The Effects of the Water, Protein and Polyphenolic Contents of four Host Plant Species on the Development and Egg Yield of Female Larvae of Gypsy Moth *(Lymantria dispar)*

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Abstract

This study was conducted to determine the effects of the water, protein and polyphenolic contents of the host plant leaves on the development and egg yield of the female larvae of a polyphagous herbivorous moth namely *Lymantria dispar* L. In a 14-day feeding experiment, *L. dispar* female larvae at the last two larval stages were fed by *Quercus cerris, Quercus infectoria*, *Quercus pubescens* and *Salix babylonica* leaves.

The larvae fed by the leaves from *Q. infectoria* and *Q. cerris* with lower water content consumed less food and had lower apparent digestibility (AD) than the ones fed by the other species (*Q. pubescens* and *S. babylonica)*. The highest efficiency for conversion of digested food into body mass (ECD) values were obtained from larvae fed by *Quercus cerris* and *Salix babylonica* as 0.63 and 0.70 respectively. These two host plant leaves also produced the lowest gallotannin content observed in the present study (4.3 % and 1.3 % respectively).

The leaves of *Quercus infectoria* and *Salix babylonica* total fenolic content of which was 8.9 % and 8.2 %, respectively caused the highest pupal death (30 % and 25 %, respectively). The results indicated that lower total fenolic and gallotannin content of the leaves of two plant species might cause higher pupal death ratio due to lower level of protection against nuclear polyhydrosis virus.

Key words: *Lymantria dispar*, *Quercus* sp., tannin, host suitability, rearing, insect food.

INTRODUCTION

Gypsy moth (*Lymantria dispar)* larvae are highly polyphagous insects feeding on approximately 300 angiosperm trees, cheifly oak species ([1-2]. The fruit trees and the forest trees especially oak species, are their principal food sources in Turkey [3]. Gypsy moth has been known to cause serious damages in the northeastern forests of the USA since 1860's [4].

The nutritive value of a food source to an animal depends on its nutrition content and antinutritive content (such as secondary compounds) [5]. Herbivory food plants' quality changes according to their vitamine or sterol contents, amino acid balance, total protein and water contents [6].

The plant phenolics such as fenolic glycosides and tannins play important roles in the herbivory food choice or selection [7]. Plant secondary metabolites are the chemical protection agents against the herbivory attacks. However, in some cases, secondary metabolites play important roles for some herbivores to locate their host plants. Therefore, some secondary metabolites act against some herbivores as chemical defensive compounds; but they may also attact some herbivores [8]. Toxic secondary metabolites may shorten the longivity of herbivores by poisoning them; therefore they may affect the total plant food ingestion [9].

In this study, it was aimed to investigate the chance in the contents of protein, water and secondary metabolites in *Quercus cerris, Quercus infectoria, Quercus pubescens, Salix babylonica* plants, offered as food onto gypsy moth (*L. dispar)* larvae and the performance of this moth on the host plants.

MATERIALS AND METHODS

L. dispar eggs were collected from the live willow tree stems growing wild along Çarşamba-Terme roadway at 5. km at May 03 in 1999. The eggs were kept at 25° C in the lab. The first larvae was to be hatched at May 27 in 1999. After hatching, the larvae transfered to the feeding cups and fed with *Q. cerris* leaves during the first, second and third larval stages. The feeding experiment were carried out with larvae at fourth and fifth larval stages in groups each having 10 larvae by feeding fresh leaves from *Q. cerris,Q. infectoria,Q. pubescens, S. babylonica*. The leaf samples were changed daily from each species. The amount of leaves given to each group of larvae and the leaf parts left uneaten were measured daily. The leaf parts left uneaten were kept in aluminium folios after weighting out. During the experiments each larva was weighed out daily. These measurements were followed until the larvae became pupa. Each pupa was placed in a separate cup. When the pupa were became mature moth, in each cup a female and a male or a female alone were placed. The egg laid in each cup were determined.

The leaf samples from the tree species with which larvae were fed were wrapped around with aluminium folios and then were dried at 50° C in an oven for five days for chemical

analyses. Dried leaf samples were ground in a laboratory mill and stored in plastic bags in refrigerator until the use.

The ground leaf samples were extracted with 50 % methanol as described by Bilgener [10]. Proanthocyanidin contents of the leaf samples were determined spectrophotometrically by a method described by Bate-Smith [11]. The method used to determine gallotannin contents of the leaf samples was described by Bate-Smith [12]. The total phenolic contents of the samples were determined by a method originally used by Swain and Hillis [13]. The protein contents of the leaf samples were measured by semi-mikro Kjeldahl method with Kjeltec Auto 1030 analyser (Tecator, Sweden). The nitrogen content of each samples obtained by Kjeldahl method was multipled with 6.25 to calculate the total protein content of the plant sample [14].

Statistical analyses

The data on the water, total protein, total phenolic, proanthocyanidin and gallotannin contents of the leaf samples and larval pupal weight, consumed total food amounts, number of eggs laid by the moths, AD, ECD, and ECI values were evaluated by one-way variance analyses (ANOVA) test. Significant differences among treatments were tested using the Duncan's Multiple Range Test. Statistical data analyses was performed by using SPSS 12 statistical software.

RESULTS

Chemical composition of the leaf samples

There were significant differences in the water contents of the leaf samples (Table 1, ANOVA, F= 927.04, p<0.001). The water contents of the leaf samples were 40.29 % in *Q. cerris*, 50.20 % in *Q. infectoria,* 52.01 % in *Q. pubescens* and 62.11 % in *S. babylonica*. The highest water content was obtained from the leaves of *S. Babylonica;* the lowest content was observed in the leaves of *Q. cerris*. The total protein contents of the leaf samples were 14.66 % in *Q. cerris*, 16.05 % in *Q. infectoria,* 15.05 % in *Q. pubescens* and 13.53 % in *S. babylonica*. Acccording to these results, the highest total protein content was obtained in the leaves of *Q. infectoria;* while *S. babylonica* leaves produced the lowest protein content. Protein content of those two species was found to be different significantly (Table 1, ANOVA, F= 118.05, p<0.001).

There were significant diffrences in the proanthocyanidin contents of the leaf samples (Table 1, ANOVA, F= 1921.79, p<0.001). The proanthocyanidin contents of the leaves from *Q. cerris*, *Q. infectoria*, *Q. pubescens* and *S. babylonica* were 5.66 %, 3.85 %, 2.19 % and 6.78 % respectively. The gallotannin content of the plant samples observed in the present study was 4.32 % for *Q. Cerris,* 4.71 % for *Q. İnfectoria*, 5.47 % for *Q. pubescens* and 1.33 % for *S. babylonica* (Table 1, ANOVA, F= 7204.20, p<0.001). These results showed that three oak species had much higher gallotannin content in leaves than that of willov leaves. Total phenolic content of the leaves of *Q. cerris, Q. infectoria*, *Q. pubescens,* and *S. babylonica* was 10.15 %, 8.90 %, 10.49 % and 8.21 %, respectively. Results from statistical data analysis revealed that host plant species were different significantly in their total phenolic content (Table 1, ANOVA, F= 1425.60, p<0.001). It was found that pupal death ratio was dropped when larvae were fed by the leaves with higher total phenolic content (Figure 1).

Table 1. The water, total protein, proanthocyanidin, gallotannin and total phenolic contents of the leaf samples

	Species	N	Mean	SE	* Significant groups	ANOVA	
						F	P
Water (%)	*Q. cerris*	14	40.29	0.33	a	927.04	<0.001
	Q. infectoria	14	50.20	0.32	b		
	Q. pubescens	14	52.01	0.16	c		
	S. babylonica	14	62.11	0.32	d		
Total Protein (%)	*Q. cerris*	14	14.66	0.13	a	118.05	<0.001
	Q. infectoria	14	16.05	0.08	b		
	Q. pubescens	14	15.05	0.08	c		
	S. babylonica	14	13.53	0.09	d		
Proantociyanidin	*Q. cerris*	14	5.66	0.06	a	1921.79	<0.001
(%)	*Q. infectoria*	14	3.85	0.06	b		
	Q. pubescens	14	2.19	0.03	c		
	S. babylonica	14	6.78	0.02	d		
Gallotannin (%)	*Q. cerris*	14	4.32	0.03	a	7204.20	<0.001
	Q. infectoria	14	4.71	0.02	b		
	Q. pubescens	14	5.47	0.02	c		
	S. babylonica	14	1.33	0.01	d		
Total phenolic	*Q. cerris*	14	10.15	0.05	a	1425.60	<0.001
(%)	*Q. infectoria*	14	8.90	0.02	b		
	Q. pubescens	14	10.49	0.01	c		
	S. babylonica	14	8.21	0.02	d		

* 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.)

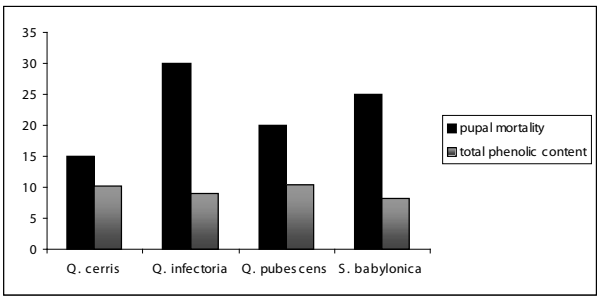

Figure 1. Relationship with total phenolic contents of the leaf samples and the pupal mortality

Feeding Experiments

The amounts of leaves consumed by the female larvae varied with the plant species; and significant differences were found among the food consumed by each species (Table 2, ANOVA, F= 1425.60, p<0.001). The larvae fed by *Q. infectoria* leaves consumed the highest food; on the contrary, the larvae fed by *S. babylonica* leaves had the lowest consumption. However; these larvae fed by *S. babylonica* had the highest apparent (AD) value; the lowest AD value was obtained from the larvae fed by *Q. cerris*. In addition, there were significant differences in the AD values of the larvae fed by different plant leaves (Table 2, ANOVA, F= 10.17, p<0.001). The larvae fed by *Q. infectoria* had an AD value statistically similar to the larvae fed by *Q. cerris* or *Q. pubescens*. AD values of the larvae were correlated with water content of the leaves (Table 3, r=0.679, p<0.01), suggesting that the enzyme activities may concern with food digestion induced by higher water content of food injected.

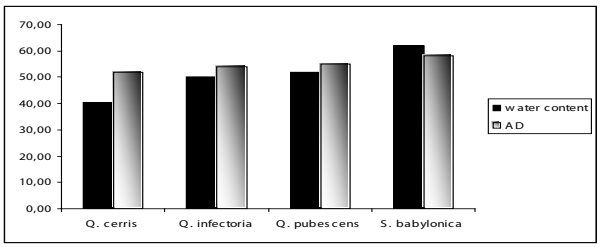

Figure 2. The correlation between the AD values of the larvae and the water contents of the food plants (AD values were mutipled by 100)

Significant differences were determined in efficiency of consumed food to biomass ECD values of the larvae fed by the leaves from different species (Table 2, ANOVA, F= 52.16, p<0.001). However, the lowest ECD value was obtained in the larvae fed by *Q. infectoria;* the highest ECD value was obtained in the larvae fed by *S. babylonica* (Table 2). The larvae fed by either *Q. infectoria* or *Q. pubescens* had similar ECD values (Table 2). Similar to ECD values, *S. babylonica* had the highest efficiency of ingested food to biomass ECI whereas *Q. Infectoria* produced the lowest ECI and significant differences were detected among host plant species in terms of this parameter (Table 2, ANOVA, F= 73.07, p<0.001). The results of Duncan's Multiple Range Test of data obtained by the larval feeding experiments releaved that the female larvae fed by the leaves from three oak species had similar ECI values (Table 2).

Table 2. The total food consuption, AD, ECD and ECI, number eggs laid and pupal weights of the gypsy moth larvae fed by the leaves from four plant species

	Plant Species	N	Mean	SE	* Significant groups	ANOVA	
						F	P
Total food consumption (mg)	*Q. cerris*	10	3910.13	42.58	a	120.22	<0.001
	Q. infectoria	10	4240.00	54.35	b		
	Q. pubescens	10	3275.00	41.45	c		
	S. babylonica	10	3123.00	52.52	d		
AD	*Q. cerris*	10	0.52	0.007	a	10.17	<0.001
	Q. infectoria	10	0.54	0.007	ab		
	Q. pubescens	10	0.55	0.007	b		
	S. babylonica	10	0.58	0.010	c		
ECD	*Q. cerris*	10	.63	0.007	a	52.16	<0.001
	Q. infectoria	10	.56	0.007	b		
	Q. pubescens	10	.58	0.007	b		
	S. babylonica	10	.70	0.012	c		
ECI	*Q. cerris*	10	.32	0.003	a	73.07	<0.001
	Q. infectoria	10	.31	0.005	a		
	Q. pubescens	10	.32	0.005	a		
	S. babylonica	10	.40	0.007	b		
Egg number	*Q. cerris*	9	71	1.52	a	88.52	<0.001
	Q. infectoria	7	91	2.41	b		
	Q. pubescens	8	93	2.66	b		
	S. babylonica	8	45	2.78	c		
Pupal weight (mg)	*Q. cerris*	10	1340.00	12.72	a	238.42	<0.001
	Q. infectoria	10	960.00	12.72	b		
	Q. pubescens	10	770.30	10.86	c		
	S. babylonica	10	1010.00	22.37	d		

* 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.)

Table 3 The Correlation matrix of water and chemical contents of the Gypsy Moth (*Lymantria dispar*) female larvae food plants and the larval food consumption parameters and the number of eggs laid by the mature moths

	Protein	PA	GT	TP	TFC	NEL	AD	ECD	ECI
Water	-0.423*	0.194	-0,655**	-0.964**	-0.621**	-0,433*	0.679**	0.419**	0.725**
Protein		-0.677**	0.750**	0.234	0.746**	0.794**	-0.344*	-0.765**	-0.758**
PA			-0.860**	-0.034	-0.147	-0.883**	0.176	0.733**	0.659**
GT				0.513**	0.448**	0.911**	0.497**	-0.832**	-0.888**
TP					0.534**	0.331	-0.625**	-0.255	-0.597**
TFC						0.478**	0.283	-0.399*	-0.525**
NEL							-0.191	-0.801**	-0.756**
AD								0.666**	0.811**
ECD									0.925**

Abbreviations: Prptein: total protein content; PA: proanthocyaninidin content; GT: Gallotannin content; TP: Total phenolic Content; TFC;: Total food consumed; NEL: Number of eggs laid; AD: Apperant digestibility; ECD: Efficiency of consumed food to bio mass; ECI: Efficiency of ingested food to bio mass.
** P< 0.01; *P< 0.05.

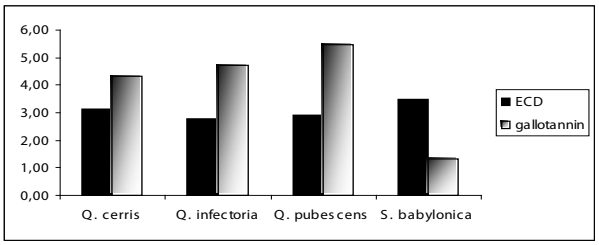

Figure 3. The correlation with the ECD values of the larvae by different plant species and gallotannin contents of their food plants leaves (Gallotannin and ECD values were mutipled by 5)

The number of eggs laid by the mature female gypsy moths were statistically correlated with the protein contents of the leaves from different plant species (Table 3, r=0.794, p<0.01). Significant differences were also found in the number eggs laid by the female moths depending on their larval food species (Table 2, ANOVA, F= 88.52, p<0.001). According to the results Duncan Multiple Range Test, *Q. infectoria* and *Q. pubescens* species produced statistically similar amount of eggs (Table 2).

The pupal weights of the gypsy moth were inversely correlated by the gallotannin contents of the leaf species with which larvae were fed. The highest pupal weights were obtained from the larvae fed by *Q. cerris* and *S. babylonica* which had lower gallotannin contents than the other two food plant species. There were statistically differences between the pupal weights according to the larval food plant species (Table 2, ANOVA, F= 238.42, p<0.001).

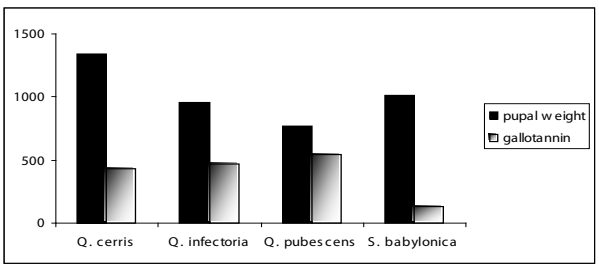

Figure 4. The relationship between the pupal weight and gallotannin contents of the food plants (Gallotannin contents were multipled by 100.)

DISCUSSION

The highest total food consumptions were observed on the larvae fed by *Q. infectoria* and *Q. cerris* leaves. In these leaves, the water contents were the lowest compared to those of *Q. pubescens* and *S. babylonica*. The AD values of the larvae fed by leaves *Q. infectoria* and *Q. cerris* were the lowest. It means that the larvae fed by *Q. infectoria* and *Q. cerris* leaves consumed more food in order to meet their nutritional requirements including water need with these highly difficult to digest food items. This result shows that water content of the plants fundamentally influences the insects in larval stage [15-16].

As shown on Table 3, the correlation between the protein content of food plants and the number of eggs laid by the larvae fed these plants' leaves, indicates that the gypsy moth larvae forage optimally [17]. It means that the gypsy moth larvae forage to maximaze the protein intake from their foods in time at least in the laboratory conditions. Therefore, the gypsy moth female larvae forage to increase their fitness [18].

Taking into account the tannin contents, plants contaning tannin up to 5 % their dry weights have been considered deterrent to herbivores [8]. The plants species used to feed the gypsy moth larvae in this study are all woody angiosperm species; therefore, it is expected to obtain their both phenolic and tannin contents to be higher than 5 %. As a polyphagous herbivorous species, the gypsy moth larvae are not totally deterred by the phenolics in their food plants; but their food consumption parameters are affected by these chemicals.

It can be speculated that there is a correlation between pupal mortality and the tannin and total phenolic contents of the plant leaf species used to feed the gypsy moth larvae. The highest pupal mortality was obtaned from the larvae fed by *Q. infectoria* leaves, followed by the larvae fed by *S. babylonica* leaves. The total phenolic contents of leaves in these two species were as 8.90 % and 8.21 %, respectively. The leaf gallotannin contents of these two species were 4.71 % and 1.33 % respectively. It was suggested that the main reason for pupal mortality is the n*uclear polihydrosis* [19]. The leaves of *Q. infectoria* and *S. babylonica* had relatively lower total phenolic and gallotannin contents; therefore, it could be suggested that the larvae fed

with the leaves from these two species had relatively higher pupal mortality.

Besides *Q. cerris* and *Q. infectoria* is different in their proanthocyanidin content (5.66 % and 3.85 %, respectively). Comparing both species in terms of pupal mortality rate, it was observed that this rate was found to be two fold higher in the larvaefed by *Q. Infectoria* than the other one. It may be concluded that proanthocyanidins provide resistance against the viral infections when the larvae became pupae.

The highest ECD values were obtained with the larvae fed by *Q. cerris* (0.63) and *S. babylonica* (0.70). The leaves of these two species had 4.32 % and 1.33 % gallotannin contents respectively. Therefore, lower the gallotannin content of the leaves; the efficiency of conversion of digested food to biomass gets higher (Table 3, $r=-0.832$, $p<0.01$). Phenolics and their oligomeric and polymeric relatives (tannins) have continued to receive attention for their regulatory functions in intra-plant, herbivore-plant [20-21-22] relationships, and also because of their beneficial effects to human and animal health [23-24-25-26].

ECI values of herbivores were given approximately as 0.3-58 % in the literature [9]. The major cause of this large variation of ECI in herbivores is large variations in digestibilty reducing tannins and other cell wall materials of food plants [27]; the ECI values obtained in this study (31-40 %) were consistent with the above values. The gypsy moth larvae fed by three oak species with higher gallotannin contents in their leaves had similar ECI values and the larvae fed by salix (*S. babylonica*) leaves with lower gallotannin contents had higher ECI values.

The highest pupal weights were obtained with the larvae fed by the leaves from two species (*Q. cerris* and *S. babylonica*) with lower gallotannin contents (4.32 % and 1.33 % respectively). This result may indicate that gallotannins in herbivory food effectively reduce the pupal weight when the larvae are fed by the food items containning them; similar result were also observed when the gypsy moth larvae were fed by an artifical diet recipe containing tannic acid (a commercial gallotannin source) (O. Yanar and M. Bilgener unpublished results).

CONLUSION

In this research, *L. dispar* female larvae were grown up by feeding them leave from four different plant species; the influence of the water, protein, proanthocyanidin, gallotannin and total phenolic contents of the leaves on larval performance and development were studied. In the future studies, by using leaves from different plant species or artifical food recipes, the factors affecting development of this species can be determined more precisely. These will contribute the understanding of food choice of polyphagous insects in general terms.

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