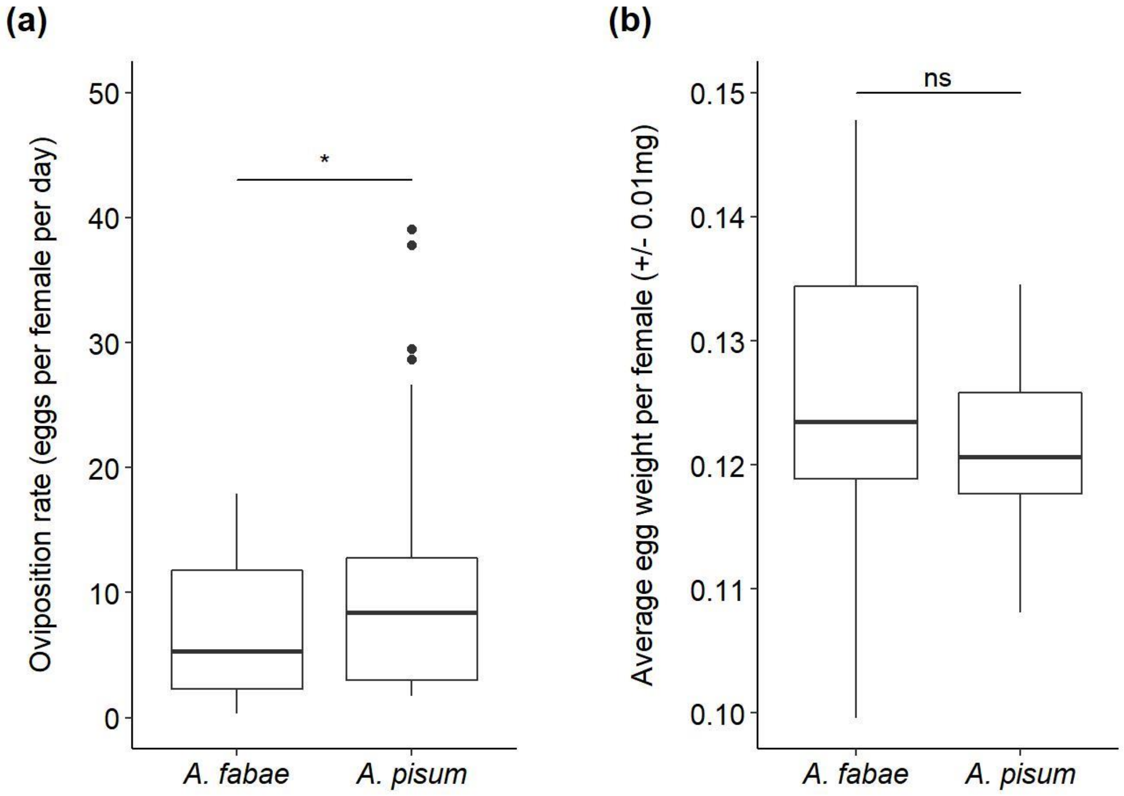
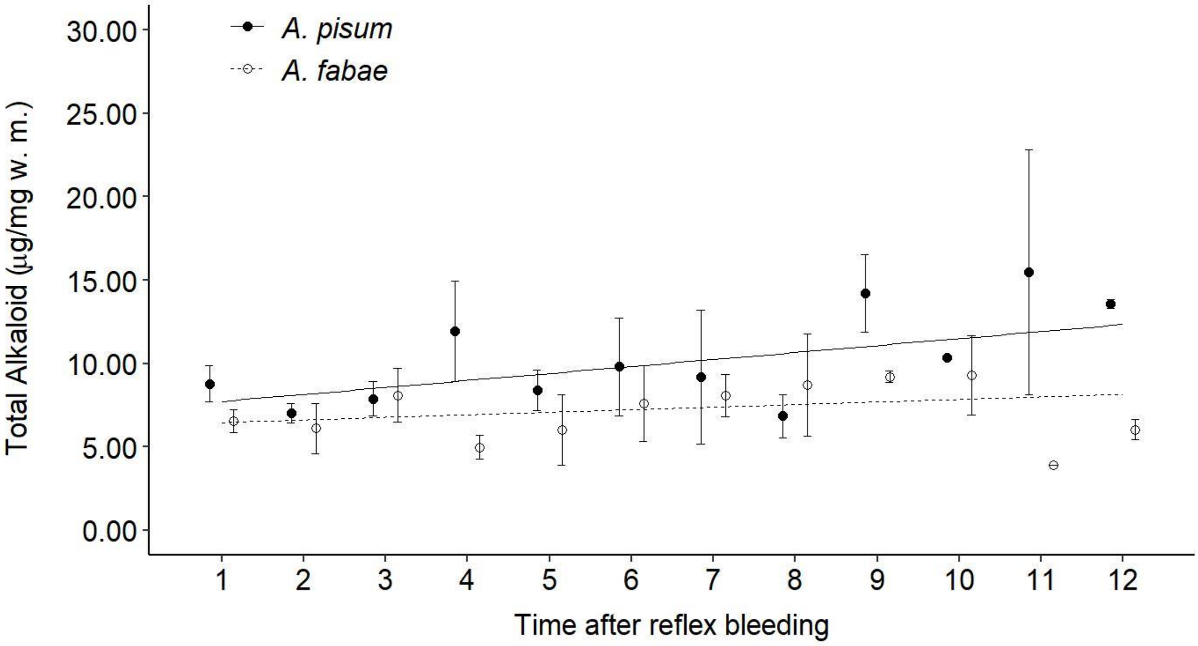

(a)
(b)
Oviposition rate (eggs per female per day)
0
10
20
30
40
50
*
A. fabae
A. pisum
Average egg weight per female (+/- 0.01mg)
0.10
0.11
0.12
0.13
0.14
0.15
ns
A. fabae
A. pisum

Figure 4:


A. pisum
A. fabae
Total Alkaloid (µg/mg w. m.)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
1
2
3
4
5
6
7
8
9
10
11
12
Time after reflex bleeding

ELECTRONIC SUPPLEMENTARY MATERIAL EXCEL FILE

TITLE	HOW DIET LEADS TO DEFENSIVE DYNAMISM: EFFECT OF THE DIETARY QUALITY ON AUTOGENOUS ALKALOIDS RECOVERY RATE IN A CHEMICALLY DEFENDED BEETLE
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TABLES	
Table S1	Conversion dataset for calculating alkaloid concentration in ladybird
Table S2	Conversion dataset for calculating alkaloid concentration in eggs

Table S1 Conversion dataset for calculating alkaloid level in ladybird

Variables				**Weight**		**GC-MS Peak Area**				**Conversion (µg nicotine equivalent /mg wet mass ladybird)**				
Aphid	Sex	Trials	Day	(g)	(mg)	Nicotine	Adaline	Adalinine	Degraded Adalinine	Adaline	Adalinine	Degraded Adalinine	Total Adalinine	Total Alkaloid
A. pisum	Female	1	0	0.01776	17.76	4249697	8596937	299862	159482	5.695	0.199	0.106	0.304	6.000
A. pisum	Female	1	1	0.02163	21.63	4909717	14884637	393015	170574	7.008	0.185	0.080	0.265	7.273
A. pisum	Female	1	3	0.0214	21.4	3839393	12066851	483798	143352	7.343	0.294	0.087	0.382	7.725
A. pisum	Female	1	5	0.01759	17.59	3476221	12975420	322906	205756	10.610	0.264	0.168	0.432	11.042
A. pisum	Female	1	8	0.02059	20.59	3115972	10459849	375005	118895	8.152	0.292	0.093	0.385	8.537
A. pisum	Female	1	12	0.01954	19.54	4679778	13641198	424975	306268	7.459	0.232	0.167	0.400	7.859
A. pisum	Female	2	0	0.01982	19.82	4852201	13747125	397597	281165	7.147	0.207	0.146	0.353	7.500
A. pisum	Female	2	1	0.01918	19.18	2975840	10432410	251737	199152	9.139	0.221	0.174	0.395	9.534
A. pisum	Female	2	3	0.01648	16.48	6481955	12993748	526508	445416	6.082	0.246	0.208	0.455	6.537
A. pisum	Female	2	5	0.01581	15.81	6071266	11496749	390456	227494	5.989	0.203	0.119	0.322	6.311
A. pisum	Female	2	8	0.01869	18.69	5328791	8250594	251933	166709	4.142	0.126	0.084	0.210	4.352
A. pisum	Female	2	12	0.01687	16.87	41168812	148551262	2692407	4437480	10.695	0.194	0.319	0.513	11.208
A. pisum	Female	3	0	0.02075	20.75	4267064	13772217	541425	568836	7.777	0.306	0.321	0.627	8.404
A. pisum	Female	3	1	0.02077	20.77	2476853	10572443	365197	449881	10.276	0.355	0.437	0.792	11.068
A. pisum	Female	3	3	0.01606	16.06	2540670	7302303	233950	285270	8.948	0.287	0.350	0.636	9.584
A. pisum	Female	3	5	0.0181	18.1	5490131	8473271	806894	525018	4.263	0.406	0.264	0.670	4.934
A. pisum	Female	3	8	0.01901	19.01	5503867	13940627	374771	424412	6.662	0.179	0.203	0.382	7.044
A. pisum	Female	3	12	0.02135	21.35	3744584	12386192	405219	585598	7.747	0.253	0.366	0.620	8.366
A. pisum	Female	4	0	0.01731	17.31	5658160	10569319	385060	266678	5.396	0.197	0.136	0.333	5.728
A. pisum	Female	4	1	0.01136	11.36	7181680	2352992	369398	225652	1.442	0.226	0.138	0.365	1.807
A. pisum	Female	4	3	0.02164	21.64	2986508	13268697	404869	331387	10.265	0.313	0.256	0.570	10.835
A. pisum	Female	4	5	0.0137	13.7	5754082	4098743	263422	276087	2.600	0.167	0.175	0.342	2.942
A. pisum	Female	4	8	0.01242	12.42	6624025	26744052	1082387	1061330	16.254	0.658	0.645	1.303	17.557
A. pisum	Female	4	12	0.01334	13.34	5740268	9364677	482823	470300	6.115	0.315	0.307	0.622	6.737
A. pisum	Female	5	0	0.01239	12.39	4513260	8910798	394633	411117	7.968	0.353	0.368	0.720	8.688
A. pisum	Female	5	1	0.01191	11.91	5529933	7654201	509429	336452	5.811	0.387	0.255	0.642	6.453
A. pisum	Female	5	3	0.01235	12.35	2368045	7319434	440578	382295	12.514	0.753	0.654	1.407	13.921
A. pisum	Female	5	5	0.01197	11.97	7041063	14564775	297100	437108	8.641	0.176	0.259	0.436	9.076
A. pisum	Female	5	8	0.01166	11.66	5912866	10930136	755065	683178	7.927	0.548	0.495	1.043	8.970
A. pisum	Female	5	12	0.01621	16.21	2431155	9178780	849550	644740	11.646	1.078	0.818	1.896	13.541
A. pisum	Female	6	0	0.01018	10.18	5651471	11379992	509983	377305	9.890	0.443	0.328	0.771	10.661
A. pisum	Female	6	1	0.01268	12.68	4499376	7943710	359809	260032	6.962	0.315	0.228	0.543	7.505
A. pisum	Female	6	3	0.01262	12.62	4513394	10144333	269248	262094	8.905	0.236	0.230	0.466	9.371
A. pisum	Female	6	5	0.01154	11.54	2965498	7458199	599064	382943	10.897	0.875	0.560	1.435	12.332
A. pisum	Female	6	8	0.011	11	5090697	5722351	220386	422476	5.109	0.197	0.377	0.574	5.683
A. pisum	Female	6	12	0.0128	12.8	1278873	8813030	604166	354996	26.919	1.845	1.084	2.930	29.849
A. pisum	Female	7	0	0.01348	13.48	4156004	11463667	604281	458012	10.231	0.539	0.409	0.948	11.179
A. pisum	Female	7	3	0.01143	11.43	5127574	6496261	257590	203038	5.542	0.220	0.173	0.393	5.935
A. pisum	Male	1	0	0.0168	16.8	3826760	5683689	362292	119295	4.420	0.282	0.093	0.375	4.795
A. pisum	Male	1	1	0.00835	8.35	4548423	5749837	608828	245682	7.570	0.802	0.323	1.125	8.695
A. pisum	Male	1	3	0.01793	17.93	5334440	7640823	483266	209562	3.994	0.253	0.110	0.362	4.356
A. pisum	Male	1	5	0.01211	12.11	5764472	13794482	1709340	606535	9.880	1.224	0.434	1.659	11.539
A. pisum	Male	1	8	0.0152	15.2	3656269	7858810	1105220	339494	7.070	0.994	0.305	1.300	8.370
A. pisum	Male	1	12	0.02362	23.62	5047493	5272811	673971	188722	2.211	0.283	0.079	0.362	2.573
A. pisum	Male	2	0	0.01541	15.41	5560239	1869640	118722	66550	1.091	0.069	0.039	0.108	1.199
A. pisum	Male	2	1	0.01332	13.32	5697899	5564154	333943	95502	3.666	0.220	0.063	0.283	3.949
A. pisum	Male	2	3	0.01813	18.13	4518956	6496802	861334	424609	3.965	0.526	0.259	0.785	4.750
A. pisum	Male	2	5	0.01744	17.44	6016897	10528566	641671	344152	5.017	0.306	0.164	0.470	5.486
A. pisum	Male	2	8	0.01622	16.22	6269418	6004246	1688444	367278	2.952	0.830	0.181	1.011	3.963
A. pisum	Male	2	12	0.01565	15.65	7706309	16648916	1402664	647778	6.902	0.582	0.269	0.850	7.752
A. pisum	Male	3	0	0.02185	21.85	24725502	27351603	2159869	1404260	2.531	0.200	0.130	0.330	2.861
A. pisum	Male	3	1	0.01873	18.73	22351529	27444592	1773223	1250722	3.278	0.212	0.149	0.361	3.639
A. pisum	Male	3	3	0.01425	14.25	5612826	9822888	1081593	588369	6.141	0.676	0.368	1.044	7.185
A. pisum	Male	3	5	0.01713	17.13	42651038	94836056	3094325	2929057	6.490	0.212	0.200	0.412	6.902
A. pisum	Male	3	8	0.01075	10.75	5990121	7093696	982093	671735	5.508	0.763	0.522	1.284	6.792
A. pisum	Male	3	12	0.01201	12.01	1881672	10347298	1586216	989675	22.893	3.510	2.190	5.699	28.593
A. pisum	Male	4	0	0.01644	16.44	6450106	3904435	255948	173377	1.841	0.121	0.082	0.202	2.043
A. pisum	Male	4	1	0.01335	13.35	5705671	10722789	706764	474741	7.039	0.464	0.312	0.776	7.814
A. pisum	Male	4	3	0.01572	15.72	2261422	4144662	383134	163073	5.829	0.539	0.229	0.768	6.598
A. pisum	Male	4	5	0.01174	11.74	5460722	6638864	882527	456347	5.178	0.688	0.356	1.044	6.222
A. pisum	Male	4	8	0.00878	8.78	2660129	11936282	1443361	872309	25.553	3.090	1.867	4.957	30.510
A. pisum	Male	4	12	0.01288	12.88	1838324	10002088	1329361	727972	21.121	2.807	1.537	4.344	25.466
A. pisum	Male	5	0	0.0102	10.2	5206735	7276134	659326	427687	6.850	0.621	0.403	1.023	7.874
A. pisum	Male	5	1	0.01147	11.47	4594187	6693337	200765	148606	6.351	0.190	0.141	0.332	6.682
A. pisum	Male	5	3	0.00772	7.72	5028186	2795017	234458	171113	3.600	0.302	0.220	0.522	4.123
A. pisum	Male	5	5	0.01099	10.99	6880492	4739974	408176	292572	3.134	0.270	0.193	0.463	3.598
A. pisum	Male	5	8	0.01156	11.56	2756124	6593360	561920	344117	10.347	0.882	0.540	1.422	11.769
A. pisum	Male	5	12	0.01157	11.57	6252047	7076086	1273999	833593	4.891	0.881	0.576	1.457	6.348
A. pisum	Male	6	0	0.00981	9.81	4309831	5096434	201807	210305	6.027	0.239	0.249	0.487	6.514
A. pisum	Male	6	1	0.01086	10.86	5753624	5163023	190057	202012	4.131	0.152	0.162	0.314	4.445
A. pisum	Male	6	3	0.0111	11.1	6249221	3737295	1248439	441836	2.694	0.900	0.318	1.218	3.912
A. pisum	Male	6	5	0.0127	12.7	6921588	7045013	411211	276782	4.007	0.234	0.157	0.391	4.399
A. pisum	Male	6	8	0.01216	12.16	3583589	2548247	197373	148540	2.924	0.226	0.170	0.397	3.321
A. pisum	Male	6	12	0.01025	10.25	1853490	6205229	437239	318730	16.331	1.151	0.839	1.990	18.321
A. pisum	Male	7	0	0.0113	11.3	5872639	5207769	371705	196992	3.924	0.280	0.148	0.428	4.352
A. pisum	Male	7	3	0.01366	13.66	3435872	3004588	303443	236916	3.201	0.323	0.252	0.576	3.777
A. fabae	Female	1	0	0.01959	19.59	5525848	7854066	364977	182794	3.628	0.169	0.084	0.253	3.881
A. fabae	Female	1	1	0.01784	17.84	3977006	7578020	442005	188161	5.340	0.311	0.133	0.444	5.785
A. fabae	Female	1	3	0.01707	17.07	2831411	8298074	877182	329937	8.584	0.907	0.341	1.249	9.833
A. fabae	Female	1	5	0.01629	16.29	5154360	13388187	1081330	457867	7.973	0.644	0.273	0.917	8.889
A. fabae	Female	1	8	0.01706	17.06	4196629	10144938	483857	319077	7.085	0.338	0.223	0.561	7.646
A. fabae	Female	1	12	0.01593	15.93	5739524	7885447	539910	283885	4.312	0.295	0.155	0.451	4.763
A. fabae	Female	2	0	0.019	19	3371487	9211753	607353	323286	7.190	0.474	0.252	0.726	7.917
A. fabae	Female	2	1	0.01471	14.71	4710414	9765946	719187	317960	7.047	0.519	0.229	0.748	7.796
A. fabae	Female	2	3	0.01744	17.44	2114932	5327345	301614	152064	7.222	0.409	0.206	0.615	7.837
A. fabae	Female	2	5	0.01573	15.73	6405770	10535949	211390	151251	5.228	0.105	0.075	0.180	5.408
A. fabae	Female	2	8	0.01614	16.14	27686443	40435219	1821724	2053120	4.524	0.204	0.230	0.434	4.958
A. fabae	Female	2	12	0.01375	13.75	30519521	62682538	1304608	1228030	7.469	0.155	0.146	0.302	7.770
A. fabae	Female	3	0	0.01735	17.35	3900589	10596939	390832	468587	7.829	0.289	0.346	0.635	8.464
A. fabae	Female	3	1	0.01801	18.01	5112869	9776458	430943	445689	5.309	0.234	0.242	0.476	5.785
A. fabae	Female	3	3	0.01938	19.38	2739656	5984647	314249	325804	5.636	0.296	0.307	0.603	6.239
A. fabae	Female	3	5	0.01556	15.56	5107611	8387284	379976	375627	5.277	0.239	0.236	0.475	5.752
A. fabae	Female	3	8	0.02122	21.22	4648511	9723721	780231	544457	4.929	0.395	0.276	0.671	5.600
A. fabae	Female	3	12	0.01406	14.06	4584783	8206348	199877	195059	6.365	0.155	0.151	0.306	6.672
A. fabae	Female	4	0	0.01418	14.18	3899253	6722671	346958	240852	6.079	0.314	0.218	0.532	6.611
A. fabae	Female	4	1	0.01192	11.92	2512408	6389235	376767	180176	10.667	0.629	0.301	0.930	11.597
A. fabae	Female	4	3	0.01489	14.89	5848039	10570142	374923	449529	6.069	0.215	0.258	0.473	6.543
A. fabae	Female	4	5	0.01074	10.74	5438752	10199276	526778	270082	8.730	0.451	0.231	0.682	9.413
A. fabae	Female	4	8	0.01237	12.37	1692548	5067328	332670	246028	12.101	0.794	0.588	1.382	13.483
A. fabae	Female	4	12	0.00935	9.35	2230516	5607295	489773	394425	13.443	1.174	0.946	2.120	15.563
A. fabae	Female	5	0	0.01085	10.85	3987747	7635482	354994	210299	8.824	0.410	0.243	0.653	9.477
A. fabae	Female	5	1	0.01059	10.59	5093926	6060051	332859	214666	5.617	0.309	0.199	0.507	6.124
A. fabae	Female	5	3	0.01512	15.12	3572210	7171753	633143	369960	6.639	0.586	0.342	0.929	7.568
A. fabae	Female	5	5	0.01243	12.43	1732083	5351721	385124	248135	12.429	0.894	0.576	1.471	13.899
A. fabae	Female	5	8	0.01216	12.16	1370934	5941632	291943	196098	17.821	0.876	0.588	1.464	19.285
A. fabae	Female	5	12	0.01201	12.01	7220303	11181653	257302	123341	6.447	0.148	0.071	0.219	6.667
A. fabae	Female	6	0	0.01153	11.53	6387229	5929423	363287	264150	4.026	0.247	0.179	0.426	4.452
A. fabae	Female	6	1	0.01019	10.19	3836538	11674688	820523	488338	14.931	1.049	0.625	1.674	16.605
A. fabae	Female	6	3	0.01449	14.49	4299791	4852784	323344	199218	3.894	0.259	0.160	0.419	4.314
A. fabae	Female	6	5	0.01125	11.25	1736750	2789744	242241	138017	7.139	0.620	0.353	0.973	8.112
A. fabae	Female	6	8	0.01467	14.67	1766463	6227650	706621	364651	12.016	1.363	0.704	2.067	14.083
A. fabae	Female	6	12	0.01205	12.05	3304144	2809083	150383	122669	3.528	0.189	0.154	0.343	3.871
A. fabae	Female	7	0	0.01203	12.03	5167919	7697228	522307	230901	6.190	0.420	0.186	0.606	6.796
A. fabae	Female	7	3	0.01212	12.12	4015786	6212882	453473	341536	6.382	0.466	0.351	0.817	7.199
A. fabae	Female	7	12	0.01334	13.34	2072088	6378204	399239	253784	11.537	0.722	0.459	1.181	12.719
A. fabae	Male	1	0	0.01433	14.33	5407321	5159692	507350	116954	3.329	0.327	0.075	0.403	3.732
A. fabae	Male	1	1	0.01247	12.47	5231821	6020490	176747	116453	4.614	0.135	0.089	0.225	4.839
A. fabae	Male	1	3	0.01293	12.93	4951455	7388121	588541	175632	5.770	0.460	0.137	0.597	6.367
A. fabae	Male	1	5	0.01505	15.05	7204531	11487489	465564	157353	5.297	0.215	0.073	0.287	5.585
A. fabae	Male	1	8	0.01503	15.03	6079074	10471506	681024	218075	5.730	0.373	0.119	0.492	6.222
A. fabae	Male	1	12	0.01484	14.84	5974498	9901682	418484	168170	5.584	0.236	0.095	0.331	5.915
A. fabae	Male	2	0	0.01415	14.15	4977127	6832845	320113	120806	4.851	0.227	0.086	0.313	5.164
A. fabae	Male	2	1	0.01445	14.45	5947613	4569079	451143	225930	2.658	0.262	0.131	0.394	3.052
A. fabae	Male	2	3	0.01239	12.39	4557105	3671759	147704	86255	3.252	0.131	0.076	0.207	3.459
A. fabae	Male	2	5	0.01094	10.94	5355050	10987202	404780	337526	9.377	0.345	0.288	0.634	10.011
A. fabae	Male	2	8	0.01166	11.66	5922563	6313147	966288	308267	4.571	0.700	0.223	0.923	5.494
A. fabae	Male	2	12	0.01948	19.48	5997917	9167524	925562	484903	3.923	0.396	0.208	0.604	4.527
A. fabae	Male	3	0	0.01144	11.44	16538698	14268076	1072299	610029	3.771	0.283	0.161	0.445	4.215
A. fabae	Male	3	1	0.01864	18.64	26804378	24966823	2491063	1402497	2.499	0.249	0.140	0.390	2.888
A. fabae	Male	3	3	0.01185	11.85	25350794	29637998	2758369	1770383	4.933	0.459	0.295	0.754	5.687
A. fabae	Male	3	5	0.01093	10.93	41472694	58649611	3131925	2429759	6.469	0.345	0.268	0.613	7.083
A. fabae	Male	3	8	0.01152	11.52	5391371	7267231	573563	567733	5.850	0.462	0.457	0.919	6.769
A. fabae	Male	3	12	0.01445	14.45	4657927	7122595	530721	507591	5.291	0.394	0.377	0.771	6.062
A. fabae	Male	4	0	0.00869	8.69	4315616	6314679	518794	325292	8.419	0.692	0.434	1.125	9.544
A. fabae	Male	4	1	0.01103	11.03	5593028	5125961	453369	320506	4.155	0.367	0.260	0.627	4.782
A. fabae	Male	4	3	0.01359	13.59	4332490	5190155	382126	233697	4.408	0.325	0.198	0.523	4.930
A. fabae	Male	4	5	0.01066	10.66	7349055	5329114	134509	160388	3.401	0.086	0.102	0.188	3.589
A. fabae	Male	4	8	0.0105	10.5	2297110	3777555	370748	180482	7.831	0.769	0.374	1.143	8.974

A. fabae	Male	4	12	0.01248	12.48	2274306	6525319	772783	483304	11.495	1.361	0.851	2.213	13.708
A. fabae	Male	5	0	0.0073	7.3	5034595	2356827	584709	330002	3.206	0.795	0.449	1.244	4.451
A. fabae	Male	5	1	0.01115	11.15	5487214	5833282	573295	269092	4.767	0.469	0.220	0.688	5.456
A. fabae	Male	5	3	0.0092	9.2	5094421	5165290	216507	184360	5.510	0.231	0.197	0.428	5.938
A. fabae	Male	5	5	0.01159	11.59	1396052	5232437	332059	238916	16.169	1.026	0.738	1.764	17.934
A. fabae	Male	5	8	0.00872	8.72	4405598	4138131	360556	205807	5.386	0.469	0.268	0.737	6.123
A. fabae	Male	5	12	0.01056	10.56	2666920	4070443	319404	237389	7.227	0.567	0.421	0.989	8.215
A. fabae	Male	6	0	0.01369	13.69	6421342	7166531	313358	218683	4.076	0.178	0.124	0.303	4.379
A. fabae	Male	6	1	0.01258	12.58	3875669	6369780	208466	146429	6.532	0.214	0.150	0.364	6.896
A. fabae	Male	6	3	0.01151	11.51	5299704	3685300	210824	198680	3.021	0.173	0.163	0.336	3.356
A. fabae	Male	6	5	0.00962	9.62	2305796	2965470	397263	265318	6.684	0.895	0.598	1.494	8.178
A. fabae	Male	6	8	0.00965	9.65	2954823	3808326	253375	227952	6.678	0.444	0.400	0.844	7.522
A. fabae	Male	6	12	0.01165	11.65	2353986	5463992	279002	172479	9.962	0.509	0.314	0.823	10.785
A. fabae	Male	7	0	0.00917	9.17	5625536	3167420	233443	146939	3.070	0.226	0.142	0.369	3.439
A. fabae	Male	7	3	0.00776	7.76	5624693	3357070	383149	188624	3.846	0.439	0.216	0.655	4.501
A. fabae	Male	7	12	0.01372	13.72	2045087	4852449	543986	254364	8.647	0.969	0.453	1.423	10.070

Table S2 Conversion dataset for calculating alkaloid level in eggs

Variables		Weight				GC-MS Peak Area				Conversion (µg nicotine equivalent /mg wet mass egg)				
Aphid	Day	Count	Total (g)	Total (mg)	Individual (mg)	Nicotine	Adaline	Adalinine	Degraded Adalinine	Adaline	Adalinine	Degraded Adalinine	Total Adalinine	Total Alkaloid
A. fabae	1	25	0.00242	2.420	0.097	1388633	2133282	239611	107934	6.348	0.713	0.321	1.034	7.382
A. fabae	1	29	0.00349	3.490	0.120	1902251	1979855	194110	112651	2.982	0.292	0.170	0.462	3.444
A. fabae	1	26	0.00345	3.450	0.133	1637307	2513651	211920	106242	4.450	0.375	0.188	0.563	5.013
A. fabae	1	25	0.00347	3.470	0.139	1626138	2376110	207315	87649	4.211	0.367	0.155	0.523	4.734
A. fabae	1	30	0.00370	3.700	0.123	345600	578660	61456	24678	4.525	0.481	0.193	0.674	5.199
A. fabae	1	26	0.00310	3.100	0.119	1248040	1244648	95101	35531	3.217	0.246	0.092	0.338	3.555
A. fabae	1	25	0.00306	3.060	0.122	2501966	2629757	135297	105912	3.435	0.177	0.138	0.315	3.750
A. fabae	1	25	0.00288	2.880	0.115	529033	1614123	125248	64872	10.594	0.822	0.426	1.248	11.842
A. fabae	1	26	0.00346	3.460	0.133	1773728	4913384	596346	349036	8.006	0.972	0.569	1.540	9.546
A. fabae	1	28	0.00325	3.250	0.116	10831184	12799750	1195803	628674	3.636	0.340	0.179	0.518	4.154
A. fabae	1	26	0.00354	3.540	0.136	3092414	8948481	935217	622106	8.174	0.854	0.568	1.423	9.597
A. fabae	1	28	0.00333	3.330	0.119	2298070	5833306	943602	571113	7.623	1.233	0.746	1.979	9.602
A. fabae	1	25	0.00351	3.510	0.140	5300947	7681958	284680	537515	4.129	0.153	0.289	0.442	4.571
A. fabae	1	38	0.00531	5.310	0.140	3781960	12296467	1084515	646603	6.123	0.540	0.322	0.862	6.985
A. fabae	1	45	0.00587	5.870	0.130	2886752	13299685	1104997	520894	7.849	0.652	0.307	0.959	8.808
A. fabae	2	30	0.00394	3.940	0.131	1443583	2420446	262604	91040	4.256	0.462	0.160	0.622	4.877
A. fabae	2	26	0.00329	3.290	0.127	12012823	11949602	1191316	567488	3.024	0.301	0.144	0.445	3.469
A. fabae	2	28	0.00341	6.020	0.215	3843620	7277543	627482	293547	3.145	0.271	0.127	0.398	3.543
A. fabae	2	31	0.00440	4.400	0.142	8662823	10928503	1359146	894797	2.867	0.357	0.235	0.591	3.458
A. fabae	2	32	0.00473	4.730	0.148	8237799	11901370	1445068	1040215	3.054	0.371	0.267	0.638	3.692
A. fabae	2	37	0.00494	4.940	0.134	2225108	9368362	904190	579170	8.523	0.823	0.527	1.349	9.872
A. fabae	2	39	0.00522	5.220	0.134	1665843	12170118	679407	536003	13.996	0.781	0.616	1.398	15.393
A. fabae	2	41	0.00593	5.930	0.145	9589853	22936273	1604784	1109236	4.033	0.282	0.195	0.477	4.511
A. fabae	3	25	0.00335	3.350	0.134	4507544	8320307	534905	695570	5.510	0.354	0.461	0.815	6.325
A. fabae	3	38	0.00476	4.760	0.125	1659056	7734507	415453	765750	9.794	0.526	0.970	1.496	11.290
A. fabae	3	31	0.00394	3.940	0.127	9965259	13205021	1309893	707748	3.363	0.334	0.180	0.514	3.877
A. fabae	3	26	0.00280	2.800	0.108	10798763	7634364	676579	415439	2.525	0.224	0.137	0.361	2.886
A. fabae	3	28	0.00322	3.220	0.115	2722489	4858016	408256	256389	5.542	0.466	0.292	0.758	6.300
A. fabae	3	27	0.00318	3.180	0.118	1116577	4920636	429511	740668	13.858	1.210	2.086	3.296	17.154
A. fabae	3	33	0.00410	4.100	0.124	2296709	7186982	568601	377576	7.632	0.604	0.401	1.005	8.637
A. fabae	3	25	0.00340	3.400	0.136	2447687	6096590	435739	401210	7.326	0.524	0.482	1.006	8.331
A. fabae	4	25	0.00310	3.100	0.124	6485031	9081984	904301	453409	4.518	0.450	0.226	0.675	5.193
A. fabae	4	30	0.00360	3.600	0.120	4443387	6776242	772621	415289	4.236	0.483	0.260	0.743	4.979
A. fabae	4	25	0.00341	3.410	0.136	9263178	9490223	958052	725545	3.004	0.303	0.230	0.533	3.537
A. fabae	4	25	0.00305	3.050	0.122	2306325	5066976	407590	323150	7.203	0.579	0.459	1.039	8.242
A. fabae	4	31	0.00430	4.300	0.139	10211759	16431538	1066644	839408	3.742	0.243	0.191	0.434	4.176
A. fabae	4	25	0.00305	3.050	0.122	9456241	9398486	805249	493908	3.259	0.279	0.171	0.450	3.709
A. fabae	5	25	0.00325	3.250	0.130	8977001	7778502	796620	558420	2.666	0.273	0.191	0.464	3.131
A. fabae	5	26	0.00297	2.970	0.114	5672487	6928110	604337	444548	4.112	0.359	0.264	0.623	4.735
A. fabae	5	25	0.00285	2.850	0.114	2245154	5106885	791513	606950	7.981	1.237	0.949	2.186	10.167
A. fabae	6	27	0.00314	3.140	0.116	10052655	10565069	1088750	658691	3.347	0.345	0.209	0.554	3.901
A. fabae	6	26	0.00254	2.540	0.098	1730241	5993421	261367	153205	13.637	0.595	0.349	0.943	14.581
A. fabae	6	40	0.00566	5.660	0.142	8907110	17531055	1159688	659976	3.477	0.230	0.131	0.361	3.838
A. fabae	6	31	0.00383	3.830	0.124	2032048	7168772	1044655	788216	9.211	1.342	1.013	2.355	11.566
A. fabae	6	25	0.00324	3.240	0.130	7305805	8746419	891374	464821	3.695	0.377	0.196	0.573	4.268
A. fabae	7	26	0.00305	3.050	0.117	5084523	8930716	1108151	546534	5.759	0.715	0.352	1.067	6.826
A. fabae	7	28	0.00317	3.170	0.113	2821820	7214492	735561	422463	8.065	0.822	0.472	1.295	9.360
A. fabae	8	28	0.00372	3.720	0.133	8505004	13370627	1618136	742158	4.226	0.511	0.235	0.746	4.972
A. fabae	8	28	0.00315	3.150	0.113	2091028	8344555	869578	500368	12.669	1.320	0.760	2.080	14.749
A. fabae	8	25	0.00359	3.590	0.144	3822596	8169744	367982	240778	5.953	0.268	0.175	0.444	6.397
A. fabae	9	25	0.00335	3.350	0.134	3091910	8563656	847616	472804	8.268	0.818	0.456	1.275	9.543
A. fabae	9	32	0.00381	3.810	0.119	2807921	8129779	854249	484032	7.599	0.798	0.452	1.251	8.850
A. fabae	10	29	0.00294	2.940	0.101	2174904	8580763	224264	122942	13.420	0.351	0.192	0.543	13.963
A. fabae	10	36	0.00502	5.020	0.139	1697388	9932221	391846	208881	11.656	0.460	0.245	0.705	12.361
A. fabae	10	26	0.00301	3.010	0.116	9077543	8916284	699272	535135	3.263	0.256	0.196	0.452	3.715
A. fabae	10	26	0.00268	2.680	0.103	3002405	4418079	780748	515959	5.491	0.970	0.641	1.612	7.102
A. fabae	11	26	0.00315	3.150	0.121	9773996	10808539	684126	578991	3.511	0.222	0.188	0.410	3.921
A. fabae	12	25	0.00357	3.570	0.143	5513752	9603336	621198	436105	4.879	0.316	0.222	0.537	5.416
A. fabae	12	27	0.00319	3.190	0.118	3298727	5527508	863922	626876	5.253	0.821	0.596	1.417	6.670
A. pisum	1	25	0.00327	3.270	0.131	1785556	2751550	140298	58385	4.713	0.240	0.100	0.340	5.053
A. pisum	1	25	0.00328	3.280	0.131	2168090	3365438	233684	101890	4.732	0.329	0.143	0.472	5.204
A. pisum	1	37	0.00436	4.360	0.118	2418593	6503288	229141	190063	6.167	0.217	0.180	0.398	6.565
A. pisum	1	25	0.00291	2.910	0.116	677585	2164432	105584	86826	10.977	0.535	0.440	0.976	11.953
A. pisum	1	27	0.00353	3.530	0.131	1007008	2875608	101215	60397	8.090	0.285	0.170	0.455	8.544
A. pisum	1	27	0.00318	3.180	0.118	535358	2398942	161026	100873	14.091	0.946	0.593	1.538	15.630
A. pisum	1	31	0.00383	3.830	0.124	1707563	2981952	106256	41267	4.560	0.162	0.063	0.226	4.785
A. pisum	1	28	0.00330	3.300	0.118	739076	1508066	56597	47169	6.183	0.232	0.193	0.425	6.609
A. pisum	1	25	0.00314	3.140	0.126	750517	2251738	72861	40491	9.555	0.309	0.172	0.481	10.036
A. pisum	1	31	0.00409	4.090	0.132	569771	1199089	56591	30161	5.146	0.243	0.129	0.372	5.518
A. pisum	1	26	0.00315	3.150	0.121	1940518	6127778	611550	848629	10.025	1.000	1.388	2.389	12.414
A. pisum	1	29	0.00349	3.490	0.120	2325276	11454358	586711	568735	14.115	0.723	0.701	1.424	15.538
A. pisum	1	32	0.00365	3.650	0.114	2996844	5944274	532447	421772	5.434	0.487	0.386	0.872	6.307
A. pisum	2	28	0.00349	3.490	0.125	619621	1209315	73055	25791	5.592	0.338	0.119	0.457	6.049
A. pisum	2	30	0.00371	3.710	0.124	693343	1516911	83374	41216	5.897	0.324	0.160	0.484	6.381
A. pisum	2	31	0.00384	3.840	0.124	2720334	7115438	467209	320480	6.812	0.447	0.307	0.754	7.566
A. pisum	2	41	0.00574	5.740	0.140	576238	1449321	74010	48087	4.382	0.224	0.145	0.369	4.751
A. pisum	2	35	0.00391	3.910	0.112	583774	1109292	59120	21650	4.860	0.259	0.095	0.354	5.214
A. pisum	2	47	0.00558	5.580	0.119	537697	1606463	54647	49515	5.354	0.182	0.165	0.347	5.701
A. pisum	2	27	0.00363	3.630	0.134	3902648	12626446	298099	499610	8.913	0.210	0.353	0.563	9.476
A. pisum	2	25	0.00313	3.130	0.125	2234810	5736690	263594	154380	8.201	0.377	0.221	0.598	8.799
A. pisum	2	25	0.00328	3.280	0.131	2143588	6080420	636688	475623	8.648	0.906	0.676	1.582	10.230
A. pisum	2	25	0.00293	2.930	0.117	6175537	10010649	541713	460939	5.532	0.299	0.255	0.554	6.087
A. pisum	3	25	0.00279	2.790	0.112	2063344	2579152	94970	67235	4.480	0.165	0.117	0.282	4.762
A. pisum	3	37	0.00464	4.640	0.125	700864	1725321	96141	54938	5.305	0.296	0.169	0.465	5.770
A. pisum	3	25	0.00306	3.060	0.122	614581	1148146	64612	30501	6.105	0.344	0.162	0.506	6.611
A. pisum	3	35	0.00445	4.450	0.127	581433	1353291	70439	44013	5.230	0.272	0.170	0.442	5.673
A. pisum	3	52	0.00673	6.730	0.129	575403	1939068	121539	63411	5.007	0.314	0.164	0.478	5.485
A. pisum	3	40	0.00483	4.830	0.121	303059	1021890	63812	14746	6.981	0.436	0.101	0.537	7.518
A. pisum	3	26	0.00265	2.650	0.102	2063087	5761491	232151	188393	10.538	0.425	0.345	0.769	11.308
A. pisum	3	56	0.00763	7.630	0.136	5543204	24931342	912295	768749	5.895	0.216	0.182	0.397	6.292
A. pisum	3	43	0.00506	5.060	0.118	3449533	16805878	1032472	659701	9.628	0.592	0.378	0.969	10.598
A. pisum	3	30	0.00369	3.690	0.123	951696	6057661	285095	157485	17.250	0.812	0.448	1.260	18.510
A. pisum	3	43	0.00569	7.020	0.163	1439286	6746169	938068	420397	6.677	0.928	0.416	1.345	8.021
A. pisum	3	28	0.00294	8.020	0.286	4332770	11878346	488542	1312779	3.418	0.141	0.378	0.518	3.937
A. pisum	3	28	0.00352	3.520	0.126	2332202	7584249	508397	423704	9.239	0.619	0.516	1.135	10.374
A. pisum	3	42	0.00467	4.670	0.111	6605778	15897806	832796	606576	5.153	0.270	0.197	0.467	5.620
A. pisum	4	25	0.00278	2.780	0.111	2807006	3535280	165215	95765	4.530	0.212	0.123	0.334	4.865
A. pisum	4	26	0.00336	3.360	0.129	601097	1008850	51583	43390	4.995	0.255	0.215	0.470	5.465
A. pisum	4	28	0.00325	3.250	0.116	4259109	8497605	377613	224588	6.139	0.273	0.162	0.435	6.574
A. pisum	4	43	0.00565	5.650	0.131	2931049	23351225	731994	437764	14.101	0.442	0.264	0.706	14.807
A. pisum	4	28	0.00347	3.470	0.124	7504101	18406626	1078289	703797	7.069	0.414	0.270	0.684	7.753
A. pisum	4	25	0.00320	3.200	0.128	1120047	8540833	423175	233663	23.829	1.181	0.652	1.833	25.662
A. pisum	4	26	0.00288	2.880	0.111	1402801	6402118	592312	506603	15.847	1.466	1.254	2.720	18.567
A. pisum	5	37	0.00435	4.350	0.118	1932381	4415298	201258	123543	5.253	0.239	0.147	0.386	5.639
A. pisum	5	26	0.00301	3.010	0.116	3127774	7385051	412144	322614	7.844	0.438	0.343	0.780	8.625
A. pisum	5	51	0.00624	6.240	0.122	1512311	10501534	441319	268219	11.128	0.468	0.284	0.752	11.880
A. pisum	5	35	0.00411	4.110	0.117	8696074	24544721	1412381	917501	6.867	0.395	0.257	0.652	7.519
A. pisum	5	27	0.00320	3.200	0.119	3529374	9792116	550557	381998	8.670	0.487	0.338	0.826	9.496
A. pisum	5	28	0.00314	3.140	0.112	5554259	12652578	544091	375662	7.255	0.312	0.215	0.527	7.782
A. pisum	5	30	0.00333	9.020	0.301	3978092	8329581	704484	682514	2.321	0.196	0.190	0.387	2.708
A. pisum	5	28	0.00293	2.930	0.105	2614564	9667693	468871	374609	12.620	0.612	0.489	1.101	13.721
A. pisum	6	25	0.00307	3.070	0.123	2654047	3407170	169612	103739	4.182	0.208	0.127	0.335	4.517
A. pisum	6	44	0.00525	5.250	0.119	5462269	13322740	631316	387993	4.646	0.220	0.135	0.355	5.001
A. pisum	6	25	0.00312	3.120	0.125	2541295	11011589	723413	438914	13.888	0.912	0.554	1.466	15.354
A. pisum	6	29	0.00363	3.630	0.125	1733874	8433644	418400	221948	13.400	0.665	0.353	1.017	14.417

A. pisum	7	31	0.00383	3.830	0.124	2421107	3689226	157576	128305	3.979	0.170	0.138	0.308	4.287
A. pisum	7	29	0.00338	3.380	0.117	6990289	12230327	751231	440505	5.176	0.318	0.186	0.504	5.681
A. pisum	7	28	0.00340	3.400	0.121	5753874	8894517	405030	265319	4.547	0.207	0.136	0.343	4.889
A. pisum	7	30	0.00381	3.810	0.127	1612563	14036978	925422	562811	22.847	1.506	0.916	2.422	25.269
A. pisum	7	44	0.00539	5.390	0.123	8225628	24264885	1056296	640827	5.473	0.238	0.145	0.383	5.856
A. pisum	8	66	0.00815	8.150	0.123	2743185	8704097	426473	299186	3.893	0.191	0.134	0.325	4.218
A. pisum	8	28	0.00316	3.160	0.113	5602835	12360580	691401	431012	6.981	0.391	0.243	0.634	7.615
A. pisum	8	30	0.00341	3.410	0.114	6248086	10933067	454706	295105	5.131	0.213	0.139	0.352	5.483
A. pisum	8	28	0.00326	3.260	0.116	2490480	7593512	355148	231634	9.353	0.437	0.285	0.723	10.076
A. pisum	9	41	0.00455	4.550	0.111	2370858	16111553	1063508	648223	14.936	0.986	0.601	1.587	16.522
A. pisum	9	30	0.00354	3.540	0.118	1984474	7718347	373816	264357	10.987	0.532	0.376	0.908	11.895
A. pisum	10	26	0.00311	3.110	0.120	2991732	8955018	446703	239508	9.625	0.480	0.257	0.738	10.362
A. pisum	11	35	0.00425	4.250	0.121	1585970	13979640	855329	557479	20.740	1.269	0.827	2.096	22.836
A. pisum	11	25	0.00245	2.450	0.098	5166024	9690303	401267	195293	7.656	0.317	0.154	0.471	8.128
A. pisum	12	36	0.00436	4.360	0.121	3550269	18703572	1169726	707107	12.083	0.756	0.457	1.212	13.296
A. pisum	12	27	0.00300	3.000	0.111	2394790	9279310	443166	231452	12.916	0.617	0.322	0.939	13.855

SUPPORTING INFORMATION: HOW DIET LEADS TO DEFENSIVE DYNAMISM: EFFECT OF THE DIETARY QUALITY ON AUTOGENOUS ALKALOIDS RECOVERY RATE IN A CHEMICALLY DEFENDED BEETLE

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Table S1 and S2

Electronic Supplementary Material Excel file (Tables S1, S2)

Table S3 Results of two-way ANCOVA analyses on the concentrations of individual alkaloids extracted from adult *A. bipunctata* after reflex bleeding

Dependent Variable	Fixed Variable	*df*	*F*	*P*
Adaline	Diet	1	0.334	0.564
	Sex	1	11.059	**0.001**
	Diet*Sex	1	0.281	0.597
	Diet*Time	1	4.162	**0.043**
	Sex*Time	1	3.423	0.066
	Diet*Sex*Time	1	0.721	0.397
Adalinine	Diet	1	1.804	0.181
	Sex	1	1.511	0.221
	Diet*Sex	1	0.001	0.980
	Diet*Time	1	9.378	**0.003**
	Sex*Time	1	9.341	**0.003**
	Diet*Sex*Time	1	3.364	0.059

Bold letters indicate a significance of (P < 0.05). Number of analyzed adult pairs of each aphid diet: A. pisum - *n = 38;* A. fabae - *n = 39. N = 154.*

Table S4 Results of two-way ANCOVA analyses on the concentrations of individual alkaloids extracted from adult *A. bipunctata* eggs after reflex bleeding

Dependent Variable	Fixed Variable	*df*	*F*	*P*
Adaline	Diet	1	1.042	0.309
	Diet*Time	1	1.288	0.258
Adalinine	Diet	1	1.367	0.245
	Diet*Time	1	0.201	0.654

Number of analyzed egg samples from each female aphid diet: A. pisum - *n = 72;* A. fabae - *n = 59. N = 131.*

Figures

Fig. S1 Scatter graphs of Pearson Correlations between analyzed alkaloid content. Undegraded and degraded adalinine for adults (a), and eggs (b). Adaline and adalinine (combination with degraded adalinine) alkaloids of adult (c), and eggs (d). Units µg nicotine equivalent/mg wet mass ladybirds. Adult *N* = 154 individuals, Eggs *N* = 131 samples

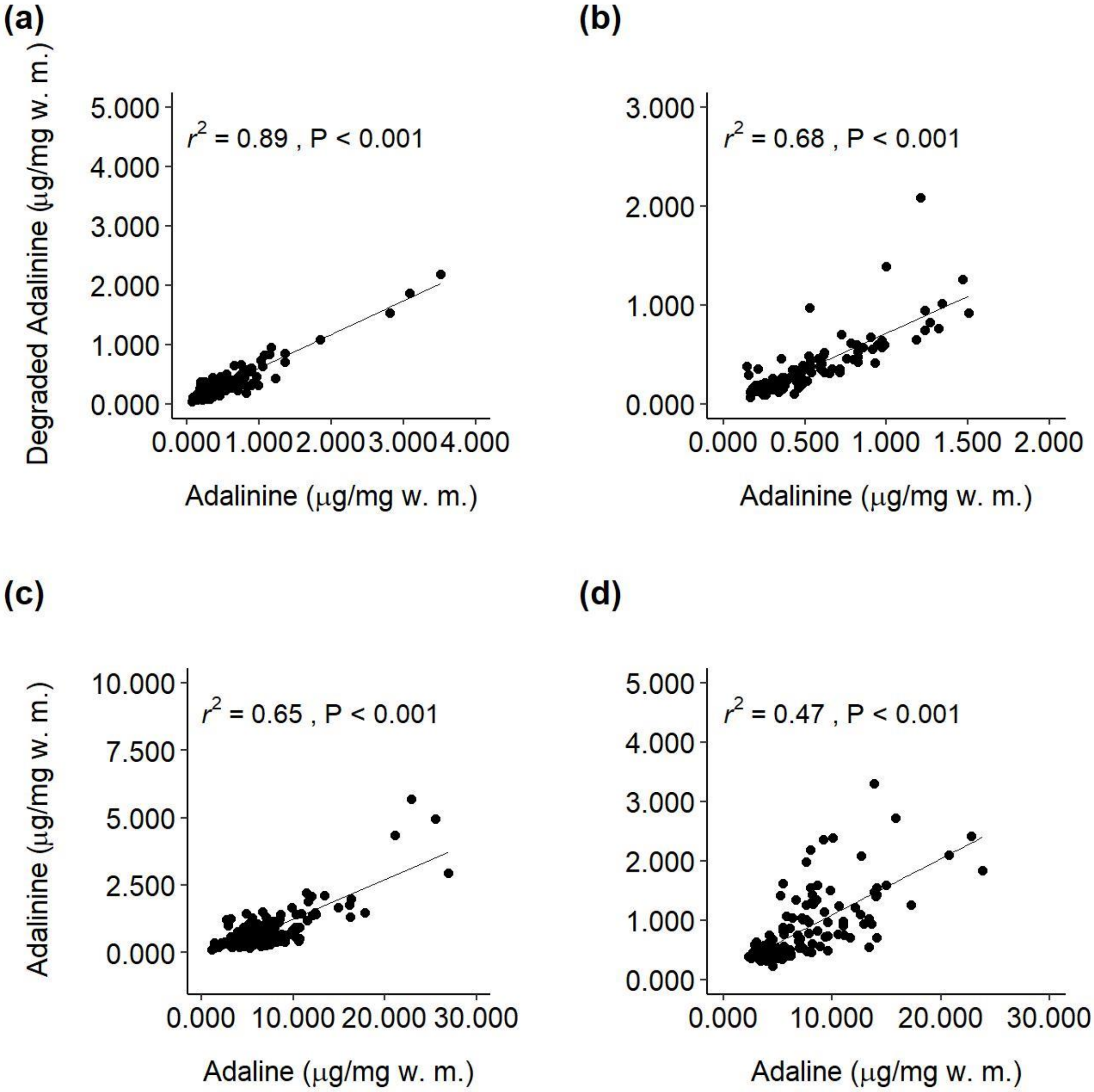

Fig. S2 Mean ±SE of adaline concentration (re)accumulation of adult *A. bipunctata* over 12 days after reflex bleeding for male (a) and female (b) adult ladybirds with different aphid diets (*A. fabae* and *A. pisum*). Unit μg nicotine equivalent/mg wet mass ladybirds. For each sampling day and treatment 6 or 7 pairs were analyzed. Pearson Correlation Analysis: Male - *A. pisum*, $r^2 = 0.26$, $n = 38$, $P < 0.001$; *A. fabae*, $r^2 = 0.19$, $n = 39$, $P = 0.0058$; Female - *A. pisum*, $r^2 = 0.091$, $n = 38$, $P = 0.066$; *A. fabae*, $r^2 = 0.28$, $n = 39$, $P = 0.31$. Data for each diet are offset horizontally to facilitate interpretation of error bars

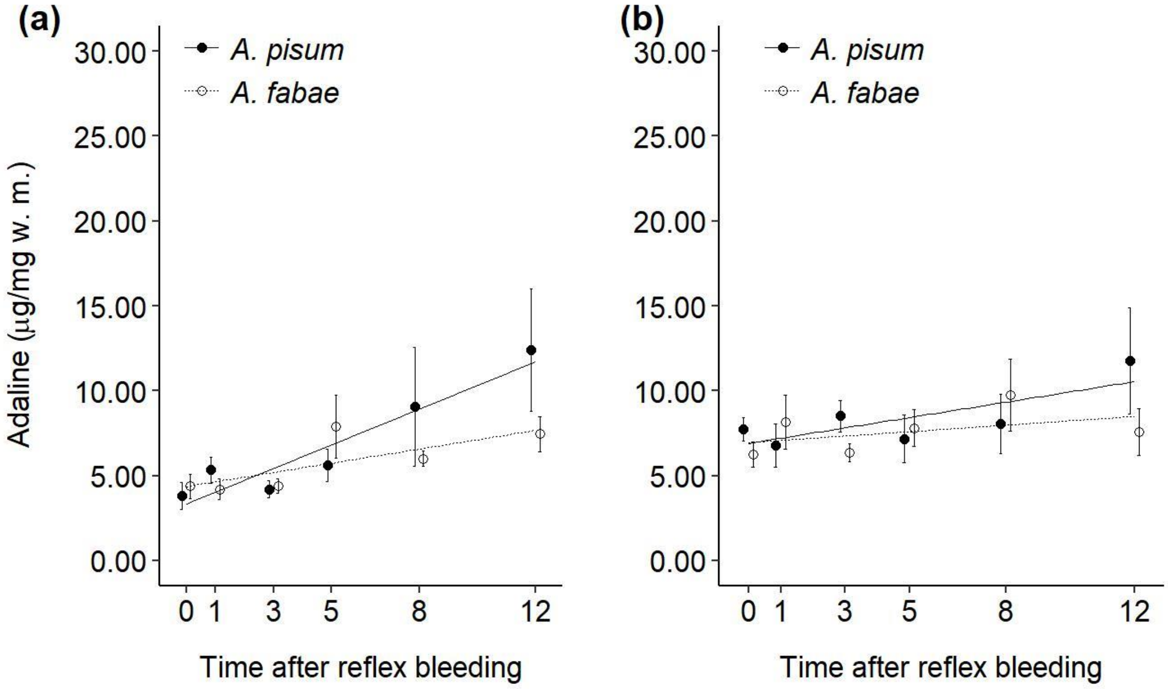

Fig. S3 Mean ±SE of adalinine concentration (re)accumulation of adult *A. bipunctata* over 12 days after reflex bleeding for male (a) and female (b) adult ladybirds with different aphid diets (*A. fabae* and *A. pisum*). Unit μg nicotine equivalent/mg wet mass ladybirds. For each sampling day and treatment 6 or 7 pairs were analyzed. Pearson Correlation Analysis: Male - *A. pisum*, $r^2 = 0.32$, $n = 38$, $P < 0.001$; *A. fabae*, $r^2 = 0.18$, $n = 39$, $P = 0.0078$; Female - *A. pisum*, $r^2 = 0.12$, $n = 38$, $P = 0.030$; *A. fabae*, $r^2 = 0.017$, $n = 39$, $P = 0.42$. Data for each diet are offset horizontally to facilitate interpretation of error bars

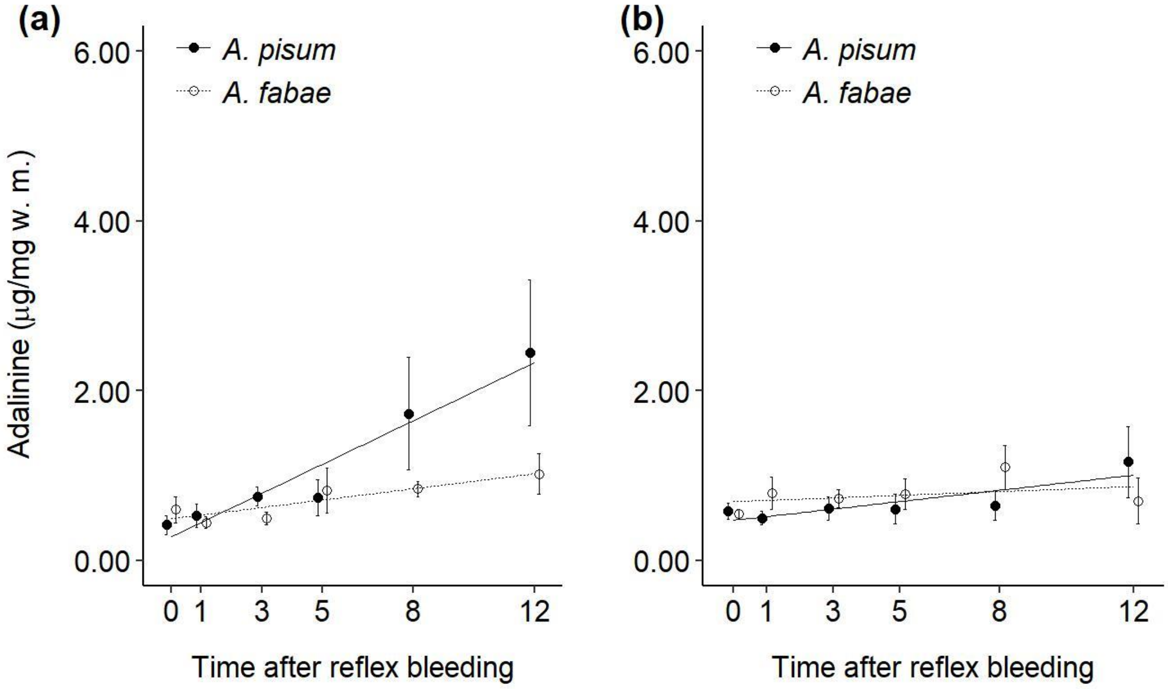

Fig. S4 Mean ±SE of individual alkaloid concentration in eggs over 12 days after reflex bleeding of females. Unit μg nicotine equivalent/mg wet mass ladybirds. (a) Adaline, (b) Adalinine. *A. pisum*; $n = 72$, *A. fabae*; $n = 59$. Pearson Correlation Analysis: Adaline - *A. pisum*, $r^2 = 0.065$, $P = 0.030$; *A. fabae*, $r^2 = 0.022$, $P = 0.26$; Adalinine - *A. pisum*, $r^2 = 0.019$, $P = 0.25$; *A. fabae*, $r^2 = 0.0038$, $P = 0.64$. Data for each diet are offset horizontally to facilitate interpretation of error bars