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Effects of dandelion partial replacement on some biological traits of the silkworm, *Bombyx mori* L. (Lepidoptera, Bombycidae)

Original Article

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Abstract:

In this study, a total of 900 specimens of univoltine polyhybrid silkworm breed were reared across early, mid, and late breeding seasons. The objective of the presented work was to assess reported cases of rearing silkworm on alternative host plants and evaluate the feasibility of partially replacing the optimal food source, *Morus alba* L., with the year-round available *Taraxacum officinale* (L.) Weber ex F.H.Wigg., while assessing larval and pupal duration, L_5 larval weight, cocoon weight, as well as larval mortality and larval–pupal intermediates (unsuccessful pupae). While the substitution showed a statistically significant effect only for the larval stage duration, different temperature ranges affected all investigated traits. The optimal values were achieved in groups bred during the mid-season (19–24.5 °C), where differences in success between the control and the treatment were minimal. Further breeding will provide clearer insights into the limitations of this food replacement strategy within the system, depending on its research purpose.

Key words:
diet, dandelion, silkworm rearing

Apstrakt:

Efekat parcijalne zamene duda maslačkom na pojedine biološke karakteristike svilene bube, *Bombyx mori* L. (Lepidoptera, Bombycidae)

Za potrebe ovog istraživanja, kroz rani, srednji i kasni period sezone, odgojeno je 900 jedinki univoltnog polihibrida svilene bube. Cilj predloženog istraživanja bio je testiranje objavljenih navoda o gajenju svilenih buba na alternativnim biljkama hraniteljkama, kao i preliminarna ocena izvodljivosti parcijalne zamene optimalnog izvora hrane, belog duda, *Morus alba* L., lako dostupnim maslačkom, *Taraxacum officinale* (L.) Weber ex F.H.Wigg. Efekat zamene praćen je na nivou bioloških karakteristika, dužine trajanja stadijuma larve i lutke, težine L_5 gusenica, težine svilenih kokona, mortaliteta gusenica i frekvencije javljanja neuspešnih međuformi larva-lutka. Zamena je pokazala statistički značajan efekat na trajanje stadijuma larve, dok su različiti temperaturni opsezi uticali na sve ispitivane karakteristike. Optimalne vrednosti postignute su u grupama gajenim u srednjem delu sezone (19–24.5 °C), kada su razlike u uspehu gajenja između tretmana i kontrole bile minimalne. Kontinuirani uzgoj će obezbediti bolji uvid u odnos prednosti i ograničenja ove izmene režima ishrane, u kontekstu krajnjeg cilja istraživanja.

Ključne reči:
ishrana, maslačak, gajenje svilene bube

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Introduction

In addition to the honeybee and the common fruit fly, the silkworm, *Bombyx mori* L. is among the most thoroughly studied insects in the world (Cermeño et al., 2022). The silkworm's economic importance extends beyond its well-known role in textile production. It also serves as a valuable model organism in scientific research, particularly in the fields of endocrinology, genetics, and immunology. Additionally, silkworms are used as bioreactors and are of increasing interest in the fields of entomophagy (insect consumption as food) and nutraceutical development (development of food products with health benefits) (Abdelli et al., 2018). Domesticated for approximately five millennia, this species exhibits significant genetic diversity, comprising numerous geographical races, hybrids, and lines. Different

breeds require specific environmental conditions (Vimala et al., 2020), reflecting the agricultural trend towards optimizing voltinism, biomass yield, silk production, and resistance to pathogens. Breeding programs, particularly advanced in China (Xiang et al., 2018), India (Sharma & Kapoor, 2020), Korea (Kim et al., 2022), Japan (Onaga, 2012), as well as Italy (Cappellozza, 2015), Bulgaria (Tzenov et al. 2022), and Romania (Pop et al., 2018) for Europe, continuously adapt to ensure optimal genetic performance. Hybridization has further widened the diversity of biological and technological traits, leading to variations in larval and pupal duration, dietary preferences, artificial diet acceptance, temperature tolerance, cocoon characteristics, and silk filament properties (Altman & Farrell, 2022).

Native to China, the silkworm is a monophagous herbivore, requiring fresh leaves of the white mulberry, *Morus alba* L., a woody species native to the Himalayan region. Due to the long-standing process of co-evolution and natural selection between silkworms and mulberry trees, moriculture and sericulture have grown in tandem (Liu et al., 2023). Understanding the mechanisms behind the mulberry-specific feeding preference has been a subject of extensive investigation, encompassing neuroregulatory, metabolomic, and genetic aspects. This feeding specialization results from an interplay of gustatory and olfactory cues, along with factors that either promote or inhibit feeding behavior (Jia et al., 2020). Mulberry leaves contain three groups of factors that promote food intake: attracting factors such as α, β-hexenal, β, γ-hexenol, citral, linalyl acetate, and linalool; a biting factor (primarily β-sitosterol) and several swallowing factors, predominantly cellulose (Song et al., 2021).

Traditional sericulture, centered around feeding silkworms with fresh mulberry leaves, has historically served as a cornerstone for both moriculture and small farm establishments, offering numerous opportunities for economic growth in many regions. However, mulberry leaves are not readily available year-round, their quality decreases as the breeding season progresses, and they are susceptible to frost damage (Urbanek Krajnc et al., 2023). This is particularly important for bivoltine and polyvoltine breeds, but also in cases in which laboratories need to establish populations outside their usual breeding timeframe. Additionally, mulberry leaves can harbor pathogens that pose a risk to silkworm colonies, even with proper disinfection protocols in place. These factors make mulberry leaves unsuitable for certain types of research and laboratory experiments that require consistent dietary conditions for the model organism (Tao et al., 2022). Consequently, extensive research has been conducted to evaluate alternative feeding options, including different varieties of mulberry, supplementation, or fortification with vitamins, but most importantly, development of artificial and semi-artificial diets that mimic mulberry leaves chemical composition for standard rearing protocols. Although progress has been made in breeding silkworm varieties capable of efficiently utilizing artificial and semi-artificial diets, there are still limitations regarding the metabolic utilization of these diets compared to mulberry leaves (Qin et al., 2020), leading to challenges such as weak larvae and low silk yields. Even when the optimal nutritional ratio and efficient feeding and growth are achieved, metabolic utilization, convenience and exploring low-cost alternatives for artificial diets remain a critical focus for researchers in the field (Dai et al., 2022).

Before interest rose in developing agar-based semi-artificial diets, the twentieth century was marked with attempts on breeding *B. mori* on alternative plants, especially in the late-summer or autumn season for bivoltine breeds or in regions unsuitable for moriculture. Lambert (1917), Samokhvalova (1958, 1959, 1971), Golanski (1959), Samokhvalova et al. (1961, 1972), Arzone & Marletto (1975), Manchev (1981), Nasreen et al. (1999) and more recently El-Shewy & Elgizawy (2017) reported experiments with several alternative host plants. Underreporting of silkworm strain specifications, geographical differences, and non-standardized environmental conditions make systematization in most publications on the subject impossible. This study aims to organize existing literature on the experimental use of alternative host plants based on the reported outcomes. Given the promising results regarding *T. officinale*, the study further focuses on comparing the biological traits and rearing performance of two feeding modalities. The study will investigate the impact of breeding season (temperature conditions and host plant quality) on rearing performance not focused on silk yield. Two diets will be tested: the preferred host plant, *M. alba*, and a combination of *M. alba* with *T. officinale*, which is readily available and easily cultivated in laboratory settings.

Materials and Methods

To systematize previous attempts at silkworm rearing on non-mulberry natural diets, existing reports on experimental trials designed as comparisons between mulberry diet and alternative host plants were retrieved via Google Scholar search engine without publication time limitations. The performed queries included the following phrases: "*bombyx mori alternative host plants*" "*silkworm*

alternative host plants" "*non-mulberry silkworm diets*", and "*silkworm adaptability*". Afterwards, the publications were treated as separate research cases based on the author or group of authors and scored by the overall reported success of the rearing program. For the experiment, the egg batches were obtained from CREA – AA (Padua, Italy), specifically double-cross (126x125) x (129x127) nr.1a, produced in June 2022, and stored at 4° C until the beginning of the trials. To investigate the impact of fluctuating temperatures during early, mid, and late season (roughly April, May, and June for univoltine breeds), the rearing was conducted at room temperature, which was recorded daily, at LD 16:8 photoperiodic conditions. Fresh mulberry leaves were collected from a local variety tree, grown without the use of pesticides, within the garden of the Faculty of Sciences and Mathematics, University of Niš, Serbia. Dandelion plants were sourced from pristine environments during the entire season. In the beginning of each experiment, 300 newly hatched larvae were carefully collected using a fine brush and transferred into perforated plastic petri dishes (10 larvae per dish). They remained in the petri dishes until the first molt was completed to secure higher humidity favorable for young larvae (Lakshminarayana et al., 2002). *Ad libitum* feeding regiment comprising mulberry (the control), and a combination of mulberry and dandelion in a 3:7 ratio (the treatment) was introduced immediately post-hatching. From the second instar onwards, the caterpillars were reared in perforated plastic containers. Prior to use, the leaves of both plants were disinfected and dried as described in Ayandokun & Alamu (2020). Mortality was monitored daily and calculated as cumulative rate (total number of dead larvae in all instars per group). All successful L_5 larvae were weighed before pupation. Container cleaning was performed as required. The number of unsuccessful pupae (larval-pupal intermediates) was recorded for each group. Upon completion of feeding, the caterpillars were transferred to wooden frames covered with muslin cloth to facilitate cocoon spinning. Pupal duration and cocoon weight were recorded accordingly. Data analysis was performed in R Studio environment for statistical computing. As all four recorded traits displayed non-normality, to reveal effects of factors (season and diet) on continuous variables (larval duration, larval weight, pupal duration, pupal weight) was subjected to the non-parametric alternative, Kruskal–Wallis analysis, with Dunn's post hoc test for pairwise comparison. The relatedness between the abovementioned factors and categorical dependent variables (occurrence of intermediates and cumulative larval mortality) was conducted through Pearson's Chi-squared test, followed by the Bonferroni post hoc test in R Studio environment.

Results and discussion

The literature survey identified 22 alternative host plants. Among these, *Scorzonera hispanica* L., *Lactuca sativa* L., and *Taraxacum officinale* (L.) Weber ex F. H. Wigg. stood out due to the significant research interest they have attracted. This conclusion is based on the total number of authors and rearing attempts associated with these plants (**Tab. 1**).

Table 1. Non-mulberry *Bombyx mori* host plants tested under laboratory conditions

Alternative host plant	Rearing performance	References*
Bougainvillea glabra Choisy	partially successful	El-Shewy & Elgizawy (2017)
Carpinus betulus L.	failed	Golanski (1959)
Cichorium intybus L.	failed	Lambert (1917)
Citrus medica L.	failed	Nasreen et al. (1999)
Cudrania triloba (Carrière) Bureau	successful	Lambert (1917)
Ficus religiosa L.	successful	Nasreen et al. (1999)
Ficus retusa L.	partially successful	El-Shewy & Elgizawy (2017)
Lactuca sativa L.	failed	Lambert (1917), Golanski (1959), Samokhvalova (1976), Nasreen et al. (1999), El-Shewy & Elgizawy (2017)
Lantana camara L.	partially successful	El-Shewy & Elgizawy (2017)
Maclura pomifera (Raf.) C.K. Schneid.	failed	Lambert (1917)

Malus domestica (Borkh.) Borkh.	failed	Nasreen et al. (1999)
Prenanthes alba L.	failed	Lambert (1917)
Pyrus communis L.	failed	Nasreen et al. (1999)
Scorzonera hispanica L.	successful	Lambert (1917), Golanski (1959), Samokhvalova et al. (1961), Samokhvalova (1959, 1976), Arzone & Marletto (1975)
Taraxacum officinale Weber ex F. H. Wigg.	partially successful	Lambert (1917), Golanski (1959), Samokhvalova et al. (1961), Samokhvalova (1971), Manchev (1981), Samokhvalova (1976)
Tilia sp.	unknown	Samokhvalova (1976)
Tragopogon porrifolius L.	successful	Lambert (1917)
Tussilago farfara L.	unknown	Lambert (1917)
Ulmus parvifolia Jacq.	partially successful	Khnournuti et al. (1965)
Ulmus pumila L.	partially successful	Lambert (1917), Golanski (1959), Samokhvalova (1976)
Urtica dioica L.	failed	Lambert (1917), Samokhvalova (1976)
Vitis vinifera L.	failed	Nasreen et al. (1999)

* – and references cited therein; **partially successful** – to either a certain Lx, with reduced economic value, high mortality, or successful if combined with other alternative host plants; **successful** – in multiple generations, selection advised.

For the experimental part of the study, dandelion was chosen as the most convenient alternative host plant due to its availability and ease of cultivation. However, previous attempts highlighted limitations, as dandelion was found suitable only for the first three instars, with stunted growth and developmental issues reported. To address this, the experimental design incorporated a partial substitution strategy, supplementing dandelion with a small amount of white mulberry.

All described modalities were successful in terms of reaching the adult stage. Overall, mean values recorded for larval duration, larval weight, pupal duration, and pupal weight, as well as the mortality rate and the percentage of unsuccessful pupae (**Tab. 2**), indicate effects of temperature variations on the development of silkworm. Lower temperatures of the early season (ranging from 19 °C to 22 °C) resulted in prolonged larval development, while higher temperatures, typical of the late season, together

Table 2. Biological traits recorded during different parts of the silkworm breeding season (mean ± SD)

Season	Diet regiment	Temp. range (˚C)	Larval stage (days)	Cumulative larval mortality (%)	L_5 weight (g)	Larval-pupal intermediates (%)	Pupal duration (days)	Cocoon weight (g)
Early	***ma***	19-22	36.50±1.68	0.06	3.61±0.32	10.64	9.90±1.60	1.77±.92
	com		37.52±1.71	0.06	3.65±0.33	12.77	10.51±1.75	1.71±.07
Mid-	***ma***	19-24.5	30.60±1.44	0.02	3.73±0.31	8.16	9.07±1.53	1.80±.07
	com		28.08±1.43	0.04	3.71±0.30	6.25	8.89±1.37	1.79±.07
Late	***ma***	21.5-32	25.92±0.80	0.08	2.92±0.34	17.39	7.86±1.22	0.70±.11
	com		25.84±2.56	0.12	1.94±0.47	20.55	7.64±0.93	0.68±.17

ma – mulberry leaves diet, com – dandelion-mulberry diet, L5 – fifth larval instar

with the large temperature fluctuations (ranging from 21.5 °C to 32 °C) led to accelerated cocooning and thus, much smaller caterpillars and cocoons. These findings align with most of the previous studies, which suggest that consistent optimal or slightly fluctuating temperatures complement the larval development (Zambrano-González et al., 2023). Additionally, these observations are consistent with expectations considering the temperate European climate (Hăbeanu et al., 2023), and the breed used for the experiments.

The mean number of days spent in the larval stage, as reported in **Table 1**, appears consistent based on the mean values for all seasons and feeding modalities. Regarding the investigated seasons, the Kruskal-Wallis test revealed significant differences for larval duration ($H=256.97$, $p<0.01$), larval weight ($H=192.29$, $p<0.01$), pupal duration ($H=28.41$, $p<0.01$), and cocoon weight ($H=107.12$, $p=0.03$). Subsequent Dunn's pairwise comparison indicated significant differences among all three season groups for each of the four continuous variables (adjusted $p<0.01$). However, Pearson's Chi-squared test found no statistically significant difference between season and larval mortality ($p=0.07$), although a significant association was observed between season and the occurrence of larval-pupal intermediates (X-squared=7.67, $p=0.02$). Further analysis using the Bonferroni post hoc test demonstrated that the mid-season group significantly differed from both the early season and late season groups ($p = 0.04$), which can potentially be explained by the lower occurrence of intermediates during the mid-season period.

Kruskal–Wallis test showed significant differences among groups based on feeding modality ($H=72.77$, $p<0.01$), which also reflects in the higher standard deviation of values on combined diet. However, further testing did not show statistically significant differences for larval weight, pupal duration, nor cocoon weight. Chi-sqaure test revealed no statistical significance for the larval mortality nor the occurrence of larval–pupal intermediates.

Based on this study, it can be concluded that the mid-season (May), characterized by slightly fluctuating temperatures within specific ranges, provides optimal conditions for silkworm growth and development. In terms of the inspected biological traits, the treatment yields results comparable to those of the control, the mulberry diet. While the early season (April, as soon as the buds open) also provides satisfactory results, it is associated with a considerably higher mortality rate compared to mid-season rearing. In contrast, late-season rearing (June) is marked by a general decrease in biomass and a noticeable difference in progress among feeding modalities. Caterpillars fed a combined diet lag by approximately 30% in terms of larval weight during late season rearing. This could be explained by the decline in dandelion quality, potential inhibitory effects on metabolism, and stress induced by high temperatures (Filippo et al., 2022).

With Europe reembracing both traditional sericulture and *B. mori* research (Marzoli et al., 2022), investigating partial replacement of mulberry with dandelion explored in this study can be of practical value, especially for laboratories trying to establish or maintain insect stocks. The standard mulberry diet can be challenging due to its seasonal availability, vulnerability to frost, and logistical constraints. The presented findings suggest that integrating dandelion into silkworm diets could offer a viable alternative, particularly during periods when mulberry is scarce, and when the breeding goal is not primarily silk yield. As suggested in the previous studies, acceptability of non-mulberry hosts can be increased through selective breeding (Kim et al., 2022). The growing interest in entomophagy, where silkworm stands out as the most promising candidate within the Lepidoptera order (Van der Fels, 2015), will require exploring the impact of non-mulberry hosts on the silkworm nutritional value. Although our study revealed satisfactory larval growth and viability on the altered diet, in respect to the most optimal breeding season, in-depth understanding of this host-plant–herbivore interaction will allow utilization of silkworm in more diverse contexts.

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