Influences of secondary metabolites on the performance of lepidopterous larvae

Achyut Aryal[1], Li-Ching Guan[2], Wei Xu[3]
Faculty of Forest and Environment Science, Albert Ludwigs University of Freiburg, Germany

ABSTRACT Plant secondary metabolites play an important role of influencing the performance of insect. The toxic secondary metabolites may have negative effect to the herbivorous insect. In this study, we tried to figure out what are the influences of secondary metabolites on growth and fitness of lepidopterous larvae. We used the larvae of *Creatonotos transiens* as test insect and caffeine as the supplement of secondary metabolites. In our experiment, female of *C. transiens* larvae had better performance on caffeine contained artificial diet. The larvae in low concentration of caffeine group had higher efficiency of conversion of food than control group. The larvae in high concentration of caffeine group eat more to compensate the energy loss in the food. It refers to high concentration of caffeine decreases the food quality. The caffeine has negative effect to herbivorous insect in general. But from our experiment, *C. transiens* female had good performance responding to low concentration of caffeine. This might be able to be the reference resource for the further research.

KEY WORDS Performance, fitness, secondary metabolites, lepidopterous larvae, caffeine, *Creatonotos transiens*

Introduction

Plant chemistry is considered to be the most important factor affecting the performance of herbivorous species. Both nutritional and secondary metabolized compounds of plant are related to the fitness of herbivorous insect. The nutritional component, such as carbon and nitrogen, are related to the insect growth and development. The secondary metabolized compounds are considered as the defensive compounds which affect the herbivore performance. Plants may use two kinds of defensive metabolites based on the plant species and apparency (Fenny, 1976). Some

1 savefauna@yahoo.com
2 unpay@hotmail.com
3 chris425616@hotmail.com

defensive metabolites are primary toxic, such as glycosides (crucifer) or caffeine, are present related with its quality. Whereas others have antifeedant and/or repellent properties, such as tannins (oak), which present related with its quantity. (Feeny, 1976; Awmack and Leather, 2002).

Growth and Fitness of herbivorous insect is directly related to food quality and secondary metabolites. In many herbivorous insects, measurements of pupal and adult weight, size, are strongly correlated with potential fitness. Both quality and quantity of defensive components of plant may directly or indirectly affect the fecundity and oviposition of herbivorous insects. Most of the secondary metabolites have negative effects on the growth and development of generalist herbivores. Higher concentration of toxic secondary metabolites and lower food quality will decrease growth and fitness of Lepidoptera insect. For some specialist herbivorous, the coevolution between plant and herbivorous leads to specialist herbivorous may adapt to the secondary metabolites (Ehrlich and Raven, 1964; Awmack and Leather, 2002).

The experiment was carried out to know the specific performance and its response to different food quality food supplements. Insects feed on a remarkably diverse list of organic substance. At the same time most species show a high degree of specificity in their choice of food. Gordon (1959) hypothesized; competition and natural selection gradually drive and bind each species to a specialized food supply that it can utilize more efficiently than any of its competitors. We are trying to find out: what amount of food is eaten by larvae? What is the response of larvae with different concentration of food specially caffeine content food.

We test the performance of *Creatonotos transiens* by feeding artificial diets which contain different concentration of caffeine. The objective of our study is to analyze influence of secondary metabolite on the performance of Lepidoptera larvae and pupae.

Materials and Methods

Source of *C. transiens*. *C. transiens* larvae were provided by Institute of Forest Zoology of Albert-Ludwigs University of Freiburg. *C. transiens* belongs to the Artiidae family and distribute in the Southeast of Asia. The larvae have a wide range of host plants. They are polyphagous species which had previously reared successfully on European substitute food plant such as *Taraxacum officinale* L (Bergomaz and Boppré, 1986). But now there is still little research about this species.

Composition and preparation of diets. The food compositions of artificial diet used in this study are listed in table 1 and 2. In this study, caffeine ($C_8H_{10}N_4O_2$, MW: 194.20 g/mol, Merck cop.) was used as secondary metabolites supplement. Three treatments were experimented for the feeding trails, control group, low concentration caffeine supplementation and high caffeine supplementation. To prepare diet of control, agar and diet powder were added into 238.16ml boiled water in 2 liter beaker and mix with mixer. After completely mixture, pour into 50 wells food container (Fig.1). The low and high concentration caffeine supplementation diets were prepared similar as control group. The 207.26ml boiled water was prepared. The dissolved caffeine were added between first and last half diet powder added, which the lower temperature doesn't destroy the caffeine.

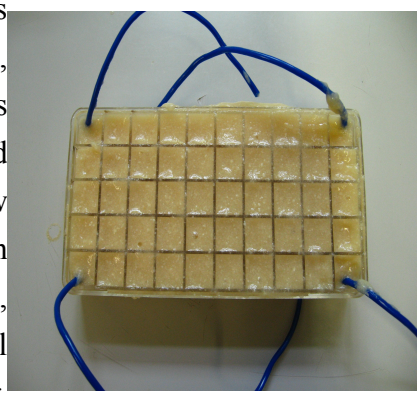


Fig. 1 50 wells food container

Table 1 Diet powder composition and agar. For simple preparation, 6 parts of I, 1 part of agar and 20 parts of water are combined. (Berrgomaz and Boppré, 1968)

Composition	Amount	
	%	g
Bean flour	15.0	75.0
Brewer's yeast	3.5	17.5
Ascorbic acid	0.7	3.5
Cholesterol	0.1	0.5
Sorbic acid	0.1	0.5
Menthyl-p-hydroxybenzoate	0.1	0.5
Streptomycin	0.08	0.4
p-formaldehyde	-	0.15
Formaldehyde (10%)	0.3	-
Germ oil with α-tocopherol	0.7	-
Agar	3.0	15.0
Water	76.0	381.0

Table 2 Three treatment of artificial diet composition for *C. transiens*

Composition	Types of food		
	Control group	Low con. Group	High con. Group
		(10^{-4} mol/l)	(10^{-5} mol/l)
Diet powder	35.57g	35.57g	35.57g
Agar	6.81g	6.81g	6.81g

Caffeine	-	30.9ml	30.9ml
Water	238.16ml	207.26ml	207.26ml

Feeding trails of *C. transiens*. To evaluate the food consumption and growth of *C. transiens* of different concentration caffeine supplement, 20 seventh instar larvae were fed individually in plastic container in each treatment. Each larva was fed with one piece of diet. All the larvae were placed in the breeding room at 22℃, with photoperiod 12:12(L:D)h condition. We changed food every day until they started to pupate. The rest food and feces were collected everyday and dried in 40℃ oven for 24 hours. Each pupa and the total rest food and feces for individual were weighted at the end of our experiment and used to evaluate insect growth, food consumption and food utilization efficiency.

Sex identification. We identify the sex of pupae by using the characteristic of pupae. The characters of female and male are shown in figure 2.

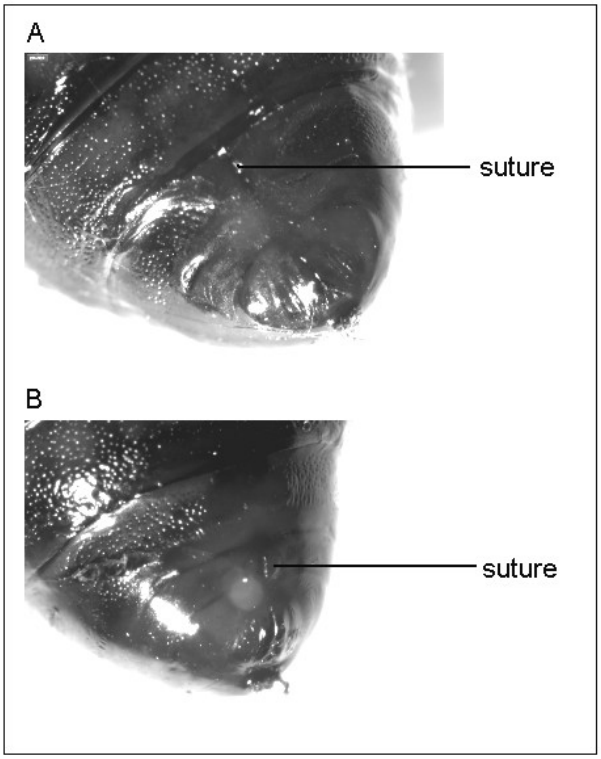


Fig. 2 Micrographs of C. transiens pupae. A. Female pupa. The indication of the suture in the third segment at the end that is female specific; B. Male pupa. The indication of the suture in the second segment at the end

Data analysis process. We used following steps to get our results. At the beginning, we collected original data of four important performance parameters of the insects in our experiment: duration of feeding period, consumption of food, dry weight of feces

and fresh pupal weight. Secondly, we calculated the index related to food quality using modified formulas from the very famous Waldbauer indices (E.C.I, E.C.D) (Waldbauer, 1968).Based on the data, we got the results of our experiment to show how the caffeine influences the performance of the insects.

Results

Performance parameters. Fig. 3 shows the deviation of these three parameters from the control group, we set the mean of these three parameters of the control group as 100% and use these levels as base line. We found that in both concentration of caffeine, the value of the intake of food, dry weight of feces and pupal weight are all higher than the control group. Larvae which taking up caffeine eat more food, produce more feces and grow better than the control group.

It seems a little bit ridiculous and unexpected. So we tried to use SPSS to show the relationship between these parameters and insect gender. From the one-way ANOVA analysis (Table 3), it shows there are significant differences of these three parameters in relation to the gender of the insects. Then we can say that gender is an influence factor for the performance of insects.

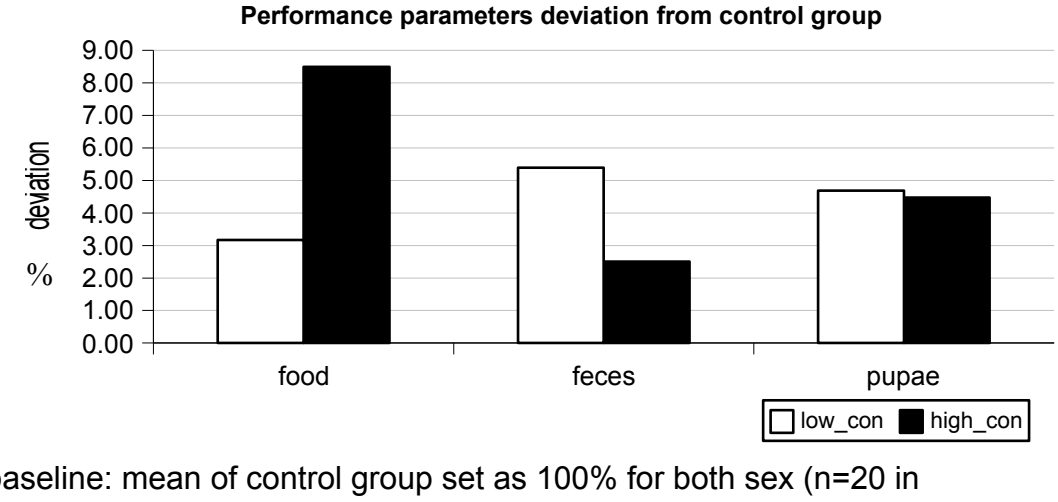


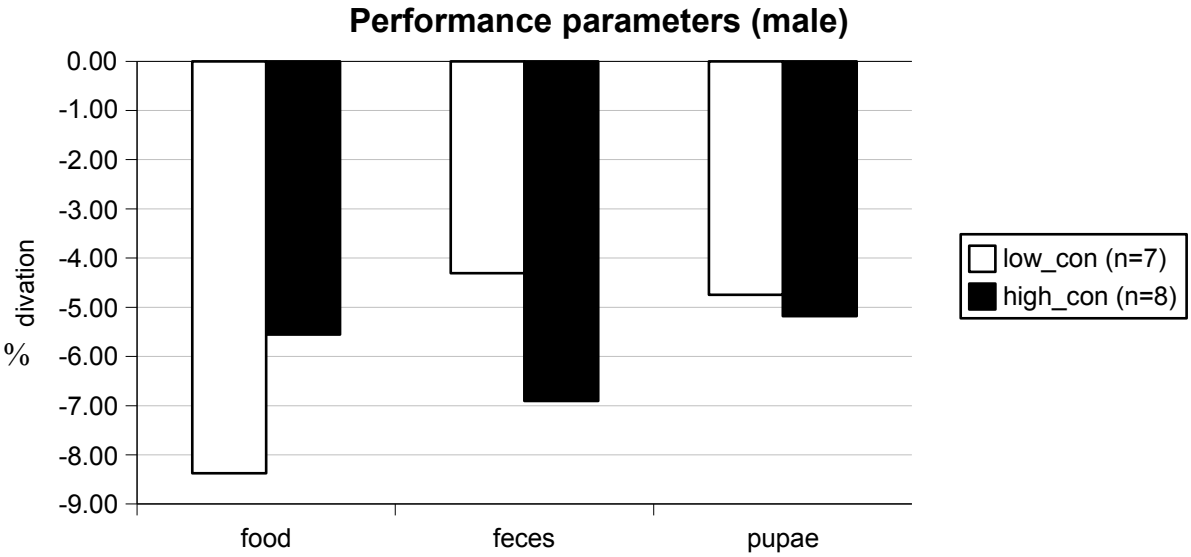
Fig. 3 performance parameters deviation from control group

Table 3 significance of three performance parameters in relation to gender (one-way ANOVA, SPSS)

ANOVA

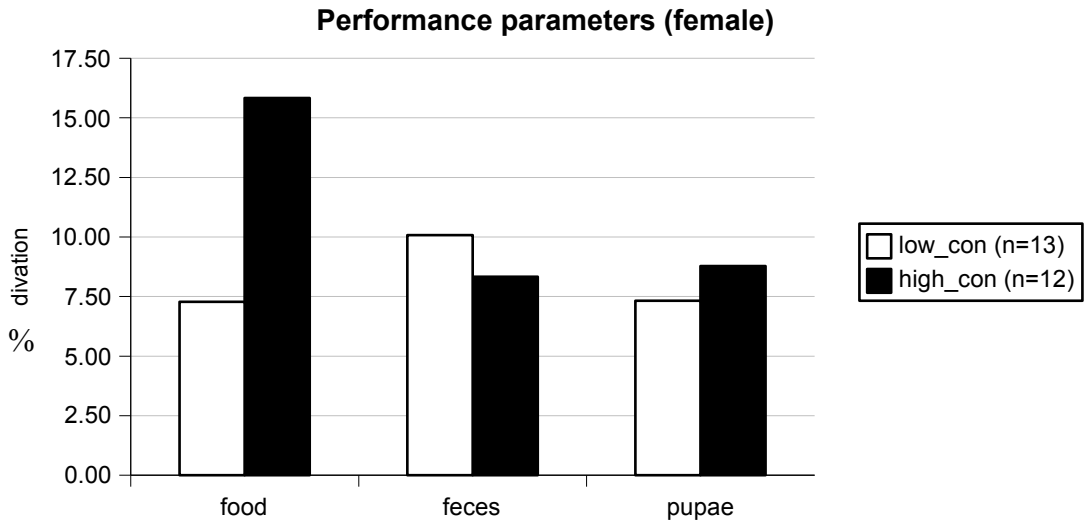
	Sum of Squares	df	Mean Square	F	Sig.
fresh_wt of pupae	.190	1	.190	37.207	.000
consumption of food	.410	1	.410	25.258	.000
dry_wt of feces	.112	1	.112	13.475	.001

Afterwards, we tried to figure out how different are males and females performance with the supplement of caffeine. We separated the data by male and female and compared their performance parameters in three groups. Compared Fig 4 and Fig 5, we found that males and females response to caffeine in a totally different way.



baseline: mean of control group set as 100% (n=11)

Fig. 4 performance parameters deviation from control group (male)



baseline: mean of control group set as 100% (n=9)

Fig. 5 performance parameters deviation from control group (female)

Duration of feeding period. We calculated the duration using the date when the pupation finished and the date when we started feeding them. There is following of duration calculation:

Duration= date of pupation finished- date of feeding started

From this graph, it is clear that the average duration in three groups is different.

Larvae in high concentration group need longer time to finish pupation steps. However, we used SPSS to check our idea and found that there is no statistically significant difference of duration in three groups (Table 4). Then we say caffeine is not an influence factor for duration of feeding period.

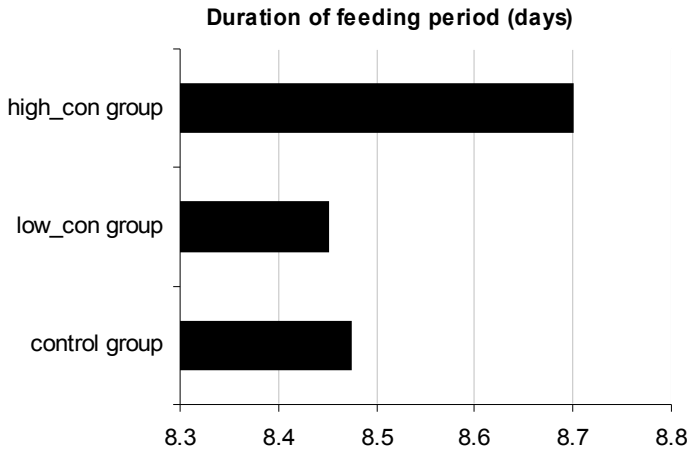


Fig. 6 average duration of feeding period for three groups

Table 4 Turkey HSD Test of average duration of feeding period of three groups (SPSS)

Tukey HSD

		Subset for alpha = .05
	1	1
Low_con group	20	8.45
Control group	20	8.50
High_con group	20	8.70
Sig.		.503

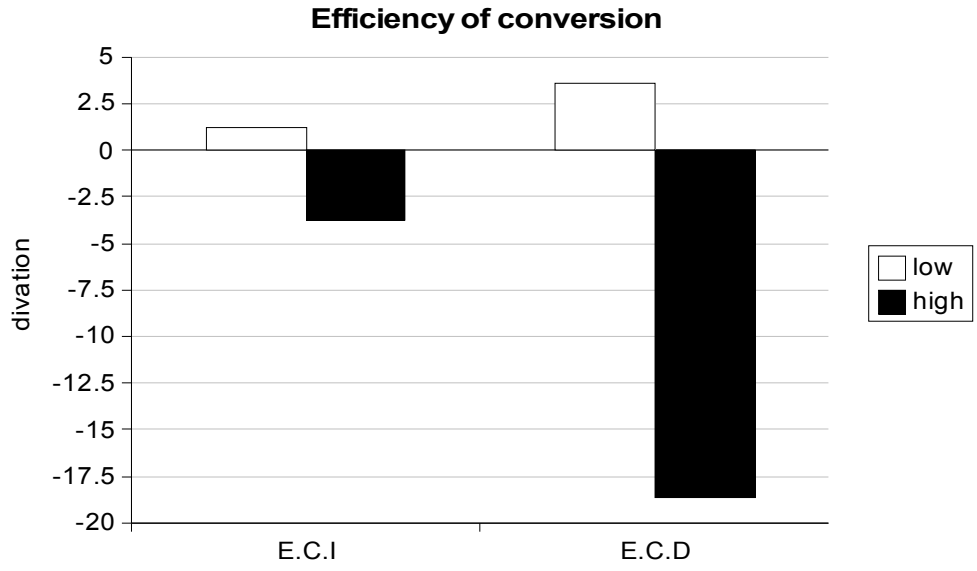
Food quality. In our calculation, we modified the Waldbauer indices of E.C.I and E.C.D as indicators of the food quality.

$$E.C.I = \frac{pupal\ weight}{weight - ingestedfood} \times 100$$

E.C.I means the efficiency of conversion of ingested food to one unit of body substance.

$$E.C.D = \frac{pupal\ weight}{wt\ \ of\ \ food\ \ ingested - wt\ \ of\ \ feces} \times 100$$

E.C.D means the efficiency of the conversion of digested food to one unit of body substance. These two indices show the efficiency of the conversion and are usually used as indicator for food quality.



baseline: mean of control group set as 100%

Fig. 7 efficiency of conversion of food to body substance

From Fig. 7, high concentration of caffeine has the lowest efficiency of conversion and larvae in this group have to eat more to compensate the energy loss in the food. We can say that high concentration of caffeine decrease the food quality and influence the fitness of insects. Low concentration of caffeine seems have better efficiency than control group, and we also found that females in low concentration of caffeine have better performance than control group in our experiment, it seems that a small amount of caffeine is helpful for the performance of the insects in our experiment, especially for females, however, we cannot find relative references for reasonable explanation.

Discussion

In our study, it was clearly demonstrated that the performance of *C. transiens* varied significantly among different concentration of caffeine, especially males and females of the larvae response to the caffeine in a different way. In addition, our result also indicates that artificial diet which has caffeine content seems have the positive effect to larvae. But the actual interaction between *C. transiens* and caffeine in natural is still unclear.

In many specialist insect, they have ability to utilize the plant secondary metabolite which some are toxic due to the coevolution. In nature, low levels of iridoid glycosides acts as a feeding stimulus for *Junonia coenia*, but larval survivorship decreases when *J. coenia* was reared on plants with increasing glycosides

concentration (Berenbaum and Zangerl, 1998). We are still not sure the host plant of *C. transiens*. And we also have no idea about the physiological function *C. transiens* between female and male.

However, we found several errors or limitation in our experiment. First, we didn't get the original data of the weight gained by the larvae during the feeding period, which is an important data used to calculate the growth rate of the insect. We used the fresh pupal weight as indicator of the growth of the insect and could cause some errors in our results. Secondly, we didn't get the accurate data about duration of feeding period due to our limitation on working hours, which may also influence the accuracy of our results. Third, when we collected the feces of the larvae, we ignored some of them when the larvae started to pupate and we are not very sure about the time of drying the feces in the oven (24hrs) is enough or not.

For further study related to our topic, we would like to suggest that one of the important things is to try to figure out the accurate caffeine concentration in natural plants and use proper concentration of caffeine when designing experiment. Another thing is to distinguish the different performance of female and male of the insect in experiment. It is better to do separate experiment for males and females. Last but not least, try do reduce the error or mistake in the experiment and make better results.

Acknowledgment

We would like to acknowledge Dr. Tim Burzlaff for his supervision in the experiment. We would also like to express our thanks to Anita and other staffs of Institute of Forest Zoology, who help us distinguish the sex of the pupae, diet preparation and other laboratory works.

Reference

Awmack CS, Leather SR. 2002. Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol*. 47:817-844.

Berrgomaz, R, Boppre M. 1986. A simple instant diet for rearing Arctiidae and other moths. *Journal of The Lepidopterists' Society*. 40(3): 131-137.

Berenbaum MR, Zangerl AR. 1998. Chemical phenotype matching between a plant and its insect herbivore. *Proc. Natl. Acad. Sci*. USA 95:13743-48

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586-608.

Feeny P. 1976. Plant apparancy and chemical defense. *Rec. Adv. Phytochem*., 10:1-40.

Gordon HT. 1959. Minimal nutritional requirements of the German roach, *Blattella germanica L.Ann.* N.Y. *Acad. Sci.* 77. 290-351.

Waldbauer GP. 1964. The consumption, digestion and utilization of Solanaceous and non-Solanceous plants by larvae of the tobacco hornworm, *Protoparce sexta* (johan.) (Lipedopter: Sphingidae). *Ent. Exp*.appl.7, 253-269.

Waldbauer GP. 1968. The consumption and utilization of food by insect. *Adv. Insect phsiol*. 5: 229-288.