

The Effect of Nutritional Quality of Some Plant's Leaf on the Feeding and Development of *Hyphantria cunea* **(Drury) (Lepidoptera: Arctiidae)**

Beran FİRİDİN^{1*} Mahmut BİLGENER² Oğuzhan YANAR¹ Nurver ALTUN² İkbal Agah İNCE¹

¹ Department of Biology, Giresun University, 28049 Giresun, Turkey

2 Department of Biology, Ondokuzmayıs University, 55060 Samsun, Turkey

ABSTRACT

The fall webworm, *Hyphantria cunea* is a polyphagous herbivorous moth, damaging forests and agricultural crops. It has a great economical importance with damaging on especially hazelnut fields in TURKEY. Effects of food quality on feeding and development in larval stage were investigated. The last instar *H. cunea* larvae were reared during 14 days-feeding experiment using the leaves of *Carpinus orientalis* Mill., *Quercus cerris* L., *Corylus maxima* Mill. and *Catalpa bignonioides* Walt. Especially, the effect of some chemical compounds present in different plant species that used in current study on the feeding and development of the larvae was analyzed. Firstly, it was determinated that *H. cunea* larvae ingested both *Q. cerris* and *C. orientalis*, had high total protein content, less than both *C. bignonioides* and *C. maxima*, and had lower total protein content than the former plant species. However, there is no relation found between the ingestion of the selected plants and development of the larvae. This might be to result from more or less limitation of phenolic compounds such as tannins on protein digestion. On the other hand, there is no significant relation between digestion efficiency of the larvae and water content of the leaf samples($r = 0.468$, $p < 0.01$). The chemical analysis demonstrated that the total phenolics and proanthocyanidin (condensed tannin) contents were higher in *Q. cerris* and *C. orientalis* than *C. bignonioides* and *C. maxima*. This may caused lower pupal mortality in the larvae fed on *Q. cerris* and *C. orientalis* than the larvae fed on *C. bignonioides* and *C. maxima*. This may be due to the protection of larvae and pupae by tannins against viral and / or bacterial infections. Furthermore, the pupation time of the fall webworm were negatively correlated by the nitrogen contents of the leaf samples of food plants (r = -0.889, p<0.01). It was determined that the larvae fed on both *C. orientalis* and *Q*. *cerris* , had high ratio of protein content, completed the last instar of larval stage in shorter time period than the other two plants.

Key words: *Hyphantria cunea*-larvae-pupa-tannin-phenolic-protein-AD-ECD-ECI

INTRODUCTION

Many herbivorous insects are rather specific in their host utilization [1,2,3,4,5-6]. This appears to be largely due to the fact that each insect species is adapted to a specific subset of a myriad of secondary compounds present in the plant kingdom. For many insect herbivores secondary metabolites of regularly used host plants are essential feeding and oviposition stimulants [5,7,8,9,10,11-12]. In contrast, secondary constituents of nonhost plants frequently act as deterrents and inhibitors for feeding and oviposition [10,13,14,15-16]. These substances may also inhibit insect growth and development [15,17]

Food quality, more than quantity, determines the distribution and abundance of many phytophagous insects[18]. Plant quality for herbivores is determined by the nutrient and water content of plant tissue as well as by the concentrations of secondary metabolites [19]. Vegetal tissues constitute lowquality food because they usually contain low levels of nitrogen and combine this nutritional poverty with poisons, digestion inhibitors or indigestible materials [18,20-21]. Thus, adults, larvae, or both, must recognize the best foods available and choose them for oviposition or feeding [22]. Consequently, many plant secondary compounds, especially tannins, effect to the insect feeding behaviour [23]. The hydroxyl groups which take place in the tannin's molecule are effective in forming complex with natural compounds, so tannins form complex with natural compounds like nourishment protein, digestion enzyme, polysaccharides such as starch, cellulose, hemicellulose and oils, nucleic acid and amino acids [24,25,26-27]. Inhibition of kinetics of digestion enzyme by tannins was studied from Bilgener [28] with pepsin, pancreatic protease, αamylase and hemicellulose. After this study, its determined that this inhibition effected from chemical structure of tannins, *pH* degree of mixture and other food polymers such as cellulose

Hyphantria cunea (Drury) with common name of fall webworm, known to feed with 636 plant species all over the world, is a polyphagous herbivore moth [29]. In respect to this characteristic of *H. cunea,* which has protection mechanisms to withstand from deterrent effects of secondary metabolites come from in host plants, is to answered an important question. *H. cunea*, has such a vast host repertoire, due to the damage is given to forest and agricultural products, on this account it has a need to investigate species about the ecological viewpoint. In this study, it was aimed to investigate the chance in the content of protein, water, crude fiber and secondary metabolites in the *leaves* of *Carpinus orientalis*, *Quercus cerris*, *Corylus maxima* and *Catalpa bignonioides* offered as food onto fall webworm (*H. cunea)* larvae and the performance of this moth on these plants.

MATERIAL AND METHODS

Field study of *H. cunea*

A polyphagous herbivore moth, *H. cunea* occur in the Terme area in the vicinity of the city of Samsun in Northern Turkey. The relative digestibility and ingestibility of these four plant species, utilized the experiment, for *H. cunea* larvae were investigated in laboratory trails. *H. cunea* larvae for the experiments were mainly collected from *C. maxima* plantations in Terme, Samsun in August 2002.

A test study for feeding experiment was performed on collected larvae in the same year. Next season, *H. cunea* larvae occurred in Terme area and collected in the season 2003. All feeding experiment performed in this season simultaneously.

Feeding trials of *H. cunea* **larvae in laboratory**

The larvae were taken to the collection pot which is large and lightened. Then, 10 larvae that reach the last instar were weighed and separated to 10 feeding pots for each plant species to utilize the experiment. Fully expanded and undamaged leaves of approximately 5 to 7 years old naturally growing plants were given to 10 feeding pots for each day and plant species. After one day, weight of the larvae, amount of eaten leaf and faeces were recorded at the feeding performance data. Feeding trial process was continued until the larvae were turned the pupae. During the experiments, larvae were kept at room temperature (19-22° C), with diffuse light for 10-12 h per day. Ingestion rate was calculated as dry food ingested per fresh weight of larvae, and apparent digestibility as the difference between food ingested and frass produced with respect to the food ingested (in both cases mg mg-1 in 24 h, expressed as a percentage of weight).Larval growth was expressed as the percentage of fresh weight gain in 24 h. For calculations of dry weight, a parallel series of leaves was dried to determine to amount of dry matter per plant species.

of the four studied plant species were dried in a freeze-drier. Total phenolic content was extracted 0.4 g leaf dry powder with 10 ml of 50 $\%$ (v/v) methanol in an ultrasonic bath for 10 min. Total phenolic content was analyzed by the Folin-Ciocalteau method [30]. An aliquot was diluted with water and assayed with Folin-Ciocalteau phenol reagent and 20 % sodium carbonate, to reach a final concentration of 1 mg ml^{-1} , and absorbance was measured at 725 nm. Condensed tannins were analyzed by the Acid-Butanol assay [31]. To compare species, direct values of absorbance at 547 nm were used, without transformation to standard equivalents, In order to calculate proanthocyanidin concentration at the samples, value of ϵ = 150 was used for cyanidin (see Waterman & Mole, 1994, for a full explanation of this procedure) [32]. Gallotannins were analyzed by Potassium iodate assay [33]. In order to calculate gallotannin concentration at the leaf specimens, a standard slope was prepared with 0.1 -1 mg m l ⁻¹ of tannic acid. The nitrogen content was quantified in leaves using the Kjeltec Auto 1030 Analyzer (Tecator, Sweden).

Analysis of crude fiber and water

Crude fiber analysis of leaf and frass samples was made according to method of Morrison [34] modified by Bilgener [28]. In order to determine the water content of leaf and frass samples, the leaves and frass samples were dried at 80˚ C during 4 days.

Larval performance

Larval performance are measured AD (Apparent Digestibility) and ECD (Efficiency of Conversion of Digested food) values that known as Nutritional Indices. AD is formulized by $[(I - F) / I]$. ECD is formulized by $[G / (I - F)$. ECI is formulized by $[G / I]$ where I = weight of food consumed, F $=$ weight of faeces produced during the feeding period, $G =$ Biomass gain of the larvae [35].

Analysis of leaf secondary chemistry

In order to relate fall webworm host utilization patterns with leaf secondary chemistry, the sample of leaves from each

Table 1. Water, crude fiber, total protein, proanthocyanidin, gallotannin, and total phenolic contents of the leaf samples in different food plant species (%)

* Different letters indicate significantly different group means (p<0.05). (The groups abbreviated by a, b, c and d have statistically significant means according to Duncan's Multiple Range Test)

			Mean	SE	*Significant groups	Anova	
	Species	N				F	P
Ingestion rate $(\%)$	C. orientalis	14	58.41	2.91	b		
	O. cerris	14	53.18	1.49	$\mathbf c$		
	C. maxima	14	67.22	2.86	a		
	C.bignonioides	14	71.04	3.00	a	1185.90	${}< 0.01$
AD	C. orientalis	14	35.25	7.56	b		
	O. cerris	14	31.26	4.42	h		
	\overline{C} . maxima	14	56.20	3.71	a		
	C.bignonioides	14	61.32	5.00	a	1464.64	${}< 0.01$
ECD	C. orientalis	14	18.35	3.41	$\mathbf c$	1733.28	
	O. cerris	14	22.43	1.46	b		
	C. maxima	14	25.46	3.38	h		
	C.bignonioides	14	34.11	1.93	a		${}< 0.01$
ECI	C. orientalis	14	8.23	1.44	$\mathbf c$		
	O. cerris	14	7.30	1.57	$\mathbf c$		
	C. maxima	14	12.28	4.75	b		
	C.bignonioides	14	17.28	2.02	a	325.14	${}< 0.01$
Pupation time	C. orientalis	14	9.00	0.44	$\mathbf c$		
	O. cerris	14	8.62	0.10	$\mathbf c$		
	C. maxima	14	10.07	0.79	h		${}_{0.01}$
	C.bignonioides	14	11.51	0.78	a	5258.63	
Pupal weight (mg)	C. orientalis	14	778.20	94.94	h		
	O. cerris	14	852.00	41.04	a		
	C. maxima	14	512.80	20.95	$\mathbf c$	3113.40	${}< 0.01$
	C.bignonioides	14	546.40	21.84	$\mathbf c$		
Number of eggs	C. orientalis	8	116.60	15.07	a		
	O. cerris	5	102.60	10.23	ab		
	C. maxima	6	85.80	7.94	$\mathbf c$	1704.98	
	C.bignonioides	6	92.60	8.26	bc		${}_{0.01}$

Table 2. Larval performance of *Hyphantria cunea* (Drury) according to the performance parameters.

*Different letters indicate significantly different group means (p <0.05). (The groups abbreviated by a, b, c and d have statistically significant means according to Duncan's Multiple Range Test)

Statistical analysis

All experimental and chemical data were analyzed using ANOVA .The data on the water, crude fiber, total nitrogen, total phenolic content, proanthocyanidin and gallotannin contents of the leaf samples and pupal weight, ingestion rate, number of eggs laid by the larvae, AD, ECD, ECI values were evaluated by one-way variance analyses test. Significant differences among parameters were tested using the Duncan's Multiple Range Test. Statistical data analyses were performed by using SPSS 10 statistical software.

RESULTS

Chemical analysis of the leaf samples

There were significant differences in the water contents of the control leaf samples (Table 1, ANOVA, $F= 1726.75$, $p<0.01$). The water contents of the leaf samples were 45.59% in

C. orientalis, 54.98% in *Q. cerris,* 46.93% in *C. maxima* and 67.30%in *C.bignonioides*. The highest water content was obtained from the leaves of *C.bignonioides and* the lowest content from the leaves of *C. orientalis*. The total protein contents of the leaf samples were 13.18 % *C. orientalis*, 13.64 % in *Q. cerris,* 11.42 % in *C. maxima*, and 11.46% in *C. bignonioides*. *Q. cerris leaves had* the highest total nitrogen content *C. maxima had* the lowest. Protein contents of those two species was found to be different significantly (Table1, ANOVA, $F= 3099.21$, $p<0.01$). There were significant differences in the proanthocyanidin contents of the leaf samples (Table 1, ANOVA, $F= 3569.69$, $p<0.01$). The proanthocyanidin contents of the control leaves from *C. orientalis*, *Q. cerris*, *C. maxima* and *C. bignonioides* were 10.89%, 8.92%, 10.73% and 2.17

%, respectively. The gallotannin content of the plant samples observed in the present study was 3.12% for *C. orientalis,* 2.52% for *Q. cerris*, 2.48% for *C. maxima,* and 0.84% for *C.* bignonioides (Table 1, ANOVA, F=715.04, p<0.01). These results showed that *C. orientalis* had much higher gallotannin content in leaves than the other plant species. Total phenolic content of the leaves of *C. orientalis, Q. cerris*, *C. maxima,* and *C.bignonioides* was 9.72%, 8.95%, 7.17%, and 6.40%, respectively. Results from statistical data analysis revealed that *C. orientalis and Q. cerris* were different significantly in its total phenolic content from the other plants. (Table 1, ANOVA, $F= 2963.67$, p ≤ 0.001).

Feeding experiments

The ingestion rates of the larvae varied with the plant species; and significant differences were found among the ingestion rates of the larvae in the plant species (Table 2, ANOVA, $F = 1185.90$, $p<0.01$). However, ingestion rate of the larvae fed on leaves of *C. maxima* and *C. bignonioides were* statistically *similar.* The highest ingestion rate of the larvae was observed on *C. bignonioides* leaves, on the contrary, the lowest on *Q. cerris* leaves. The larvae fed on *C. bignonioides* had the highest apparent digestibility (AD) value and the larvae fed on *Q. cerris* had the lowest. In addition, there were significant differences between in the AD values of the larvae fed on *C. orientalis*, *Q. cerris and C. maxima*, *C. bignonioides* leaves $(Table 2, ANOVA, F= 1464.64, p<0.01).$

Significant differences were determined in ECD values of the larvae fed on the leaves from each plant species (Table 2, ANOVA, $F= 1733.28$, $p<0.01$). However, the lowest ECD value was obtained in the larvae fed on *C. orientalis;* the highest ECD value was obtained in the larvae fed on C. bignonioides (Table 2). The larvae fed on either *Q.cerris* or *C.maxima* had similar ECD values (Table 2).

On the other hand, *Q.cerris* produced the lowest ECI whereas C. bignonioides had the highest ECI and the ECI values belonging to the larvae were detected statistically significant in each host plant (Table 2, ANOVA, $F = 325,14$ p<0.01). The results of Duncan's Multiple Range Test of data obtained by the larval feeding experiments revealed that the larvae fed on the leaves of *C. orientalis and Q. cerris* had statistically similar ECI values (Table 2)*.*

The number of eggs laid by the females were statistically correlated with the protein contents of the leaves from different plant species (Figure 1, r=0.801, p<0.01). Significant differences were also found in the number of eggs laid by the females depending on their larval food sources (Table 2, ANOVA, F= 1704.98, p<0.001). Females fed on *C. orientalis and Q. cerris in the larval stage produced statistically similar amount of eggs* (Table 2).

The pupal weights of the fall webworm were inversely correlated by the nitrogen contents of the leaf specimens that larvae fed on (Figure 1, $r=0.998$, $p<0.01$). The highest pupal weights were obtained from the larvae fed on the leaves of *Q. cerris* and *C. orientalis* which had higher nitrogen contents than the other two food plant species. There were statistically differences between the pupal weights according to the larval food plant species (Table1, ANOVA, $F= 3113.32$, $p<0.01$).

Figure 1. Relationship with total protein content of the leaves in food plants and number of egg of the larvae (total protein contents were multiple by 10 and pupal weight reduced 10 times)

Figure 2. Relationship with total protein content of the leaves in food plants and pupation time of the larvae

The pupation time of the fall webworm were negatively correlated by the nitrogen contents of the leaf samples of food plants (Figure 2, $r = -0.889$, $p < 0.01$). On the other hand the pupation time of the fall webworm were not correlated by the water contents of the leaf specimens that larvae fed on (Figure $2, r= 0.680, p<0.01$).

The AD values of the larvae were negatively correlated by the proanthocyanidin and total phenolic contents of the leaf specimens that larvae fed on (Figure 3, $r = -0.821$, $p < 0.01$; $r =$ -0.780 , $p<0.01$ respectively).

The AD and ECD values of the larvae were not correlated by the crude fiber contents of the leaf leaf samples of food plants (Figure 4, $r = 0.248$, $p < 0.01$; $r = 0.579$, $p < 0.01$ respectively).

Pupal mortality rates of the larvae were 62%, 37%, 28%, 14% in the larvae fed by *C. bignonioides, C. maxima, Q. cerris,* and *C. orientalis* respectively (Figure 5).The pupal mortality of the fall webworm were negatively correlated by the total phenolic and gallotannin contents of the leaf samples of food plants (Figure 6, r= -0.945, p<0.01; r= -0.974, p<0.01 respectively).

The ingestion rate of the larvae were negatively correlated by the total protein and total phenolic contents of the leaf samples of food plants (Figure 7, $r = -0.973$, $p < 0.01$; $r = -0.787$, p<0.01 respectively).

Figure 3. Relationship with AD values of the larvae and total phenolic content, proanthocyanidin content of the leaf samples

Figure 4. Relationship among AD and ECD values of the larvae and crude fiber content of the leaf samples

Figure 5. Pupal mortality rate of the H. cunea according to the plant species.

Figure 6.Relationship among total phenolic content, gallotannin content of the leaf samples and pupal mortality of the larvae

Figure 7. Relationship with total phenolic content of the leaf samples and ingestion rate of the larvae

Figure 8. Relationship with gallotannin content of the leaf samples and ECD values of the larvae

The ECD values of the larvae were negatively correlated by the gallotannin contents of the leaf specimens that larvae fed on (Figure 8, $r = -0.979$, $p < 0.01$). The AD values of the larvae were not correlated by the water contents of the leaf leaf samples of food plants (Figure 9, $r = 0,468, p<0.01$). Similar to ECD and gallotannin, The ECI values of the larvae were negatively correlated by the proanthocyanidin contents of the leaf specimens that larvae fed on (Figure 10, $r = -0.951$, $p < 0.01$)

Figure 9. Relationship with water content of the leaf samples and AD values of the larvae

Figure 10. Relationship with proanthocyanidin content of the leaf samples and ECI values of the larvae

DISCUSSION

Phenolics and their oligomeric and polymeric relatives (tannins) continue to draw attention for their regulatory functions in intra-plant, herbivore-plant [36, 37- 38]. In present study, the highest ECD values were obtained with the larvae fed by C. *maxima and C. bignonioides* and were as 25.46 and 34.11 % respectively. The leaves of these two species had 2.48 and 0.84 % gallotannin contents respectively. Therefore, lower the gallotannin content of the leaves lead higher the efficiency of conversion of digested food to biomass (Figure 8, $r = -0.979$, p<0.01). As shown on Figure 1, there was a correlation between the protein content of food plants and the number eggs laid by the larvae $(r = 0.801, p<0.01)$ that fed these plant leaves indicates that *H. cunea* larvae forage optimally [39]. Similar results found that reared by female larvae of *Lymantria dispar* [40]. This means that *H. cunea* larvae forage to maximize the protein intake from their foods in time at least in the laboratory conditions. Therefore, *H. cunea* larvae forage to increase their fitness [41].

There seems to be a correlation between pupal mortality and the tannin and total phenolic contents of the leaves used to feed the gypsy moth larvae(Figure 6, $r = -0.945$, $p < 0.01$; $r =$ -0.974, p<0.01 respectively). The highest pupal mortality was obtained with the larvae fed by *C. maxima* leaves and was followed by the larvae fed by *C. bignonioides* leaves. The total phenolic contents of these two species were as 17.17% and 6.40% in the leaves respectively. Gallotannin content of the leaves of these two species were as 2.48% and 0.84 % respectively. It was suggested that the principal pupal mortality was due to the nuclear polyhedrosis virus infection [42]. The leaves of *C. maxima* and *C. bignonioides* had relatively smaller total phenolic and gallotannin contents; therefore, the larvae fed with the leaves from these two species had relatively higher the pupal mortality.

Leaf water content provides a surprisingly useful index of larval growth performance of a variety of phytophagous (leafchewing) Lepidoptera [43]. It is considered that there was a relation between pupation time and water content of the leaf samples. Because , the pupation time was short in the larvae fed by plants that had high water contents, except to the larvae fed by *C. bignonioides* (Figure 2, $r=0.843$, $p<0.01$).. It is known that the iridoids are utilized by caterpillars via sequestration [44]. Catalpol known as an iridoid in the leaves of *C. bignonioides*, might be prolong this time despite higher water content of the leaves. This result shows that water content of food plants fundamentally influences the insects in larva stage [45].

The highest pupal weights were obtained with the larvae fed by the leaves from two species (*Q. cerris* and *C. orientalis*) with higher protein contents (13.64% and 13.18% respectively). This result may indicate that protein in herbivory food effectively increase the pupal weight when the larvae are fed by the food items containing them.

It is determined that the AD values of the larvae declined depending on the total phenolic contents of the leaf samples (Figure 3, $r = -0.780$, $p < 0.01$). In contrary, it was found that the phenolic contents of the food plants didn't effect considerably on digestion efficiency of *Paropsis atomaria* [46]. In literature, the AD values that related by the water content[43] but not water content of the leaf samples in the study (Figure 9, $r=$ 0.468 , $p<0.01$).

As a general rule, it is accepted that phenolic, especially tannins, contents of food plants higher than 5 % by dry weight are deterrent to the most specialist herbivorous animals to feed on plants [47]. As a polyphagous herbivorous species, *H. cunea* are not totally deterred by the phenolics in their food plants as well as their ingestion rate parameters not affected by these chemicals.

The major cause of this large variation of ECI in herbivores is large variations in digestibility reducing tannins and other cell wall materials of food plants [48]. In this study, it is determined that the ECI values of the larvae decrease, while the proanthocyanidin (condensed tannin) contents of the leaf samples increase (Figure 10, $r=$ -0.951, $p<0.01$). AD and ECD values of the larvae not affected by. crude fiber of plant samples indicating that cell wall materials of food plants is not effective on ECI values of the larvae

CONCLUSION

In present study, *H. cunea* larvae were grown up by feeding from the leaves of four different plant species; the influence of the water, protein, crude fiber, proanthocyanidin, gallotannin and total phenolic contents of the leaves on larval performance and development were studied. Larval feeding and development of the fall webworm influenced directly or indirectly from the some leaf chemical components. In the future studies, by using leaves from different plant species or artificial food recipes, it can be determine more precisely any of the factors such as water, crude fiber, protein and polyphenolic contents of the foods are the most effectively influencing on the development and performance of *H. cunea*. The study will contribute the understanding of food choice of polyphagous insects in general terms. Especially, the effect of catalpol known as an iridoid in the leaves of *C. bignonioides* has to be investigated on the feeding and development of insect herbivores.

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