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Research Article

# Nutritional indices of the willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae) on different host plants

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**Abstract:** Nutritional indices of the willow leaf beetle, *Plagiodera versicolora* Laicharting, third instar larvae and adults were studied on four host plants including *Salix alba* L., *Salix aegyptica* L., *Populus caspica* Bornm. and *Populus alba* L. at 22 ± 2 ºC, 70 ± 10% R.H., and a photoperiod of 16:8 h (L: D). The results showed that the highest consumed food by the larvae and adults (148.5 ± 18.0 and 175.21 ± 4.51 mg dry weight, respectively) was on *P. caspica*. The larvae fed on *S. alba* and *S. aegyptica* had the highest efficiency of conversion of ingested food (ECI) (2.3 ± 0.33 and 2.23 ± 0.67%, respectively) and efficiency of conversion of digested food (ECD) (2.5 ± 0.35 and 2.41 ± 0.72%, respectively). Approximate digestibility (AD) of larvae was the highest on *P. caspica* and *P. alba* (98.51 ± 0.25 and 98.14 ± 0.1%, respectively). The lowest relative consumption rate (RCR) of the larvae and adults was on *S. alba* (0.14 ± 0.001 and 0.13 ± 0.006 mg/mg/day, respectively). ECI and ECD values of the adults fed on various host plants were not significantly different. Adults fed on *P. caspica* had the highest values of RCR (0.59 ± 0.01 mg/mg/day), relative growth rate (RGR) (0.02 ± 0.008 mg/mg/day) and AD (98.72 ± 0.24%). These results demonstrated the higher feeding performance of the willow leaf beetle on *P. caspica* and its poorer performance on *P. alba*.

**Keywords:** Nutritional indices; willow leaf beetle; *Plagiodera versicolora*; host plants

## Introduction

Due to increasing request for wood and woody products as well as declining of forest areas in Iran (Nordman *et al*., 2005), the cultivation of fast growing trees is being developed as a key goal of forest management (Daryaei *et al*., 2008). Also, because of producing a renewable feedback for bioproducts and enlarging rural profits, short-rotation woody crops such as poplars and willows are being industrialized as a supportable method (Nordman *et al*., 2005; Coyle *et al*., 2006).

The willow leaf beetle, *Plagiodera versicolora* Laicharting (Coleoptera: Chrysomelidae), is an expert herbivore of white poplar, aspens and willows, which can make important defoliation by feeding on host plants leaves both as larvae and adults (Ostry *et al*., 1989). It is reported that fresh leaves of host plants are preferred by *P. versicolora* adult more than full-grown ones (Raupp and Denno, 1983; Ikonen, 2002; Wait *et al*., 2002). However, the larvae prefer to feed on older leaves in groups (Çanakçıoğlu and Mol, 1998). This species is distributed in northern Africa, America, Europe, and Asia (Çanakçıoğlu and

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Mol, 1998; Aslan and Ozbek, 1999; Urban, 2005; Ishihara and Ohgushi, 2006), and northern and central provinces of Iran (Abaii, 2000).

One of the main tools to study the insect-plant interactions is the examination of consumption and utilization of host plants by insect herbivores (Scriber and Slansky, 1981). The required chemical components by an organism are named nutrition which is necessary for its growth, development, reproduction, energy supply and tissue preservation (Chapman, 1998). The importance of keeping the balance of nutrients is cleared in most insects (Dadd, 1985). In addition, growth, reproduction, diapause or migration is the factors that could affect the required nutrients of insects (Chapman, 1998; Nation, 2001). On the other hand, insects' ability to digest consumed food, converting it into biomass as well as the relative growth rate could have remarkable influence on the insects' performance (Price *et al.,* 1980). The host plants which are more appropriate for insect herbivores' growth and reproduction are intensively preferred by them (Futuyma and Moreno, 1988; Jaenike, 1990; Via, 1990). For instance, it is recommended that the salicylate-rich willows are preferred by larvae of beetles because they can preserve themselves from natural enemies on these host plants (Pasteels *et al*., 1988; Denno *et al*., 1990). Nutritional indices are calculated to evaluate insect growth and food utilization efficiency (Hare, 1998). Consumption of diet components, digestion efficiency and alteration of food to the insects' biomass are the factors that could be indicated by nutritional indices in order to deliver useful information on different influences of compounds or total food (Bhat and Bhattacharya, 1987). Understanding insects' response to host plants would be conceivable by examining the nutritional indices (Lazarevic and Peric-Mataruga, 2003).

Previously, effect of different host plants including *Salix aegyptica* L. (Malpighiales: Salicaceae) (musk willow), *S. alba* L. (Malpighiales: Salicaceae) (white willow), *Populus caspica* Bornm. (Salicales: Salicaceae) (caspian poplar) and *P. alba* L. (Malpighiales: Salicaceae) (white poplar) was determined on digestive proteolytic activity of *P. versicolora* (Zibaee and Hajizadeh, 2013). However, as no studies have been carried out on nutritional indices of *P. versicolora*, the goal of the current research was to evaluate how different host plants could affect the nutritional indices of *P. versicolora* adults and larvae. The outcome of such studies will provide helpful information for designing a successful pest management program against *P. versicolora*.

## Materials and Methods

### Host plants

Four host plants including *Salix alba*, *Salix aegyptica*, *Populus caspica* and *Populus alba* were used in this experiment. Leaves of the host plants were provided from Guilan University campus, Rasht (Guilan province, Iran) in summer 2013.

### Insect rearing and experimental conditions

The adults *P. versicolora* were collected from infested leaves of the examined host plants in Rasht, Guilan province in summer 2013. The adults and third instar larvae were used to determine the effect of different host plants on their nutritional indices. Twenty adults (10 males and 10 females) were placed in separate transparent plastic jars (6 × 8 cm), as oviposition containers, which had a hole covered by a fine muslin cloth for ventilation. After hatching, the neonate larvae were divided into five replicates (15 larvae in each replicate) and then placed in transparent plastic jars containing the leaves of test host plants. To provide moisture to the leaves, a piece of wet cotton was placed in each container and the leaves were replaced with fresh ones every day. The insect rearing was conducted under laboratory condition at 22 ± 2 °C, 70 ± 10% RH, and a photoperiod of 16:8 (L:D), until the third instar larvae appeared.

The third instar larvae and adults were left to starve for 12 hours before starting the experiment. The weights of provided and remained leaves after feeding were recorded daily. Larval weights (i. e. one-day-old third instar larvae) were recorded daily before and after feeding until they reached the pupal stage. The adults' weights were also measured before and after feeding on different host plants for 3 days regularly. The frass produced by larvae and adults was collected and weighed at the end of each day. Dry weight of the larvae, adults and their produced frasses as well as dry weight of the leaves were measured after drying at 60 °C for 48 hours. The following formulae (Waldbauer, 1968) were used to calculate nutritional indices of *P. versicolora*:

Approximate digestibility (AD) = (E - F)/E × 100

Efficiency of conversion of ingested food (ECI) = (P / E) × 100

Efficiency of conversion of digested food (ECD) = P / (E - F) ×100

Relative consumption rate (RCR) = E/($W_0$ × T)

Relative growth rate (RGR) = P / ($W_0$ × T)

where, T= feeding period (day), P= dry weight gained larvae or adults (mg), E = dry weight of food consumed (mg), F= dry weight of frasses produced (mg), $W_0$= initial weight of larvae or adults (mg).

**Statistical analysis**

Normality of the data was tested by Kolmogorov-Smirnov method. To find out significant differences, data were analyzed by one-way ANOVA using statistical software Minitab ver. 16.0 (Minitab Inc. 1994). Statistical differences among the means were assessed using the least significant difference test (LSD test, $p < 0.05$).

## Results

The highest dry weight of food consumed was in the larvae fed on *P. caspica* (148.5 ± 18.0 mg) ($F = 24.62$; df = 3, 16; $P < 0.05$). The dry weight of frasses produced by larvae was highest on *S. aegyptica* (6.48 ± 0.25 mg) and lowest on *P. alba* (1.6 ± 0.07 mg) ($F = 190.45$; df = 3, 15; $P < 0.05$). Dry weight gained of third instar larvae was highest (3.2 ± 1.28 mg) on *P. caspica* and lowest (0.37 ± 0.07) on *P. alba* ($F = 3.68$; df = 3, 15; $P < 0.05$) (Table 1).

Nutritional indices of larvae fed on the four host plants are shown in Table 2. The highest value of AD ($F = 69.00$; df = 3, 15; $P < 0.01$) was recorded in the larvae fed on *P. caspica* (98.51 ± 0.25%) and *P. alba* (98.14 ± 0.1%). The obtained data demonstrated that larvae fed on *S. alba* and *S. aegyptica* had the highest values of ECI (2.3 ± 0.33 and 2.23 ± 0.67%, respectively) ($F = 3.61$; df = 3, 15; $P < 0.05$) and ECD (2.5 ± 0.35 and 2.41 ± 0.72%, respectively) ($F = 3.89$; df = 3, 15; $P < 0.05$) compared with those fed on the other two host plants. However, the lowest values of ECI and ECD (0.44 ± 0.1 and 0.45 ± 0.1%, respectively) were in the larvae reared on *P. alba*. Our results showed that RCR value was highest ($F = 8.56$; df = 3, 15; $P < 0.05$) on *P. caspica* (0.78 ± 0.16 mg/mg/day) and lowest on *S. alba* (0.14 ± 0.005 mg/mg/day). Relative growth rate (RGR) was highest ($F = 4.06$; df = 3, 16; $P < 0.05$) on *P. caspica* (0.01 ± 0.007 mg/mg/day) (Table 2).

The highest dry weight of food consumed was recorded in adults fed on *P. caspica* (175.21 ± 4.51 mg) ($F = 90.09$; df = 3, 15; $P < 0.05$) and the lowest on *S. alba* (46.37 ± 2.24 mg). The dry weight of frasses produced by adults was not significantly different on the four host plants tested. The highest weigh gained of adults was on *P. caspica* (7.17 ± 2.57 mg) ($F = 4.6$; df = 2, 11; $P < 0.05$), however, this value was zero when adults were reared on *P. alba* (Table 3).

The nutritional indices of adults showed that AD value was the highest ($F = 21.94$; df = 3, 15; $P < 0.05$) on *P. caspica* (98.72 ± 0.24%) and the lowest on *S. alba* (92.67 ± 1.01%). There was no significant difference in ECI and ECD values in adults fed on *S. alba*, *S. aegyptica* and *P. caspica*. The highest value of RCR (0.59 ± 0.01 mg/mg/day) ($F = 131.37$; df = 3, 15; $P < 0.05$) and RGR (0.02 ± 0.008 mg/mg/day) ($F = 4.46$; df = 3, 11; $P < 0.05$)

was observed in adults fed on *P. caspica*. The adults reared on *S. alba* and *S. aegyptica* showed the lowest level of RCR (0.13 ± 0.006 and 0.16 ± 0.007 mg/mg/day, respectively) and RGR (0.004 ± 0.001 and 0.004 ± 0.002 mg/mg/day, respectively) (Table 4).

**Table 1** Mean (± SE) weights of food consumed, frass produced and weight gained of *Plagiodera versicolora* third instar larvae fed on various host plants under laboratory conditions.

| Host plants | E (mg) | F (mg) | P (mg) |
|---|---|---|---|
| *Salix alba* | 34.75 ± 0.67c | 2.59 ± 0.13b | 1.02 ± 0.14b |
| *Salix aegyptica* | 90.10 ± 4.51b | 6.48 ± 0.25a | 2.02 ± 0.61ab |
| *Populus caspica* | 148.50 ± 18.00a | 2.07 ± 0.09b | 3.20 ± 1.28a |
| *Populus alba* | 86.54 ± 2.56b | 1.60 ± 0.07c | 0.37 ± 0.07c |

E: dry weight of food consumed (mg), F: dry weight of frass produced (mg), P: dry weight gained (mg). The means followed by different letters in each column are significantly different **(**LSD test, $P < 0.05$**).**

**Table 2** Mean (± SE) nutritional indices of *Plagiodera versicolora* third instar larvae fed on various host plants under laboratory conditions.

| Host plants | AD (%) | ECI (%) | ECD (%) | RCR (mg/mg/day) | RGR (mg/mg/day) |
|---|---|---|---|---|---|
| *Salix alba* | 92.51 ± 0.46b | 2.30 ± 0.33a | 2.50 ± 0.35a | 0.14 ± 0.001c | 0.003 ± 0.0004b |
| *Salix aegyptica* | 92.70 ± 0.53b | 2.23 ± 0.67a | 2.41 ± 0.72a | 0.42 ± 0.03b | 0.009 ± 0.003ab |
| *Populus caspica* | 98.51 ± 0.25a | 1.62 ± 0.46ab | 1.65 ± 0.46ab | 0.78 ± 0.16a | 0.010 ± 0.007a |
| *Populus alba* | 98.14 ± 0.1a | 0.44 ± 0.1b | 0.45 ± 0.1b | 0.36 ± 0.02bc | 0.001 ± 0.0003b |

AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food, RCR: Relative consumption rate, RGR: Relative growth rate.
Means followed by different letters in each column are significantly different (LSD test, $P < 0.05$).

**Table 3** Mean (± SE) dry weight of adults, food consumed, frass produced and weight gained of *Plagiodera versicolora* adults fed on various host plants under laboratory conditions.

| Host plants | E (mg) | F (mg) | P (mg) |
|---|---|---|---|
| *Salix alba* | 46.37 ± 2.24d | 3.44 ± 0.53a | 1.48 ± 0.33b |
| *Salix aegyptica* | 66.95 ± 3.41c | 2.40 ± 0.11a | 0.97 ± 0.14b |
| *Populus caspica* | 175.21 ± 4.51a | 2.26 ± 0.47a | 7.17 ± 2.57a |
| *Populus alba* | 141.60 ± 13.8b | 3.25 ± 0.23a | 0 |

E: dry weight of food consumed (mg), F: dry weight of frass produced (mg), P: dry weight gained (mg). Means followed by different letters in each column are significantly different (LSD test, $P < 0.05$).

**Table 4** Mean (± SE) nutritional indices of *Plagiodera versicolora* adults fed on various host plants under laboratory conditions**.**

| Host plants | AD (%) | ECI (%) | ECD (%) | RCR (mg/mg/day) | RGR (mg/mg/day) |
|---|---|---|---|---|---|
| *Salix alba* | 92.67 ± 1.01c | 3.18 ± 0.64a | 3.43 ± 0.69a | 0.13 ± 0.006c | 0.004 ± 0.001b |
| *Salix aegyptica* | 96.40 ± 0.07b | 2.36 ± 0.85a | 2.45 ± 0.88a | 0.16 ± 0.007c | 0.004 ± 0.002b |
| *Populus caspica* | 98.72 ± 0.24a | 3.99 ± 1.38a | 4.05 ± 1.4a | 0.59 ± 0.01a | 0.020 ± 0.008a |
| *Populus alba* | 97.60 ± 0.35ab | _ | _ | 0.39 ± 0.04b | _ |

AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food, RCR: Relative consumption rate, RGR: Relative growth rate.
Means followed by different letters in each column are significantly different (LSD test, $P < 0.05$).

## Discussion

The process of herbivore host reception and suitability depends on physiological features and chemical characteristics of the host plants (Foss and Rieske, 2003). In fact, the host plant that insects pass their larval stages on it, does influence the ability of the insects to store energy (Liu *et al*., 2007). It is noticeable that the body weight is an important fitness index of insect population dynamics (Liu et al., 2004). According to the results of this study, among different host plants tested, the lowest weight gained of third stage larvae was detected on *P. alba*, suggesting its low-nutritive value for this pest. Also, it is well understood that the amount of food utilization is dependent on the efficiency of digested food to convert into biomass (Batista Pereira *et al.*, 2002). Although the amount of food consumed by the larvae fed on *P. alba* was approximately in an equal level compared to *S. aegyptica*, yet it had the lowest ECD. Also, amount of frass in larvae was lowest on *P. alba*, which is not in agreement with the findings of Daryaei *et al*. (2008), who reported the highest dry weight of frass produced by *Lymantria dispar* L. (Lymantriidae: Lepidoptera) fourth instar larvae when fed on *Populus euramericana triplo*. Such difference can be due to either variation in host plants or differences in the insect species. Data showed that the highest weight of food consumed and highest larval weight gained were in the larvae fed on *P. caspica*, indicating the palatability of food source for *P. versicolora*.

The current study showed that the highest ECI value of larvae was recorded on *S. alba* and *S. aegyptica* which can be attributed to the high ability of the larvae in converting the ingested food to body mass. In addition, higher ECD in the larvae reared on *S. alba* and *S. aegyptica* indicated the higher efficiency in modifying the digested food into growth. Furthermore, the larvae fed on *S. alba* had the lowest value of RCR with a high value of ECI. So, it could be concluded that *S. alba* was a suitable host plant for larvae, which could be due to some biological serving as feeding stimulants.

In our experiment, larvae showed the lowest ECI and ECD when fed on *P. alba*, suggesting less larval efficiency to convert the eaten food to body matter. However, the larvae fed on *P. alba* showed a high level of approximate digestibility. So, these larvae probably were forced to devour more food in order to obtain adequate nutrient. In general, the amount of food utilization could be strongly influenced by the digestibility of eaten food and the efficiency of conversion of digested food into biomass (Batista Pereira *et al*., 2002). Additionally, Lazarevic *et al.* (2004) reported that the activity of digestive enzymes affects feeding indices especially ECI and ECD values of insects. Therefore, one reason for differences in ECI and ECD values can be due to the differences in enzymatic activities in response to primary and secondary host plant metabolites. It is clear that such disagreements can be due to variation in the nutritional value of the host plant for the insect species.

Among various host plants, although amount of frass produced was not significantly different on four host plants, the highest food consumed and weight gained of *P. versicolora* adults was recorded on *P. caspica*, suggesting that this host was more suitable plant for adults than the other host species.

It could be accepted that the feeding performance of a herbivore insect is influenced by plant secondary metabolites that affect the digestive process (Chown and Nicolson, 2004), and the nutritional requirements of an insect that are directly associated with body biomass (Phillipson, 1981). In the current research, *P. versicolora* adults that fed on *P.alba* did not have weight gained, therefore we could not record ECI, ECD and RGR values on this host. In an earlier study, Zibaee and Hajizadeh (2013) found that trypsin-like enzyme activity of *P. versicolora* adults was lowest on *P. alba*, which can be related to the presence of protease inhibitors in this host plant (Saikia *et al*., 2011). Therefore, it can be concluded that *P. alba* is an unsuitable host for feeding of *P. versicolora* adults.

It is obvious that high RCR and RGR values are related to adult weight increase, and this weight gained could have a direct relation with ECI. In the current study, adults fed on *P. caspica* showed the highest values of ECI, RCR and RGR, suggesting that *P. caspica* was more nutritive for adults than other host plants tested.

The obtained data demonstrated that among four host plants examined, the amount of AD, in both larvae and adults, was low when the insect was reared on *S. alba*. According to Mattson (1980), the index AD has a close relationship with the nitrogen content of a host plant. Ikonen (2002) reported that the younger leaves of some willows with a high content of nitrogen are more nutritious for leaf beetles. Furthermore, Mattson (1980) and Scriber (1984) reported that the plants which have a high rate of nitrogen are preferred by numerous insects and nitrogen content could be as a proper indicator for assessing the plant quality. So, we suggest that *S. alba* has probably more nitrogen content than the other host plants. Although the larvae and adults could feed on *P. alba* adequately, they were seemingly unable to convert the eaten food into body matter. Thus, it could be well understood that *P. alba* is an unsuitable host plant for both larvae and adults.

In conclusion, the highest values of larval/adult weight gained and the RGR index, as well as high ECI and ECD values of willow leaf beetle were detected on *P. caspica*, hence, this plant is likely more nutritive and suitable host for feeding of this pest. In addition, since the lowest ECI and ECD values as well as the lowest weight gained for the third instar larvae were on *P. alba*, and no weight gained was observed by the adults fed on this host species, it could be suggested as the most unsuitable host for *P. versicolora*. Finally, more examinations on nutritional indices of *P. versicolora* on different species of its host plants, especially willows and poplars are required in order to understand the role of nutrition in growth and development of this pest. The obtained data will be helpful in designing new procedures to manage forest pests like *P. versicolora*.

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# شاخص‌های تغذیه‌ای سوسک برگخوار بید *Plagiodera versicolora* (Coleoptera: Chrysomelidae) روی میزبان‌های گیاهی مختلف

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**چکیده:** شاخص‌های تغذیه‌ای لاروهای سن سوم و حشرات کامل سوسک برگخوار بید، *Plagiodera versicolora* Laicharting، روی چهار میزبان گیاهی شامل بیدسفید .*Salix alba* L، بیدمشک *Salix aegyptica* L.، صنوبر *Populus caspica* Bornm و سپیدار .*Populus alba* L در شرایط آزمایشگاهی (دمای ۲ ± ۲۲ درجه ی سلسیوس، رطوبت نسبی ۱۰ ± ۷۰ درصد و دوره‌ی نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی) مطالعه شد. نتایج نشان داد که بیش‌ترین مقادیر وزن غذای خورده شده لاروهای سن سوم و حشرات کامل (به‌ترتیب ۱۸/۰ ± ۱۴۸/۵ و ۴/۵۱ ± ۱۷۵/۲۱ میلی‌گرم) روی صنوبر بود. لاروهای تغذیه کرده روی بیدسفید و بیدمشک بیش‌ترین بازدهی تبدیل غذای بلعیده شده (ECI) (به ترتیب ۰/۳۳ ± ۲/۳ و ۰/۶۷ ± ۲/۲۳ درصد) و بازدهی تبدیل غذای هضم شده (ECD) (به ترتیب ۰/۳۵ ± ۲/۵ و ۰/۷۲ ± ۲/۴۱ درصد) را داشتند. بیشترین شاخص تقریبی هضم شوندگی (AD) لاروها روی صنوبر و سپیدار (به‌ترتیب ۰/۲۵ ± ۹۸/۵۱ و ۰/۱ ± ۹۸/۱۴ درصد) بود. کم‌ترین نرخ مصرف نسبی (RCR) لاروها و حشرات کامل روی بیدسفید (به‌ترتیب ۰/۰۰۱ ± ۰/۱۴ و ۰/۰۰۶ ± ۰/۱۳ میلی‌گرم/میلی‌گرم/روز) بود. مقادیر ECI و ECD حشرات کامل تغذیه کرده بر روی میزبان‌های مختلف گیاهی تفاوت معنی‌داری نداشت. حشرات کامل تغذیه کرده روی صنوبر بیش‌ترین مقادیر RCR (۰/۰۱ ± ۰/۵۹ میلی‌گرم/میلی‌گرم/روز)، نرخ رشد نسبی (RGR) (۰/۰۰۸ ± ۰/۰۲ میلی‌گرم/میلی‌گرم/روز) و AD (۰/۲۴ ± ۹۸/۷۲ درصد) را داشتند. نتایج نشان داد که سوسک برگخوار بید روی صنوبر بیش‌ترین و روی سپیدار کم‌ترین کارایی تغذیه‌ای را در مقایسه با سایر میزبان‌های مورد آزمایش داشت.

**واژگان کلیدی:** شاخص‌های تغذیه‌ای، سوسک برگخوار بید، *Plagiodera versicolora*، گیاهان میزبان