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Nitrogen Utilization Pattern and Degradation Capability of Some Plant Secondary Metabolites by *Agelastica alni* L. (Coleoptera: Chrysomelidae)

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ABSTRACT

An oligophagous leaf beetle, *Agelastica alni* (L., 1758) (Coleoptera: Chrysomelidae), often occurs in outbreak densities and utilizes both alders and willows in Europe. It was reported on alder and hazel in Turkey and its outbreaks on alder occur almost every year in the middle and eastern Black Sea regions of the country where hazel plantations cover huge areas. Nitrogen utilization, and degradation of some plant secondary metabolites by *A. alni* larvae fed with leaves of alder (*Alnus glutino*sa (L.) Gaertn. ssp. g*lutinosa*), babylon willow (*Salix babylonica* L.) and hazel (*Corylus avellana* L.) with varying nutritional status were investigated. Larvae of this species were fed leaves of these plants that had been fertilized (NPK) and not been fertilized. Larval nitrogen utilization was determined to calculate the nitrogen utilization efficiency (NUE). The effects of some secondary metabolites of leaves of the plants on the digestion efficiency of *A. alni* larvae were tested. It was determined that *A. alni* larvae balanced its nitrogen utilization to some degree, despite the increase of nitrogen content of the leaves. Although the nitrogen content changed from 2,97% to 4,33 in hazel, from 2,47 % to 3,68 in babylon willow, from 4,27 % to 5,34 in young alder, from 4,04 % to 4,66 in older alder, the efficiency of nitrogen utilization of larvae changed from 16,54% to 12,77, from 11,92 % to 13,86, 22,72 % to 24,53, and 21,09 % to 23,71, respectively. In addition, the correlation between the changed N content of the treated leaves and the nitrogen utilization of the larvae that fed on them was negative (except from the larvae fed on unfertilized leaves of *C. avellana*) in all plants and treatments. The statistical analysis indicated that the approximate digestibility (AD) of the larvae was not affected by these secondary compounds ($r^2 < 0.7$, $P < 0.05$). On the other hand, the degradation ratio of proanthocyanidin (condensed tannin) and total phenolics by *A. alni* larvae was determined as 33-81%. These results lead to the interpretation that metabolites had no measurable effects on *A. alni* larvae, or alternatively, the larvae utilized regulatory mechanisms to overcome the undesirable effects of these compounds. This study also indicated that larvae of *A. alni* have developed nitrogen homeostasis, which may involves a decrease of predation risk.

Key Words: Agelastica alni, larva, phenolic, nitrogen homeostasis, proanthocyanidin, AD, NUE

INTRODUCTION

Food intake, utilization, and allocation play central roles in the survival, growth, development and fecundity of insects (Slansky, 1993). For example, food consumption and nutrient utilization efficiency can be increased when larvae are fed low quality food resulting in final biomass comparable to larvae fed high quality food (Slansky & Feeny, 1977). Knowledge of insect responses to these nutritional challenges is important for the understanding insect-plant interactions, insect lifestyles, and other

aspects of insect biology. Many herbivorous insects are rather specific in their host utilization (Smiley, 1978; Bernays & Graham, 1988; Jaenike, 1990; Kolehmainen *et al*., 1994; Baur & Rank, 1996; Roininen *et al.,* 1999). This appears to be largely due to the fact that each insect species has 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 (Matsuda & Matsuo, 1985; Rank, 1992; Soetens & Pasteels, 1994; Gross & Hilker, 1994/1995; Kolehmainen *et al.,* 1994; Orians *et al.,* 1997; Roininen *et al.,* 1999). In contrast, secondary constituents of non-host plants frequently act as deterrents and inhibitors for feeding and oviposition (Kraft & Denno, 1982; Matsuda & Senbo, 1986; Kelly & Curry, 1991; Gross & Hilker, 1994/1995; Ikonen *et al.,* 2001). These substances may also inhibit insect growth and development (Lindorth *et al*., 1988; Kelly & Curry, 1991). Food quality, more than quantity, determines the distribution and abundance of many phytophagous insects (White, 1993). Vegetal tissues constitute low-quality food because they usually contain low levels of nitrogen and this nutritional poverty can be combined with toxins, digestion inhibitors or indigestible materials (Mattson, 1980; White, 1993; Zamora *et al*., 1999). Thus, adults, larvae, or both, must recognize the best foods available and select them for oviposition or feeding (Bernays & Chapman, 1994). Consequently, many plant secondary compounds, especially tannins, can affect insect feeding behaviour (Harborne, 1977). The hydroxyl groups which comprise a component of the tannin molecule are effective in forming complexes with natural compounds, so tannins can combine with natural compounds such as proteins, digestion enzymes, polysaccharides such as starch, cellulose, hemicellulose (Loomis, 1974; Mole & Waterman, 1987; Price *et al*., 1980) and oils, nucleic acids and amino acids (Takechi & Tanaka, 1987). Inhibition of the kinetics of digestion enzymes by tannins was associated with the chemical structure of tannins, *pH*, and other food polymers such as cellulose (Bilgener, 1988).

Foliar nitrogen level is frequently an important factor influencing the performance of herbivorous species (Mattson, 1980; Slansky & Scriber, 1985; Slansky, 1993). Herbivore species that are able to efficiently utilize scarce resources may be more competitive compared with species that lack this ability. Adaptive responses by herbivores to low nutrient levels include increased consumption, digestibility of nutrients and efficiency of nutrient utilization (Slansky & Wheeler, 1989). In general, foliar N content is an appropriate indicator to measure plant quality for herbivorous insects, because many herbivorous insects prefer and perform best on plants with high N contents (Mattson, 1980; Scriber, 1984). On the other hand, in herbivorous insects, many studies have examined N utilization efficiency to maintain body N content with varying food qualities; in these studies ingested food was artificially manipulated by fertilization or by using artificial diets (Slansky & Feeny, 1977; Wheeler & Halpern, 1999; Simpson & Raubenhaimer, 2001; Lee *et al*., 2002, 2004). However, it is still unknown to what extent herbivorous insects can maintain their body-element composition when there is natural variation in host plant quality (Kagata & Ohgushi, 2006).

Herbivores are often classified as mono-, oligo- and polyphagous, or as generalists and specialists. Several meanings have been attached to these terms by different

authors (Symons & Beccaloni, 1999), since no universal criterion that distinguishes specialists from generalists exists. The terms species-, genus- and family specialist (Barone, 1998) are used for herbivorous species feeding on a single plant species, genus or family. They represent a potentially useful set of host specificity definitions. Many European leaf beetle species utilize both betulaceous and saliceous species as their hosts (Tischler, 1977; Baur and Rank, 1996; Ikonen *et al*., 2001). In some of them conspecifics associated with alternative host species are differently adapted and form distinct host races (Kreslavsky *et al*., 1981).

An oligophagous leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae), often occurs in outbreak densities and utilizes both alders and willows (Tischler, 1977; Koch, 1992; Baur & Rank, 1996). *A. alni* was reported on alder and hazel in Turkey (Çanakçıoğlu & Mol, 1998), and its outbreaks on alder occur almost every year in the middle and eastern Black Sea regions of the country where hazel plantations cover huge areas. The present study examined the feeding performance of *A. alni* larvae fed on leaves of *Alnus glutin*osa (L.) Gaertn. ssp. g*lutinosa, Salix babylonica* L. and *Corylus avellana* L., and body N content with varying nutritional status of the leaves for these plants, under laboratory conditions. Thus, we determined the effect of high and low nitrogen levels of alder, willow and hazel leaves on larval consumption, digestibility, and nitrogen utilization. We also determined the effect of some host plant secondary metabolites on digestion efficiency of the larvae and the larval ability to withstand these metabolites. In addition we discussed the function of some secondary compounds of these plants in nitrogen homeostasis of this insect.

MATERIAL AND METHODS

Small branches of alder (*Alnus glutin*osa (L.) Gaertn. ssp. *glutinosa*), babylon willow (*Salix babylonica* L.) and hazel (*Corylus avellana* L.) were grown in erlenmayer flasks filled with distilled water for control and treatment groups. Older and young small branches of alder were obtained from the branches of two alder trees 16 and 6 years old, respectively. Water-soluble fertilizer was applied to the water so branches could absorb nutrients. A group branches were placed into 10 h and the fertilized water were pulverized to leaves of another group branches three times in a day. Fertilized water contained 10 mg/ml A-crop [18–18–18 NPK; Agro altın, Samsun-TURKEY]. Each branch was grown for 9 days and leaves were cut from these plant parts as needed. Treatments were included to determine the effect of fertilizer on insect performance.

Larval food utilization efficiencies

Second instar *A. alni* larvae were obtained from wild populations feeding on *A. glutin*osa ssp. *glutinosa,* trees in the vicinity of Trabzon and Giresun provinces in the Eastern Blacksea Region of Turkey, on June 2006 and 2007. Third-instar larvae (n = 120) were reared individually in petri dishes (9 x 3 cm) at room temperature (22° C) and L15: D9 h photophase. The larvae were fed leaves cut down the mid-ribs. One set of leaf halves was used for estimation of initial percent dry mass of the leaf and the other half was fed to the larvae. Leaves were replaced after consumption exceeded roughly 60% of the leaf-half by visual inspection. Frass was removed daily. Frass,

uneaten leaf halves, and unconsumed leaf material were dried for 2 days at 40 ° C. A Sartorios MA-30 balance (± 0.1 mg) was used for leaf fresh mass measurements, weighing larvae, pupae and frass, and determining all remaining masses. All dry mass determinations were made within several seconds of removing the sample from the heated oven. Insect nutrition data are typically expressed with a series of ratios that describe the impact of diet quality on various insect performance parameters, such as consumption and digestibility (Waldbauer, 1968; Slansky & Scriber, 1985). Food consumption was calculated as dry food ingested, and approximate 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. A parallel series of leaves was dried to determine to amount of dry matter per plant species. Larval food utilization efficiencies were calculated to determine the digestibility and nitrogen utilization. Feeding performances measure Approximate Digestibility (AD) values that are known as nutritional indices. AD is formulized by [(I – F) / I] where I = weight of food consumed, F = weight of faeces produced during the feeding period, and G = biomass gain of the larvae (Waldbauer, 1968). Larval nitrogen utilization efficiency (NUE) was also determined and was formulized by [G / I] where G = assimilated nitrogen by the larvae and I = consumed nitrogen by the larvae (Tabashnik, 1982).

Analysis of leaf chemistry

In order to relate leaf beetle host utilization patterns with leaf secondary chemistry, the samples of leaves from each of the four experimental plant species were freeze-dried. Total phenols were extracted from 0.4 g leaf dry powder with 10 ml of 50 % (v/v) methanol in an ultrasonic bath for 10 min. Total phenols were analysed by the Folin-Ciocalteau method (Swain & Hillis, 1959). 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 analysed by the Acid-Butanol assay (Bate-Smith, 1975). To compare species, direct values of absorbance at 547 nm were used, without transformation to standard equivalents. In order to calculate proanthocyanidin concentration in the samples, the value of Є = 150 was used for cyanidin (see Waterman & Mole, 1994, for a full explanation of this procedure). Gallotannins were analysed by the Potassium Iodat assay (Bate-Smith, 1977). In order to calculate gallotannin concentration of the leaf specimens, a standard slope was prepared with 0.1-1 mg ml^{-1} of tannic acid. The nitrogen content was quantified in leaves and frass samples using the Kjeltec Auto 1030 Analyzer (Tecator, Sweeden). Crude fiber analysis of leaf and frass samples was made according to the method of Morrison (1972) modified by Bilgener (1988). In order to determine the water content of leaf and frass samples, the leaves were dried at 80° C over 4 days.

Statistical analysis

The data on the water, crude fiber, total nitrogen, total phenolic, proanthocyanidin and gallotannin contents of the leaf samples and pupal weight, consumed total food amounts by the larvae, AD and NUE values of the larvae were evaluated by a one-way variance analyses (ANOVA) test. Significant differences among treatments were tested using the Duncan's Multiple Range and Tukey tests. The degradation ratio of

secondary metabolites of larvae was tested by a *t*-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= 624.08, p<0.05). The water contents of the leaf samples were 72.38 % in young alder, 53.67 % in older alder, 84.67 % in babylon willow, and 60.08 % in hazel. The highest water content was obtained from the control leaves of babylon willow and the lowest content from the control leaves of older alder. In general, fertilizer treatments did not increase the water contents of the leaves, but the leaves of the hazel especially from the pulverizing and absorbing treatments had significantly greater (37,61% and 15,24%) water content compared to the leaves from the control.

Significant differences were found between crude fiber contents of the control leaf samples. Crude fiber contents of the control leaves of young alder, older alder, babylon willow, and hazel were 39.58, 45.52, 32.18, and 41.21 %, respectively (Table 1, ANOVA, F= 627.79, p<0.001).

The total nitrogen contents of the control leaf samples were 4.27 % in young alder, 4.04 % in older alder, 2.47 % in babylon willow, and 2.97 % in hazel. Young alder leaves had the highest total nitrogen content and babylon willow had the lowest. Nitrogen content of those two species was found to be significantly different (Table1, ANOVA, F= 128.04, p<0.001). Fertilizer treatments increased the nitrogen levels of leaves in three plant species. The total nitrogen contents were increased 6.79% and 25. 06 in young alder, 5.94% and 15.34 in older alder, 14.17% and 48.98 in babylon willow , 40.40% and 45.79 in hazel for absorbing and pulverizing, respectively. Fertilizer pulverizing more significantly increased the nitrogen levels of plant leaves. Foliar nitrogen concentration of the hazel was significantly greater in both treatments compared with the control.

There were significant differences in the proanthocyanidin contents of the control leaf samples (Table 1, ANOVA, F= 921.79, p<0.001). The proanthocyanidin contents of the control leaves from young alder, older alder, babylon willow, and hazel were 8.43, 7.36, 7.62 and 8.65%, respectively. Total phenolic content of the control leaves of young alder, older alder, babylon willow, and hazel was 11.22, 11.02, 11.61, and 8.76%, respectively. Results from statistical data analysis revealed that hazel was different significantly in its total phenolic content and changing of this parameter via fertilization (Table 1, ANOVA, F= 1413.50, p<0.001). These results showed that hazel and young alder leaves had significantly decreased total phenolic content with fertilizing compared to the leaves of the older alder and willow. Significant differences were also found in the crude fiber contents among control leaf samples.

Feeding experiments

The amounts of leaf tissues consumed by the larvae varied with the plant species, and significant differences were found among the food consumed by the larvae

Table 1. Water, crude fiber, proanthocyanidin, total phenolic, and total nitrogen contents of the leaf samples with varying nutritional status (%).

	Not Fertilized	Absorbing	Pulverizing
Young alder			
Total nitrogen	4,27 (0,74)^{a}z	4,56 (0,32)y	5,34 (0,62)x
Total phenolic	11,22 (0,28)^{a}x	9,23 (0,28)z	10,85 (0,16)y
Proanthocyanidin	8,43 (1,98)^{a}x	8,31 (2,23)xy	8,26 (1,98)y
Crude fiber	39,58 (4,36)^{b}xy	37,25 (2,84)y	40,12 (5,72)x
Water	72,38 (6,13)^{b}x	68,41 (9,35)y	61,96 (4,44)z
Older alder			
Total nitrogen	4,04 (0,18)^{a}z	4,28 (0,29)x	4,66 (0,66)y
Total phenolic	11,02 (0,36)^{a}xy	10,29 (0,81)y	11,07 (1,10)x
Proanthocyanidin	7,36 (1,67)^{c}x	8,31 (2,01)y	7,45 (1,51)z
Crude fiber	45,52 (2,37)^{a}z	48,62 (4,26)y	54,20 (4,95)x
Water	53,67 (3,61)^{d}y	60,21 (5,63)x	58,25 (9,38)x
Babylon willow			
Total nitrogen	2,47 (0,30)^{c}z	2,82 (0,24)y	3,68 (0,42)x
Total phenolic	11,61 (1,04)^{a}y	10,49 (0,69)z	12,17 (0,92)x
Proanthocyanidin	7,62 (2,32)^{b}x	5,78 (1,34)z	7,45 (1,60)y
Crude fiber	32,18 (3,45)^{c}y	35,84 (2,56)x	31,68 (3,12)y
Water	84,67 (3,61)^{a}x	80,11 (5,63)x	68,25 (9,38)y
Hazel			
Total nitrogen	2,97 (0,43)^{b}y	4,17 (0,25)x	4,33 (0,38)x
Total phenolic	8,76 (1,00)^{b}x	7,45 (1,00)z	7,48 (1,10)y
Proanthocyanidin	8,65 (3,51)^{a}xy	7,02 (1,93)y	9,55 (4,20)x
Crude fiber	41,21 (4,15)^{b}x	40,10 (5,68)x	43,59 (6,35)x
Water	60,08 (4,96)^{c}z	69,24 (10,54)y	82,68 (7,01)x

Different letters indicate significantly different group means (α = 0.05). (The groups among treatments abbreviated by x, y, z and the groups among plant species abbreviated by a,b,c,d have significantly different means according to Duncan's Multiple Range Test). (Results are means ± standard deviation, n=9).

in each plant species (Table 2, ANOVA, $F= 1425.60$, $p<0.05$). The highest leaf consumption by the larvae was observed on older alder leaves; by contrast, the lowest were found on babylon willow leaves. The larvae fed on control leaves of hazel and babylon willow had the lowest approximate digestibility (AD) values and the larvae fed on young alder had the highest. In addition, there were significant differences in the AD values of the larvae fed on control leaves of different plants (Table 2, ANOVA, $F= 20.16$, $p<0.05$). The AD values of the larvae fed on control leaves of hazel and willow were statistically similar. AD values of the larvae fed on control leaves among plant species were not correlated with water content of the control leaves (Fig. 1, $r = - 0.341$, $p<0.05$), suggesting that the enzyme activities may concern with food digestion induced by higher water content in relation to hydrolysis of polymers by hydrolytic enzymes results in free monomers. There were statistically significant differences between the pupal weights depending on the larval food plant species (Table 2, ANOVA, $F= 178.32$, $p<0.05$). On the other hand, the pupal weights of the beetle were inversely correlated with the nitrogen contents of the control leaf specimens

Table 2. Larval consumption of leaf tissue, digestion efficiency and pupal weights of *Agelastica alni* L. on different host plants.

Treatments	Total leaf consumed (mg) (dw)	Pupal weight (mg) (dw)	AD
A. glutinosa ssp. *glutinosa* (6 years old)			
Not Fertilized	34.44(5.04)^{b} y	5.93(1.12)^{a} y	0.40(0.06)^{a} z
Absorbing	32.0(11.20) y	7.22(0.09) x	0.50(0.05) y
Pulverizing	40.56(6.63) x	7.01(1.28) x	0.57(0.04) x
A. glutinosa ssp. *glutinosa* (16 years old)			
Not Fertilized	74.2(10.34)^{a} x	6.04(0.04)^{a} x	0. 37(0.05)^{a} y
Absorbing	57.2(12.4) y	6.27(1.85)x	0.45(0.05)x
Pulverizing	40.74(3.78) z	6.21(2.02)x	0.46(0.04)x
Salix babylonica			
Not Fertilized	20.16(1.76)^{c} y	4.04(0.07)^{c} z	0.18(0.03)^{b} y
Absorbing	22.2(5.29) y	5.21(1.54)y	0.25(0.04)x
Pulverizing	32.64(12.1) x	7.14(0.08)x	0.21(0.02)xy
Corylus avellana			
Not Fertilized	36.8(14.0)^{b} x	4.54(1.23)^{b} y	0.18(0.04)^{b} y
Absorbing	26.66(8.37) y	3.27(1.89)z	0.20(0.04)y
Pulverizing	14.58(4.5) z	4.88(0.03)x	0.27(0.04)x

Abbreviation: dw.= Dry weight (Results are means ± standard deviation of the values, n=9). Different letters indicate significantly different group means (α = 0.05). (The groups among treatments abbreviated by x, y, z and the groups among plant species abbreviated by a,b,c,d have significantly different means according to Duncan's Multiple Range Test)

among plant species that larvae fed upon (Fig. 2, r=0.988, p<0.05). The highest pupal weights were obtained from the larvae fed on the leaves of older alder and young alder which had higher nitrogen contents than the other two food plant species. The performance of the larvae varied among the different fertility treatments (Table 2). Pupal weight and AD values were higher when the larvae fed on fertilized leaves in all plant species. However, total leaf tissue consumed by the larvae decreased with fertility treatments in older alder and hazel. The nitrogen utilization efficiency (NUE) values of the larvae did not differ significantly among the fertility treatments in all plant species (Table 3, ANOVA, p<0.05). Throughout the experiment, the N utilization of the larvae for the control group ranged from 22.72 to 24.53%, from 21.27 to 23.71%, from 12.42 to 14.86% and from 16.54 to 12.77% when feeding on young alder, older alder, babylon willow, and hazel, respectively. This range was relatively smaller than that of the treated leaves. In fact, the nitrogen content variations among the treated leaves of these plants were 4,27 % to 5,34, 4,04 % to 4,66, 2,47 % to 3,68, 2,97% to 4,33, respectively. Although there was no significant correlation between the changed N content of the treated leaves and the nitrogen utilization of the larvae that fed on them, the correlation coefficient was negative (except from the larvae fed on unfertilized leaves of *C. avellana*) in all plants and treatments (Table 4). These results imply that the N utilization by the larvae was independent of foliar N content of the host plants, indicating strong N homeostasis of the *A. alni* larvae.

The AD values of the larvae were not significantly correlated with proanthocyanidin (except from the larvae fed on *S. babylonica*) and total phenolic content of the leaves

in all plants. These results demonstrate that the digestion efficiency of the larvae was independent of these secondary plant chemicals (Table 5, $r^2 < 0.7$, $p<0.05$). Degradation rates of proanthocyanidin and total phenolics by the larvae were statistically important depending on to the plant species and treatment types. These rates were higher in the larvae fed on young *A. glutinosa* ssp. *glutinosa* and older *A. glutinosa* ssp. *glutinosa* than the larvae fed on *S. babylonica* and *C. avellana*.

Table 3. The effect of varying nitrogen content of treated leaves on the nitrogen utilization efficiency (NUE) values of the larvae.

Treatment	Number of specimen	NUE	Treatment	Number of specimen	NUE
young *A.glutinosa* ssp. *glutinosa*			older *A.glutinosa* ssp. *glutinosa*		
		1			1
Not Fertilized	9	22.72	Pulverizing	9	21.09
Absorbing	9	24.02	Not Fertilized	9	21.27
Pulverizing	9	24.53	Absorbing	9	23.71
Significance		0.78	Significance		0.52
S. babylonica			*C. avellana*		
Pulverizing	9	11.92	Absorbing	9	12.77
Not Fertilized	9	12.42	Pulverizing	9	15.77
Absorbing	9	14.86	Not Fertilized	9	16.54
Significance		0.28	Significance		0.30

Different numbers indicates significantly different groups that divided using the number of 1,2,3 according to Tukey honestly significantly different Test (Confidence intervaal: 95%).

Table 4. Correlation between leaf nitrogen content and nitrogen utilization efficiency (NUE) values of the larvae.

Control and treated leaf of the plant	Correlation between Leaf nitrogen vs. NUE	
	Correlation coefficient (r)	r square (r^2)
Young *A. glutinosa* ssp. *glutinosa* (nf.)	-0.7983	(0.6373)
Young *A. glutinosa* ssp. *glutinosa* (abs.)	-0.7221	(0.5215)
Young *A. glutinosa* ssp. *glutinosa* (pulv.)	- 0.7569	(0.5729)
Older *A. glutinosa* ssp. *glutinosa* (nf.)	-0.4940	(0.2441)
Older *A. glutinosa* ssp. *glutinosa* (abs.)	-0.4558	(0.2078)
Older *A. glutinosa* ssp. *glutinosa* (pulv.)	- 0.3410	(0.1163)
Salix babylonica (nf.)	-0.7700	(0.5930)
Salix babylonica (abs.)	-0.6236	(0.3889)
Salix babylonica (pulv.)	-0.8407	(0.7068)
Corylus avellana (nf.)	0.5337	(0.2849)
Corylus avellana (abs.)	-0.6007	(0.3609)
Corylus avellana (pulv.)	-0.7850	(0.6163)

Abbreviations: nf: not fertilized leaf; abs: leaf fertilized by absorbing; pulv leaf fertilized by pulverizing. (n=9).

DISCUSSION

Larval performance on fertilized leaves

Slansky & Feeny (1977) examined the growth efficiency of the cabbage butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae), when fed different host Brassicaceae species or *Brassica oleracea* with varying fertilization levels. They demonstrated that while host plant N varied from 1.48 to 6.11%, insect N varied from 7.11 to 9.40%. The slope of their regression line was 0.02 (95% upper CL =0.12), indicating strong N homeostasis in the butterfly. Fox and Macauley (1977) examined the growth of a leaf beetle *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae), on several *Eucalyptus* species. They demonstrated that while host plant N ranged from 0.49 to 1.85%, insect N varied only from 4.6 to 8.6%. The slope of the regression line was 0.23 (95% upper CL =0.44). Kagata & Ohgushi (2006) examined the performance and N content of the willow leaf beetle, *Plagiodera versicolora* (Laicharting) (Coleoptera: Chrysomelidae), when fed leaves of host willow, *Salix eriocarpa* with varying nutritional status. They found that foliar N content ranged from 1.91 to 2.56%, the N content of the leaf beetle ranged from 11.05 to 11.40%. They demonstrated that there was no significant correlation between the standardized N content of the willow leaves and N content of the leaf beetle. The slope of regression line was 0.02 (95% upper CL =0.11). Kagata & Ohgushi (2006) claimed that the result of Fox and Macauley (1977) indicated that *P. atomaria* had also N homeostasis. Ohmart et al. (1985) found that there was a foliar N concentration threshold between 1.2% and 1.7% above which *P. atomaria* larval growth and development were optimum and independent of foliar N concentration since they were able to regulate N intake and utilization by altering consumption and nitrogen utilization efficiency (NUE). We found that NUE values of the larvae were not affected significantly among the fertility treatments in all plant species (Table 3, ANOVA, $p<0.05$). The nitrogen content variation of the treated leaves of young alder, older alder, babylon willow and hazel species was 4,27 % to 5,34, 4,04 % to 4,66, 2,47 % to 3,68, 2,97% to 4,33, respectively. On the other hand, the N utilization efficiency of the larvae ranged from 22.72% to 24.53, from 21.09 to 23.71, from 11.92 to 13.86 and from 16.54 to 12.77 when feeding on each of these species, respectively. Despite the correlation coefficient was negative (except from the larvae fed on unfertilized leaves of *C. avellana*), there was no significant correlation between the changed N content of the treated leaves and the nitrogen utilization efficiency of the larvae that fed on them (Table 4, $r^2 \leq 0.7$, $p<0.05$). There were no important effects of varying nitrogen content of treated leaves on the NUE values of the larvae (Table 3). According to these results the NUE of the larvae was independent of foliar N content of the host plants, indicating improved N homeostasis of the *A. alni* larvae. The performance of the *A. alni* larvae varied among the treated leaves of three different plant species. Total leaf consumption by the larvae fed with fertilized leaves decreased 46% in older alder and 60% in hazel. Generally, AD values and pupal weights were higher when the larvae were fed with fertilized leaves in all plant species in this study (Table 2). Fertilizer treatments, especially pulverizing raised the AD values by 17% in young alder, 9% in older alder and 9% in hazel. AD values of willow were increased by 7 and 3% for absorbing and pulverizing. It was also found

that insect larvae feeding leaf tissue with higher nitrogen content resulted in pupae that were higher in weight than larvae that were fed tissue lower in nitrogen content, indicating their fitness was glowingly affected from nitrogen level of the leaf tissues.

Plants contaning tannin comprising up to 5% their dry weights have been considered deterrent to herbivores (Harborne, 1994). The plant species used to feed the beetle larvae in this study were all woody angiosperm species; therefore, it is expected that both their phenolic and tannin contents should be higher than 5%. As an oligophagous herbivorous species, *A. alni* larvae are not totally deterred by the phenolics in their food plants; moreover, their food consumption and digestion parameters are not affected by these chemicals. Fox & Macauley (1977) reported that the growth rate of *P. atomaria* larvae was directly related to N concentration in *Eucalyptus* foliage and not affected at all by a wide range in concentrations of phenols. The results of the chemical analysis of the leaves demonstrated that in general the total phenolics and proanthocyanidin levels of the control leaves were high in all the all tree species. The AD values of the larvae were not affected by proanthocyanidin and total phenolic contents of the control leaves in all plants. These results demonstrated that the digestion efficiency of the larvae was independent from these secondary plant chemicals (Table 5, $r^2 < 0.7$, $p < 0.05$). On the other hand the degradation ratio of proanthocyanidin and total phenolics by *A. alni* larvae was 33-81% (Table 6). There may be different explanations for the wide range of degradation ratio. In particular, babylon willow and hazel phenols, including condensed tannins, as reported Takechi and Tanaka (1987), couldn't efficiently bind protein. Larval gut pH was not sufficient for binding tannins with proteins. The effect of gut pH on this metabolic reaction was explained for a mammal in great detail from Bilgener (1988). Retention time of food particles through the larval gut was too short for the biochemical complexes that had been formed. An other possible reason was the larvae fed on the leaves of willow and hazel couldn't detoxify these compounds very effectively as well as the larvae that fed on alder leaves. Consequently, this result leads to the interpretation that either the secondary metabolites show post-digestive effects on *A. alni* larvae or the larvae utilize regulatory mechanisms to overcome the inappropriate effects of these compounds from the host on their digestion system.

Larval performance on unfertilized leaves

The larvae fed with the leaves of older alder and hazel showed the highest total food consumption. Water contents of the leaves of these plants were the lowest, and crude fiber contents were the highest compared to those of young alder and willow. Water content of the plants fundamentally influences insects in the larval stage (Scriber, 1977; Scriber and Slansky, 1981). This means that the larvae fed on the leaves of older alder and hazel, consumed more food in order to meet their nutritional requirements including water need with these highly difficult to digest food items high in crude fiber. On the other hand, the AD values of the larvae fed with the leaves of older alder and hazel were the lowest but there was no correlation between the AD values of the larvae and the water content of the plant's leaves (Fig. 1, $r = - 0.341$, $p < 0.05$). The pupal weights of the beetle were inversely correlated with the nitrogen contents of the leaf specimens that larvae fed upon (Fig. 2, $r = 0.988$, $p < 0.05$).

The highest pupal weights were obtained from larvae that fed on the leaves of older and young alder which had higher nitrogen contents and is a more preferred host than the other two food plant species. There were statistically significant differences between the pupal weights depending on the larval food plant species. Phenolics and their oligomeric and polymeric relatives (tannins) have continued to receive attention for their regulatory functions in intra-plant, herbivore-plant relationships (Bernays *et al*., 1983; Lindroth *et al*., 1988; Lempa *et al*., 2004). Insect feeding behaviour can be affected from many plant secondary compounds including phenolics like tannins (Harborne, 1977). Surprisigly, the leaf beetle larvae fed on hazel leaves with lowest total phenolic content had the lowest AD values. However, the leaf beetle larvae fed on two alder leaves with higher total phenolic contents had similar higher AD values in this study. This result may imply that total phenolics as a food at least for this species do not effectively reduce the AD values when the larvae fed on the food items containing high total phenolics.

Table 5. Correlation between leaf secondary compounds content vs. Approximate digestibility (AD) values of the larvae.

Leaf specimen of plant species	Proanthocyanidin vs. AD		Total phenolics vs. AD	
	Correlation coefficient (r)	r square (r^2)	Correlation coefficient (r)	r square (r^2)
Young *A. glutinosa* ssp. *glutinosa*	-0.2387	(0.0570)	-0.2029	(0.0412)
Older *A. glutinosa* ssp. *glutinosa*	0.0412	(0.0017)	-0.1577	(0.0249)
Salix babylonica	-0.8485*	(0.7201)*	0.1126	(0.0127)
Corylus avellana	-0.3910	(0.1529)	0.01	(0.0001)

(*$p<0.05$).

Table 6. Degradation rates of proanthocyanidin and total phenolics in *A. alni* larvae fed with control and treated leaves (%).

Treated leaves of plant species	Control	Absorbing	Pulverizing
	Proanthocyanidin		
Young *A. glutinosa* ssp. *glutinosa*	62.29**	62.34**	72.70**
Older *A. glutinosa* ssp. *glutinosa*	81.76**	72.11**	69.59**
Salix babylonica	37.35*	35.85**	23.85**
Corylus avellana	43.43*	53.31**	35.88**
	Total phenolic		
Young *A. glutinosa* ssp. *glutinosa*	59.77**	58.42**	75.40**
Older *A. glutinosa* ssp. *glutinosa*	68.16**	58.92**	57.92**
Salix babylonica	33.96**	35.47*	54.02**
Corylus avellana	37.06**	43.81*	41.35**

* $P<0.05$, ** $P<0.01$ (Data were evaluated according to *t*- test between the materials excreted and intake from the larvae).

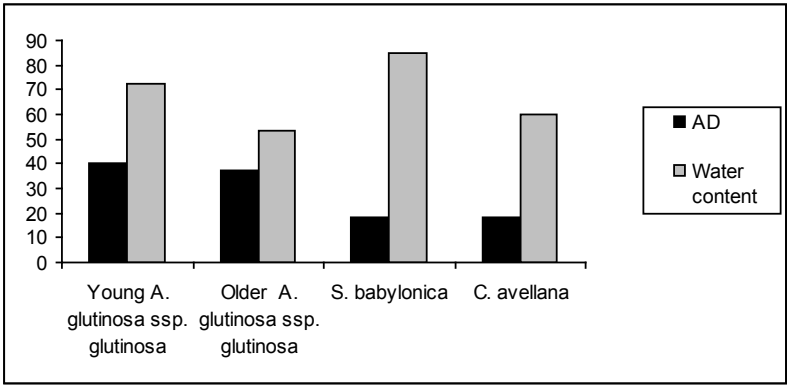

Fig. 1. Relationship with water content of not fertilized leaves in food plants and the AD values of the larvae.

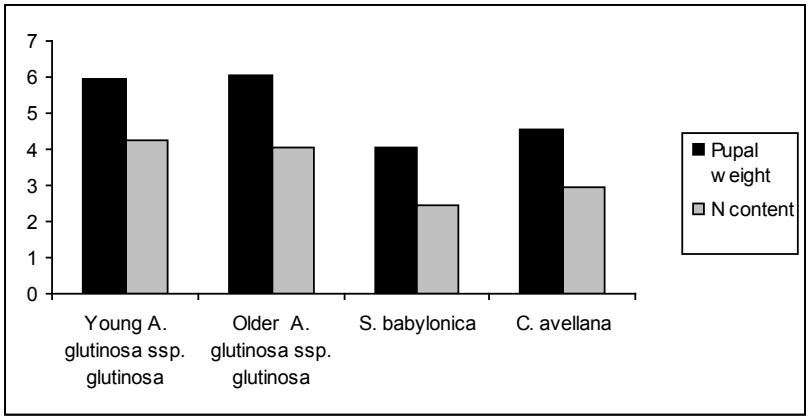

Fig. 2. Relationship with nitrogen content of the not fertilized leaves in food plans and the pupal weight of the larvae

CONCLUSIONS

In this research, *A. alni* larvae were reared by feeding on varying quality leaves of plant species; the influence of the nitrogen levels, water, crude fiber, proanthocyanidin and total phenolic contents of the leaves on larval performance were studied. Firstly, we found that the larvae of *A. alni* have a developed nitrogen homeostasis, which may involves a decrease of the predation risk. This study lead to the interpretation that either the secondary metabolites may show post-digestive effects on *A. alni* larvae or the larvae utilize regulatory mechanisms to overcome the inappropriate effects of these compounds from the host on their digestion system. In addition, despite the significant change of nitrogen level in hazel leaves depending on fertilizing, larval nitrogen utilization, feeding performance and digestion efficiency indicated that larvae of this species utilized leaves of alder with greater nitrogen utilization efficiency.

There were differences between alder and willow, alder and hazel according to the degradation of some plant's secondary compounds by the larvae fed on them. In future studies, by using leaves from different plant species or artifical that diet formulations, the factors affecting feeding and development of this species can be determined more precisely. These will contribute to the understanding of food choice and utilization of oligophagous insects in general terms.

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