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Effect of Different Sowing Seasons, Growth Stages, Leaf Positions, and Soybean Varieties on the Growth of *Clanis bilineata tsingtauica* Mell Larvae

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Abstract: *Clanis bilineata tsingtauica* Mell (Lepidoptera: Sphingidae, CBT), as a traditional edible insect, is becoming popular in China due to its high nutritional value, but production needs to be improved to meet the expanding market. In the present study, CBT eggs were artificially inoculated on soybean leaves to evaluate the effects of leaf position, growth stage, sowing season, and soybean variety on CBT larval growth, respectively. The results showed that (1) the larval weight and survival rate were poorly correlated, so they could represent two different larval growth and development indicators for CBT. The 21-day-old larval weight was significantly different between the sowing seasons and between soybean growth stages, which was suitable as a key indicator for evaluating CBT larval rearing factors. (2) Compared with autumn-sown soybeans, the weight of 21-day-old larvae feeding on V6 stage (sixth trifoliolate) leaves of summer-sown soybeans was significantly higher, with an average increase of 44.7%. (3) Under autumn sowing conditions, the weight of 21-day-old larvae feeding on soybeans in the V6 stage was significantly higher than those fed on soybeans in the R3 stage (beginning pod), increasing by 33.9%. (4) Under summer sowing conditions, the weight of 21-day-old larvae feeding on the third-top leaf (the third leaf from the top of the soybeans' main stem) was significantly higher than those feeding on the third-bottom leaf (the third leaf from the bottom of the soybeans' main stem) at V6 stage by 35.7%. Similar results also appeared in autumn sowing; the average weights of 21-day-old larvae feeding on the third-top leaf increased significantly by 29.9% compared to those feeding on the third-bottom leaf. Moreover, the survival rate of larvae fed with the third-top leaf was significantly higher than that of those fed with the third-bottom leaf at the V6 stage in autumn sowing. Leaf position is the main factor affecting the survival rate of larvae. (5) Under summer sowing conditions, the weights of larvae fed with the third-top leaf of the susceptible-soybean varieties NN89-29 and NN1138-2 were significantly higher than that of those fed with the third-bottom leaf of these varieties. This difference was significantly reduced with autumn sowing. In conclusion, the CBT eggs inoculated on the third-top leaf of NN89-29 and NN1138-2 at the V6 stage in summer sowing could achieve maximum larval yield.

Keywords: *Clanis bilineata tsingtauica* Mell; evaluation indicator; sowing season; soybean growth stage; leaf position; soybean variety

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1. Introduction

With the global population rapidly increasing and changing socio-demographics, more attention is being paid to food nutrition, especially protein [1,2]. Insects are the most diverse species on earth, making them an alternative source to traditional protein for more environmentally sustainable production [3,4]. Insects are extremely efficient at converting organic matter into animal protein and food energy [5]. In addition, the high nutritional value and low cost of insects make them suitable as human food and animal feed [6,7]. In addition to their high protein content, insects are also rich in trace minerals and vitamins [8,9], such as iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, and zinc [10]. Furthermore, insects can use their specialized metabolic systems to convert secondary metabolic products into compounds with therapeutic properties useful to mankind [11]. For instance, the black soldier fly, *Hermetia illucens* (Linnaeus, 1758), produces inducible antibacterial peptides that have in vitro activity against *Helicobacter pylori* (Marshall and Goodwin) [12].

C. bilineata tsingtauica (Lepidoptera: Sphingidae, CBT), as a leaf-chewing insect, exhibits a strong preference for feeding on soybeans [13]. The history of these larvae being edible can be traced back to the Qing Dynasty, three hundred years ago, in China [14]. The meat of CBT larvae, delicious and rich in nutritional value, is very popular with local consumers in China [15]. The protein content of CBT larvae is of up to 66% dry weight, which is a good source of high levels of protein [16]. The larval content of vitamin E reaches 98.00 mg/kg, alongside the content of vitamin B1 and B2 at 0.22 and 6.80 mg/kg, respectively [17]. At present, CBT larvae can be processed into different types of common food, including freeze-dried, fried, fresh meat, and canned fresh meat [18].

Traditionally, the primary method of obtaining CBT larvae has been through capturing them in the wild. However, with the promotion of CBT and recognition from consumers, relying solely on manually capturing wild larvae has become insufficient to meet the market's growing demand for CBT [19]. Therefore, people have begun to explore more systematic and sustainable methods of artificial rearing [20,21]. Due to a lack of artificial feed in current CBT rearing, using soybean plants for rearing in net houses and fields, which are covered with nets on the top and surrounded by nets to prevent larvae from escaping and being preyed upon by natural enemies, have become the most economical and effective methods [21]. Moreover, through reasonable cultivation management measures, not only soybeans but also a considerable amount of CBT can be harvested [20]. Farmers collect mature larvae from the fields in autumn and place them in greenhouses (25 °C, 70% RH) to overwinter [17]. The greenhouse temperature is then increased the following spring to allow the pupae to metamorphose into moths for mating and laying eggs. They place the insect eggs on the egg card and then use a stapler to secure them on the back of soybean leaves [19]. After hatching, the larvae feed freely until the fifth instar for harvesting. This process not only ensures the quality of the CBT larvae, but also greatly improves the efficiency and yield of rearing. The income from CBT rearing is much higher than that from growing soybeans, with a notable 108% increase in income compared to ordinary soybean fields [20].

Plants have evolved a diverse array of defensive mechanisms against biotic and abiotic stresses [22]. Herbivorous insects use diverse feeding strategies to obtain nutrients from their host plants, and plants respond to herbivory with the production of toxins and defensive proteins that target physiological processes in insects [23]. When plants are attacked by insects, they quickly respond by secreting toxic compounds at the insect feeding sites, leading to insect diapause or death [24]. In order to evaluate the resistance of different varieties of plants to insects, methods such as antibiosis, antixenosis, and tolerance identification are usually used to evaluate the strength of plant resistance to insects [25–27].

Different leaf positions on plants exhibit varied insect-resistance levels. The induction of soybean defense against herbivory *Spodoptera cosmioides* predominantly relies on the leaf position and plant genotype [22]. The expression level of the ANNEXIN1 gene, which mediates the systemic defense in plants attacked by herbivorous insects, varies at different leaf positions [28]. The expression of Bt toxin varies among different leaf positions of cotton during the same growth period [29]. Furthermore, the upper soybean trifoliates display higher *S. cosmioides* larvae consumption and growth rates than the lower soybean trifoliates [22]. The potato leafhopper, *Empoasca fabae* (Harris), prefers to oviposit on the newest (i.e., apical) part of leaves or stem tissue over older tissue in soybean plants [30]. The selection of leaf positions seems to affect the survival rate of the larvae. Feeding from leaves in different positions has varied impacts on the growth of the rice leaf roller, *Cnaphalocrocis medinalis* (L.) [31].

Host plants show different resistances to herbivorous insects in different growth stages of plants. *Chrysanthemum* sp. resistance against *Frankliniella occidentalis* was significantly affected by different growth stages [32]. The reproductive rate of aphids feeding on the winter wheat leaves at the ear pregnancy stage was higher than on the leaves of the flowering and filling stages [33]. The larval weight of cotton earworm feeding on soybean leaves prebloom after 12 days was significantly higher than that of larvae fed during the bloom or pod-fill stage [34].

The soybean's genotype plays a decisive role in the plant's resistance to insects. Based on the level of resistance, soybean varieties can be roughly classified as either insect resistant or susceptible [35]. In traditional soybean breeding, highly insect-resistant varieties are usually selected. However, when evaluating soybean varieties for rearing CBT, the criteria are reversed. Therefore, to better identify and screen the plants for herbivorous insects' rearing, we have introduced the concept of plant adaptability to insects, which is based on the definition of plant resistance to insects. Plant adaptability to insects means that the host plant can quickly increase their larval weight and improve the larval survival rate.

The CBT larvae rearing is expected to be affected by leaf position, growth stage, soybean genotype, and sowing season, factors that are likely determinants of larval growth rate. Under spring sowing conditions, low larval yield was caused by excessively high planting density, whereas appropriately increasing insect density and planting density in autumn sowing can increase larval yield [36]. Meanwhile, different suitable cultivars should be chosen for different seasons, according to local climatic and environmental factors [37]. In summary, precise sowing seasons, combined with corresponding cultivation factors, are of primary influence in increasing production, reducing costs, and ensuring larval health.

The market prospects for CBT larvae rearing are very good [18]. At present, the production of CBT larvae is at approximately 30,000 tons per year, with an output value of CNY 4.5 billion (about USD 620 million), but this cannot meet the annual consumption demand of 100,000 tons per year [38]. Furthermore, insufficient exploration of the optimal growth conditions for CBT larvae has resulted in the inability to rapidly increase larval yield [36]. The leaf-feeding insect-sensitive soybean varieties, NN1138-2 and NN89-29, along with the resistant variety, Lamar, were used as the test materials in the present study. The effects of leaf positions, growth stages, sowing seasons, and soybean varieties on CBT growth were compared through forced feeding in mesh bags. The purposes of this study are to (1) screen the evaluation indicators of soybean adaptability to CBT larvae; (2) compare the differences in larval rearing between the summer and autumn sowing seasons, and propose season-specific varieties and corresponding egg inoculated leaf positions for different sowing seasons; (3) investigate the effects and interactions of different soybean growth stages and leaf positions on the larval growth in autumn, as well as the interactions between soybean varieties and egg–inoculated-leaf positions. Based on the above, we aim to lay the foundations for screening the adaptability of soybean varieties to CBT in the future.

2. Materials and Methods

2.1. Test Insects

The eggs of *C. bilineata tsingtauica*, laid on the same day, were obtained from the professional rearing base in Lianyungang, Jiangsu Province, China. These eggs were artificially sterilized and used in experiments.

2.2. Experimental Conditions and Materials

The experiments were carried out at the Wanjiang Base of Nanjing Agricultural University (118°37′ 22′′ E and 31°33′ 4′′ N), Dangtu County, Ma'anshan City, Anhui Province, China. The field temperature, humidity, light intensity, and sunshine duration from 15 May 2022 to 12 October 2022 were monitored using automatic insect monitoring (AIM) which was developed by Pherobio Technology Co., Ltd. (Yangling, China) [39]. AIM was installed in the soybean test field, with its accompanying meteorological-environment monitor (Figure 1A). The experimental fields were subjected to conventional agricultural management. Insecticides were prohibited during the experiments. Net houses (length: 6 m; width: 4 m; higth: 2 m) were used, and all were spliced with a steel pipe skeleton structure covered with white nylon mesh (40 mesh screen) (Figure 1B). Small mesh bags (15 cm × 20 cm) with a one-sided opening and a drawstring were used to pick up the eggs, which were replaced with a large mesh covering (30 cm × 40 cm) 15 days after inoculating the eggs (Figure 1C). In the meantime, we refer to this larval-rearing method as a bagging isolation feeding method. In previous experiments, we found that the CBT larvae have weak activity abilities before the third instar and only feed near the leaves where eggs are inoculated (Figure 1D). Hence, the choice of leaf position to inoculate eggs has a significant effect on larval yield. For this, we chose the third-top leaf and third-bottom leaf which were stuffed into the bag containing the eggs to inoculate the eggs, respectively. The highly susceptible-soybean varieties NN89-29 [36] and NN1138-2 were used as test varieties, and the insect-resistant variety Lamar [40] as the control variety to feed the larvae.

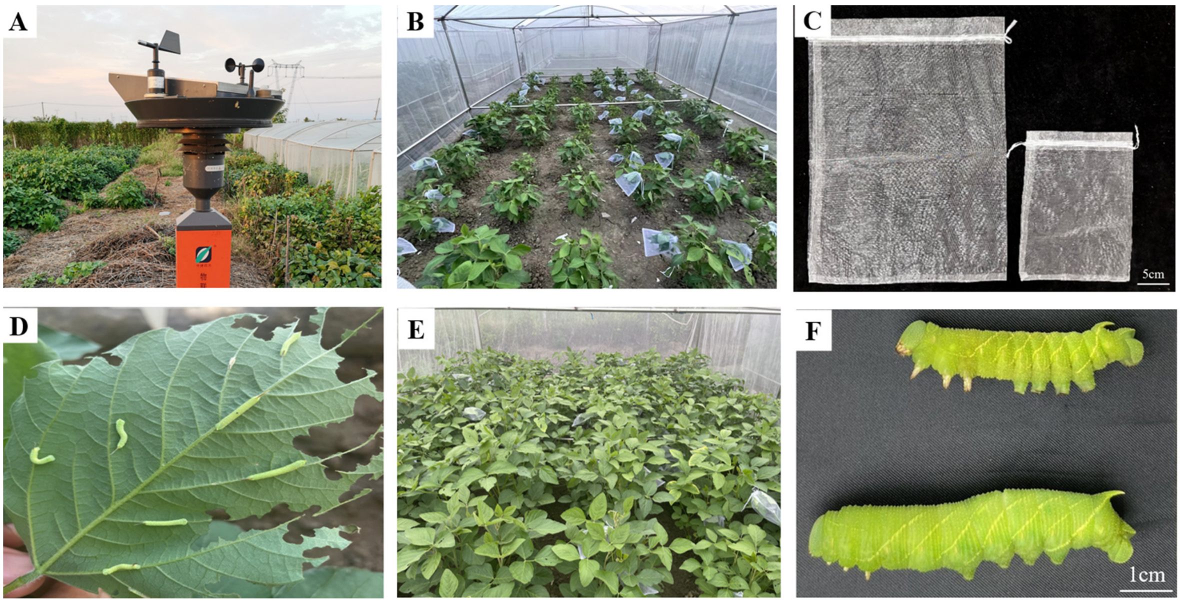

Figure 1. The cultivation mode of soybean and rearing process of CBT larvae in the experiments. (**A**) Automatic insect monitoring (ATM) with a meteorological-environment monitor in the soybean test field; (**B**) the internal structure of the net houses (length: 6 m; width: 4 m; height: 2 m) with soybeans planted in autumn sowing; (**C**) large mesh bags (30 cm × 40 cm) and small mesh bags (15 cm × 20 cm); (**D**) 9-day-old CBT larvae after eggs were inoculated artificially in the soybean; (**E**) soybean planted in the net house in summer sowing, and larvae fed on leaves in the mesh bag; (**F**) 21-day-old larvae fed on different leaf positions of NN1138-2, harvested on 28 July 2022, with the lower larvae inoculated at the third-top leaf and the upper larvae inoculated at the third-bottom leaf.

2.3. Evaluation of CBT-Rearing Factors under Summer Sowing Condition

The experiment was arranged in a completely randomized design. Six treatments were evaluated, composed of combinations of three soybean varieties (NN89-29, NN1138-2 and Lamar) and two soybean leaf positions (the third-top leaf and the third-bottom leaf). Nine replications were used per treatment. The soybeans were planted with 0.6 m × 0.6 m spacing in the net houses with 54 holes. The soybean seeds were sown using dibbling on 6 June 2022 in the summer sowing experiment. The planting density was reduced to three plants in each hole when soybeans grow their first compound leaves. The CBT eggs were inoculated artificially in the V6 stage (sixth trifoliolate) [41]. Two bags of eggs (15 eggs per bag) were inoculated at the third-top leaf and the third-bottom leaf in each hole on 7 July, respectively. If the leaf was consumed completely by larvae, the larvae were transferred to another leaf at the same leaf position of the same hill (Figure 1E). The first weighing was carried out on the ninth day after the eggs hatched and consisted of five larvae with similar growth. We replaced the small mesh bags with large mesh bags to meet the increasing food consumption of larvae on the third weighing (Figure 1C), and then, every three days thereafter, until a total of five weighings were performed. At the fifth weighing, the larvae were harvested which was on 28 July 2022 (Figure 1F).

2.4. Evaluation of CBT-Rearing Factors under Autumn Sowing Condition

The experiment was arranged in a completely randomized design. Twelve treatments were considered, composed of combinations of two soybean growth stages (V6 stage and R3 stage) [41], three soybean varieties (NN89-29, NN1138-2, and Lamar), and two soybean leaf positions (the third-top leaf and the third-bottom leaf). Nine replications were used per treatment. The same sown and inoculated treatments of summer sowing were employed. The soybean seeds were sown on 18 July 2022 during autumn sowing. Then, eggs were inoculated artificially at the V6 stage and R3 stage on 19 August and 13 September, respectively. The first weighing was carried out on the ninth day after the eggs hatched and retained five larvae with similar growth, and then, every three days thereafter, until a total of five weighings were performed. The larvae were harvested on 4 October 2022.

2.5. Statistical Analyses

The experimental data was recorded using Microsoft Excel (2013) software and was analyzed using analysis of variance (ANOVA), with multiple comparisons and a correlation analysis using SAS 9.4 software (SAS Institute 2013, Singapore). The ANOVA was run using the PROC GLM process, and multiple comparisons were completed using Duncan's method. Three-way ANOVA (sowing seasons, leaf positions, and soybean varieties) were used to analyze both the different-day-old and 21-day-old larval weight in the soybeans' V6 stage. Additionally, three-way ANOVA (growth stages, leaf positions, and soybean varieties) was used to analyze both the different-day-old and the 21-day-old larval weight in autumn sowing. Tables and graphs were made in Microsoft Excel, Microsoft Word (2013) and GraphPad Prism 8.0.2. Average larval weight = plot-larval weight/plot-larval number. Larval survival rate = (plot-larval number/the 9-day-old retained plot-larval number) × 100%.

3. Results

3.1. Meteorological Conditions for the CBT Rearing of Summer and Autumn Sowing Seasons

In the summer sowing trial, the temperature gradually increased, while the temperature gradually decreased during autumn sowing. The average temperature during summer sowing was 31.6 °C, which was higher than the average temperature during autumn sowing (28.7 °C) (Figure 2A). The average humidity was more stable in summer sowing than in autumn sowing. The average humidity recorded around 13 August was as low as 66.4% in autumn sowing, followed by a fast increase (Figure 2B). The average light intensity was slightly lower in summer sowing than in autumn sowing, with the highest light intensity recorded reaching 11.3 thousand lux around 13 August 2022 (Figure 2C). The average sunshine duration was longer during summer sowing than in autumn sowing. And, the average sunshine duration overall little during the summer sowing trial, whereas it gradually decreased in the autumn sowing trial (Figure 2D).

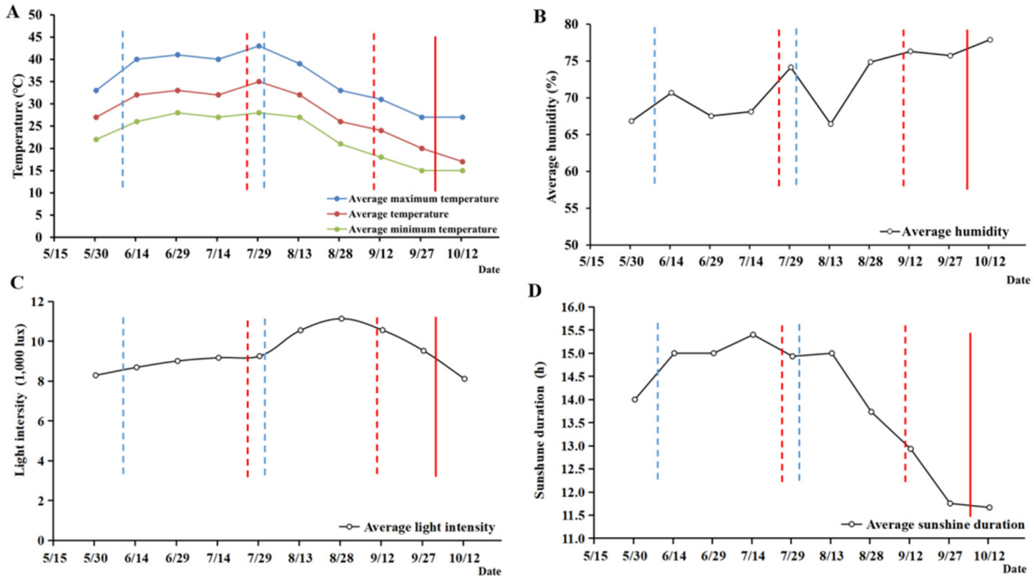

Figure 2. The trend of average meteorological factors every 15 days during summer and autumn sowing. (**A**) Temperature; (**B**) humidity; (**C**) light intensity; (**D**) sunshine duration. The insect-rearing duration at V6 stage for inoculating eggs during summer sowing is shown between the blue dotted lines, and the duration for inoculating eggs at V6 stage during autumn sowing is shown between the red dotted lines. The red solid line indicates the end of the test of eggs inoculated at R3 stage in autumn sowing.

3.2. Effects of Different Rearing Factors on the Growth of Different-Day-Old CBT Larvae

3.2.1. Effects of Different Leaf Positions and Soybean Varieties at V6 Stage in Different Sowing Seasons on the Growth of CBT Larvae

The weight of different-day-old CBT larvae feeding in the V6 stage showed significant differences among the sowing seasons, varieties, and leaf positions. The differences between leaf positions and among varieties overall gradually increased with larvae growth. The maximum *F*-value of leaf positions and varieties both occurred for 21-day-old larvae, which were 67.9 ($p < 0.01$) and 93.3 ($p < 0.01$), respectively (Table 1). Only the survival rate of 18-day-old larvae was significantly different between sowing seasons, while the others were not significantly different. Survival rates of 12-, 15-, 18-, and 21-day-old larvae were significantly different between leaf positions, with the highest *F*-value at 15-day-old larvae ($F = 11.7$, $p < 0.05$). Only the survival rate of 12-day-old larvae showed significant differences among varieties. The weight of 21-day-old larvae was significantly different between the sowing seasons, among varieties, and between leaf positions, making it suitable as a key evaluation indicator to compare differences among CBT-rearing factors in the V6 stage. Compared to the larval weight, the larval survival rate was easily affected by larval ages. In other words, the differences among the levels of rearing factors are not easily detected using the larval survival rate.

Significant differences for all ages regarding larval weights were observed among the interactions between the sowing season and leaf position, with a maximum *F*-value for the 15-day-old larvae ($F = 41.2$, $p < 0.01$). The interactions between sowing season and variety had a significant effect on larval weight, except in 9-day-old larvae, with a maximum *F*-value at 15-day-old larvae ($F = 10.1$, $p < 0.05$). The larval weight at all ages were significantly affected by interactions between leaf position and variety, with a maximum *F*-value for 18-day-old larvae ($F = 18.2$, $p < 0.01$). And, the interactions of sowing season, leaf position, and variety had a significant effect on the weight of 12-, 15-, 18-, and 21-day-old larvae, with a maximum *F*-value for 18-day-old larvae ($F = 12.6$, $p < 0.01$). The larval survival rates at all ages were significantly affected by interactions between the sowing season and leaf position, with a maximum *F*-value for 15-day-old larvae ($F = 13.5$, $p < 0.05$). However, the interactions between sowing season and variety, and leaf position and variety, both had an insignificant effect on survival rate. The interaction of sowing season, leaf position, and variety only had a significant effect on the survival rate of 15- and 18-day-old larvae.

Table 1. Three-way analysis of variance of different sowing seasons, leaf positions, and varieties for the different-day-old larvae growth indicators of eggs inoculated at the soybean V6 stage.

Source of Variation	*DF*	LW9	LW12	LW15	LW18	LW21	LSR12	LSR15	LSR18	LSR21
SS	1	37.2 **	178.4 **	126.3 **	169.7 **	119.3 **	2.5	1.8	5.8 *	0.2
LP	1	34.2 **	28.1 **	66.6 **	63.1 **	67.9 **	9.8 *	11.7 *	7.0 *	4.9 *
V	2	18.5 **	16.6 **	42.1 **	73.9 **	93.3 **	3.5 *	0.8	0.7	1.7
SS × LP	1	5.3 *	15.3 *	41.2 **	32.9 **	19.7 **	6.2 *	13.5 *	13.3 *	9 *
SS × V	2	2.5	9.5 *	10.1 *	7.5 *	5.6 *	1.5	0.7	0.2	0.9
LP × V	2	3.3 *	6.9 *	15.8 **	18.2 **	12.9 **	0.2	1.7	0.2	2.8
SS × LP × V	2	1.2	5.5 *	10.9 **	12.6 **	5.6 *	1.1	3.4 *	4.9 *	1.9
Error	24									

LW: Larval weight; LSR: larval survival rate—the number following LW and LSR represents the numbers of days after eggs hatched—SS: sowing season; LP: leaf position; V: variety. The values in the table are *F*-values; * represents 0.05 level of significance; ** represents significance at 0.01 probability level; *DF*: degrees of freedom.

3.2.2. Effects of Different Leaf Positions, Soybean Growth Stage, and Soybean Varieties on the Growth of CBT Larvae in Autumn Sowing

Different soybean varieties and leaf positions had significant effects on the larval weights of all ages in the autumn sowing group. The maximum *F*-value of variety was found for 21-day-old larvae ($F = 119.6$, $p < 0.01$), and the maximum *F*-value of leaf position appeared for 9-day-old larvae ($F = 36.4$, $p < 0.01$). The soybean growth stage had no significant effect on the weights of 9-day-old larvae, but had a significant effect on the others, with a maximum *F*-value for 21-day-old larvae ($F = 81.6$, $p < 0.01$) (Table 2). Only the survival rate of 21-day-old larvae was significantly different at different growth stages of soybeans. Being fed from leaves at different positions had a highly significant effect on the survival rate of larvae, and the maximum *F*-value occurred for 15-day-old larvae at 25.2 ($p < 0.01$). No significant differences for all ages of larvae's survival rates were found among soybean varieties. Interestingly, all rearing factors had highly significant effects on the 21-day-old larval-weight *F*-value. So, the 21-day-old larval weight could be a key indicator for comparing the differences among the levels of CBT-rearing factors in autumn sowing.

Table 2. Three-way analysis of variance of different growth stages, leaf positions, and varieties on the different-day-old larvae growth indicators of eggs inoculated during autumn sowing.

Source of Variation	*DF*	LW9	LW12	LW15	LW18	LW21	LSR12	LSR15	LSR18	LSR21
GS	1	2.9	4.8 *	19.9 **	13.5 *	81.6 **	3.1	3.6	1.8	6.6 *
LP	1	36.4 **	17.2 **	14.7 *	10.4 *	34.4 **	13.3 *	25.2 **	16.9 **	15.9 *
V	2	5.5 *	20.1 **	28.3 **	41.3 **	119.6 **	1.6	0.6	0.2	0.3
GS × LP	1	0.08	0.1	0.3	0.1	3.1	2.6	1.9	1.3	0.6
GS × V	2	7.3 *	12.6 **	9.6 *	7.9 *	13.4 **	1.3	0.4	0.3	1.3
LP × V	2	0.03	0.3	0.7	1.2	3.1	1.4	1.5	0.5	0.6
GS × LP × V	2	1.6	2.9	1.7	0.7	0.9	0.2	1.3	2.6	3.7 *
Error	24									

LW: Larval weight; LSR: larval survival rate—the number following LW and LSR represents the numbers of days after eggs hatching—GS: growth stage; LP: leaf position; V: variety. The values in the table are *F*-values. * represents 0.05 level of significance; ** represents significance at 0.01 probability level. *DF*: degrees of freedom.

In autumn sowing, the interactions between the soybean growth stage and leaf position, the same as the interactions between the leaf position and variety, had no significant effect on larval weight and larval survival rate. Nevertheless, the interactions between soybean growth stage and variety had a significant effect on all ages of larvae's weight, with a maximum *F*-value for 21-day-old larvae ($F = 13.4$, $p < 0.01$). No significant difference in larval survival rate with the interaction of the soybean growth stage and variety was observed. The interactions among soybean growth stage and leaf position and variety only had a significant effect on the 21-day-old larvae's survival rate (Table 2).

3.2.3. Correlation between Larval Weight and Larval Survival Rate

Significant positive correlations were observed among different larval weights in the V6 stage (Table 3). The correlation coefficient between the weight of 18- and 21-day-old larvae, as well as the weight of 15- and 18-day-old larvae, was the same and the highest. And, the correlation of larval weights tended to increase with the increase of larvae-infestation days, indicating that the stability of the indicator of larval weight in the late larval stage was good. There were also significant positive correlations among all the survival rates of larvae fed in the V6 stage, with the highest correlation coefficient between the survival rates of 15- and 18-day-old larvae ($r = 0.83$; $p < 0.01$). The survival rate of the 12-day-old larvae was significantly positively correlated with the weights of all-day-old larvae. The survival rate of 15-day-old larvae had a positive correlation with the weight of the 15- and 21-day-old larvae, though with a small correlation coefficient ($r = 0.21$ and 0.19, respectively). Except for the above, the correlations between other-day-old larval survival rate and larval weight did not reach a significant level (Table 3).

Table 3. Correlation analysis among larval growth indicators.

Indicator	LW9	LW12	LW15	LW18	LW21	LSR12	LSR15	LSR18	LSR21
LW9		0.64 **	0.57 **	0.42 **	0.39 **	0.29 *	0.33 *	0.32 *	0.30 *
LW12	0.74 **		0.77 **	0.67 **	0.65 **	0.01	0.13	0.14	0.14
LW15	0.79 **	0.86 **		0.78 **	0.75 **	0.15	0.04	0.06	0.08
LW18	0.76 **	0.86 **	0.92 **		0.85 **	0.04	−0.04	−0.03	−0.01
LW21	0.71 **	0.77 **	0.84 **	0.92 **		0.12	0.01	0.01	−0.01
LSR12	0.19 *	0.20 *	0.25 *	0.23 *	0.25 *		0.73 **	0.73 **	0.54 **
LSR15	0.15	0.16	0.21 *	0.17	0.19 *	0.70 **		0.81 **	0.75 **
LSR18	0.12	0.16	0.17	0.14	0.15	0.54 **	0.83 **		0.90 **
LSR21	0.11	0.11	0.15	0.13	0.13	0.47 **	0.68 **	0.72 **	

LW: Larval weight; LSR: larval survival rate—the number following LW and LSR represents the numbers of days after eggs hatching—* represents 0.05 level of significance; ** represents significance at 0.01 probability level. The lower left part shows the correlation among average growth indicators of summer and autumn sowing season's V6 stage, and the right upper part shows the correlation among average larval growth indicators of V6 and R3 growth stages in autumn sowing.

Significant positive correlations were observed among all larval weights in autumn-sown soybean (Table 3), with the highest correlation coefficient being found between larval weights at 18 and 21 days ($r = 0.85$; $p < 0.01$). Significant positive correlations of the larval survival rate were also observed, with the highest correlation coefficient between the 18- and 21-day-old larval survival rate ($r = 0.90$; $p < 0.01$). Significant positive correlations were found among 9-day-old larval weight and all larval survival. The highest correlation arose between 9-day-old larval weight and 15-day-old larval survival rate ($r = 0.33$; $p < 0.05$). Whilst the correlations among other larval weights and all larval survival rates did not reach significant levels (Table 3).

3.3. Effects of Different Rearing Factors on the Weight of 21-Day-Old CBT Larvae

3.3.1. Effects of Different Sowing Seasons, Leaf Positions, and Soybean Varieties on 21-Day-Old CBT Larval Weight with Eggs Inoculated in Soybean V6 Stage

The weight of 21-day-old larvae feeding on soybeans in the V6 stage during summer sowing was significantly higher than in autumn sowing, increasing by an average of 44.7% (Figure 3A). Additionally, the average 21-day-old larval weight, feeding on the third-top leaf, significantly increased by 35.7% compared with the third-bottom leaf in the V6 stage (Figure 3B). No significant difference in the 21-day-old larval weights between the susceptible-soybean varieties NN89-29 and NN1138-2 was found. Meanwhile, the weights of 21-day-old larvae feeding on NN89-29 and NN1138-2 both were significantly higher than that of those feeding on the insect-resistant variety, Lamar (Figure 3C).

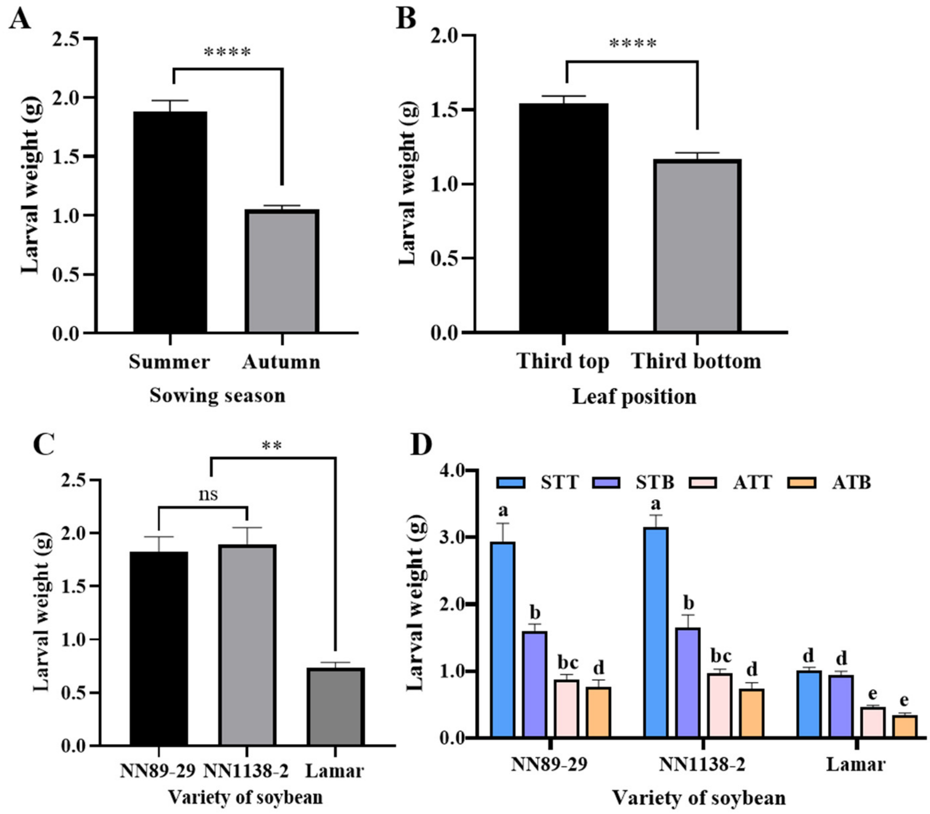

Figure 3. Effects of different sowing seasons, leaf positions, and soybean varieties on 21-day-old larvae's weight which eggs inoculated at V6 stage. (**A**) Sowing season; (**B**) leaf position; (**C**) soybean variety; (**D**) effect of different treatments of sowing seasons and leaf positions on the weights of 21-day-old larvae reared with different soybean varieties in V6 stage; STT, STB: In summer sowing soybean V6 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively; ATT, ATB: In autumn sowing soybean V6 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively. ns, ** and ****, which represent insignificant difference and represents significance at 0.01 and 0.0001 probability level, respectively. Different lowercase letters in subfigure D indicate significant differences of $p < 0.05$.

The weight of 21-day-old larvae, in the V6 stage during summer sowing, fed on the third-top leaf of NN89-29 and NN1138-2, were significantly different than other trail treatments. However, the weight of 21-day-old larvae feeding on Lamar was only affected by the sowing season and was not affected by the leaf positions (Figure 3D). In a word, the weight of 21-day-old larvae feeding on the third-top leaf of NN89-29 and NN1138-2 was significantly higher than that of those undergoing other treatments during summer sowing.

3.3.2. Effects of Different Growth Stages, Leaf Positions, and Soybean Varieties on 21-Day-Old CBT Larval Weight in Autumn Sowing

Under autumn sowing conditions, the 21-day-old larval weight in the V6 stage was significantly higher than in the R3 stage by 33.9% (Figure 4A). Compared with the third-bottom leaf, the average weight of the 21-day-old larvae feeding on the third-top leaf could significantly increase by 29.9% (Figure 4B). No significant difference between the 21-day-old larval weights of the susceptible-soybean varieties, NN89-29 and NN1138-2, and both were significantly higher than that of the insect-resistant variety, Lamar (Figure 4C). The 21-day-old larvae's weights, of those fed with the susceptible varieties NN89-29 and NN1138-2 at the third-top leaf in the V6 stage to inoculate eggs, were significantly higher than those undergoing the other treatments. However, there was little difference in the 21-day-old larvae's weights among different treatment combinations of the resistant variety, Lamar (Figure 4D).

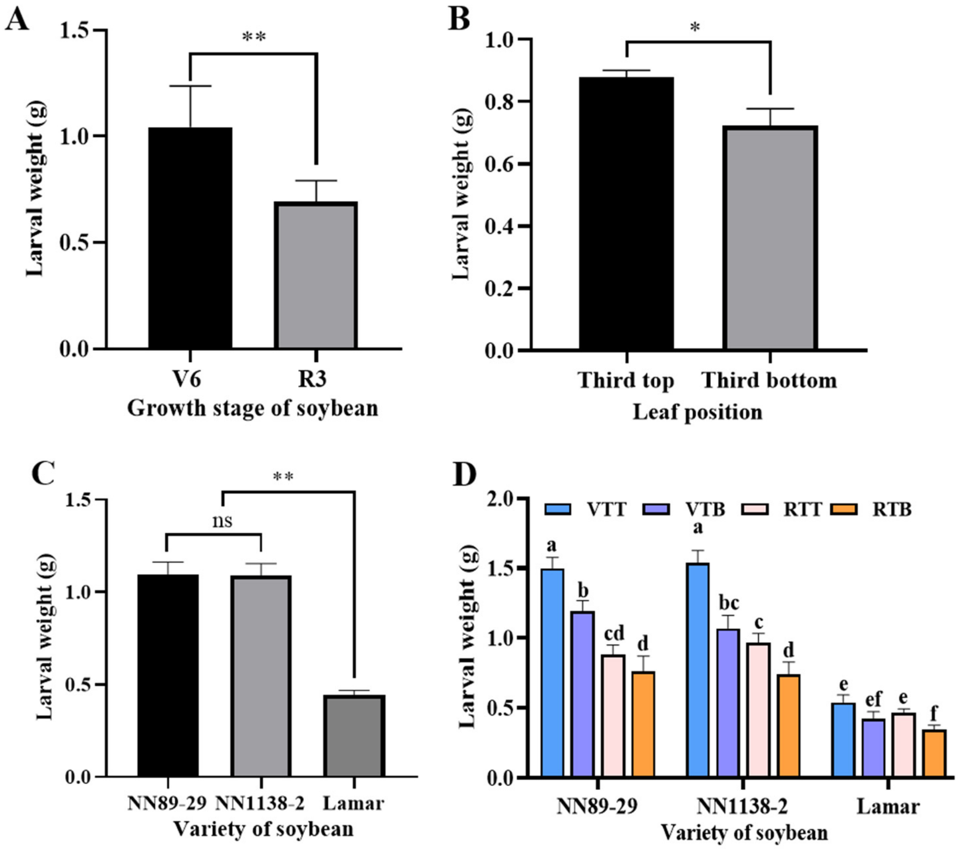

Figure 4. Effects of different growth stages, leaf positions, and soybean varieties on 21-day-old larvae's weight in autumn sowing group. (**A**) Growth stage; (**B**) leaf position; (**C**) variety; (**D**) effects of different treatments of growth stages and leaf positions on the weights of 21-day-old larvae reared with different soybean varieties in autumn sowing; VTT, VTB: in autumn sowing soybean group V6 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively; RTT, RTB: in autumn sowing soybean group R3 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively. ns, * and **, which stand for not significant difference and represents significance at 0.05 and 0.01 probability level, respectively. Different lowercase letters in subfigure D indicate significant differences of $p < 0.05$.

3.4. Trends of Different-Day-Old CBT Larval Weight of Each Treatment

In summer sowing, the differences in larval weights between different leaf positions of NN89-29 and NN1138-2 at V6 stage gradually increased with the numbers of rearing days' growth. In addition, the weights of larvae fed on the third-top leaf were higher than those feeding on the third-bottom leaf (Figure 5A,B). No significant difference was observed in the weight of the larvae fed from Lamar leaves in different positions and with increasing rearing days (Figure 5C). Under autumn sowing conditions, when eggs were inoculated at the V6 stage, the differences in larval weights fed from leaves in different positions of NN89-29 and NN1138-2 also gradually increased with the rearing-day-number growth. But, the degree of increase was significantly weaker than that of the summer sowing group (Figure 5D,E). Just like the summer sowing group, the weight of larvae fed from leaves in different positions of Lamar had no significant difference in increasing rearing days in autumn sowing (Figure 5F). Under autumn sowing conditions, the changing trends of weight of larvae fed by NN89-29 and NN1138-2 in the R3 soybean stage were similar to those in the V6 stage, while the magnitude of change in the R3 stage was weaker than in the V6 stage (Figure 5G,H). The weights of larvae fed from leaves in different positions of Lamar still were not affected by increasing rearing days (Figure 5I). Interestingly, the larval weights feeding on Lamar in the R3 stage were basically the same as in the V6 stage (Figure 5F,I).

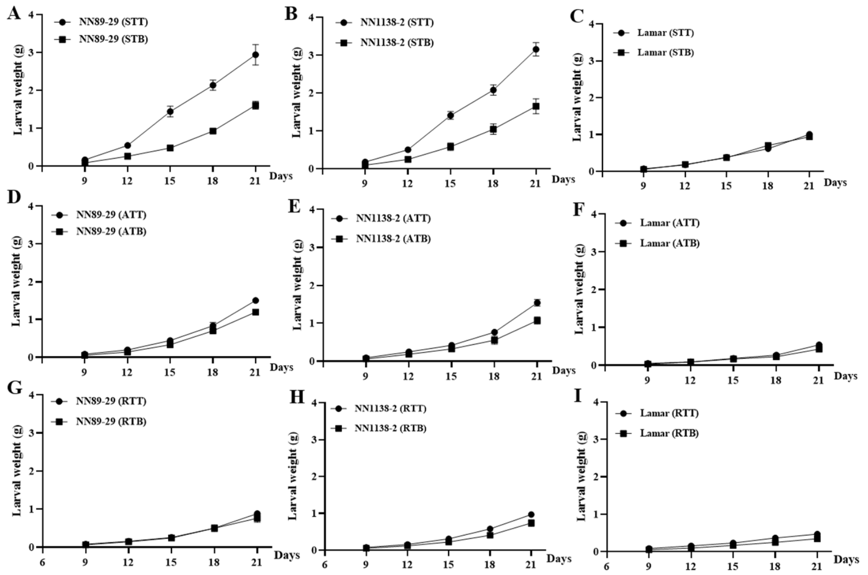

Figure 5. The trends of CBT larval weight with different treatments of leaf positions, varieties under different soybean growth stages, and sowing seasons. (**A**–**C**) In summer sowing, the eggs inoculated at different leaf positions of NN89-29, NN1138-2, and Lamar in soybean V6 stage, respectively; (**D**–**F**) in autumn sowing, the eggs inoculated at different leaf positions of NN89-29, NN1138-2, and Lamar in soybean V6 stage, respectively; (**G**–**I**) in autumn sowing, the eggs inoculated at different leaf positions of NN89-29, NN1138-2, and Lamar in soybean R3 stage, respectively; STT, STB: in summer sowing soybean V6 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively; ATT, ATB: in autumn sowing, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively; RTT, RTB: in autumn sowing soybean R3 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively.

3.5. Trends of Different Day-Old CBT Larval Survival Rate of Each Treatment

The larval survival rate was not significantly affected by different leaf positions in the summer-sown group's V6 stage. Under autumn sowing conditions, the larval survival rates were significantly higher at the third-top leaf than at the third-bottom leaf both in the V6 and R3 stage (Figure 6A–C). The different-day-old larval survival rates were affected by interactions between the sowing season and leaf position at the V6 stage. A large decrease was found in the larval survival rate of those of the third-bottom leaf in the V6 stage in the autumn sowing group (Figure 6D–G). In conclusion, leaf position was the main factor affecting the survival rate of larvae.

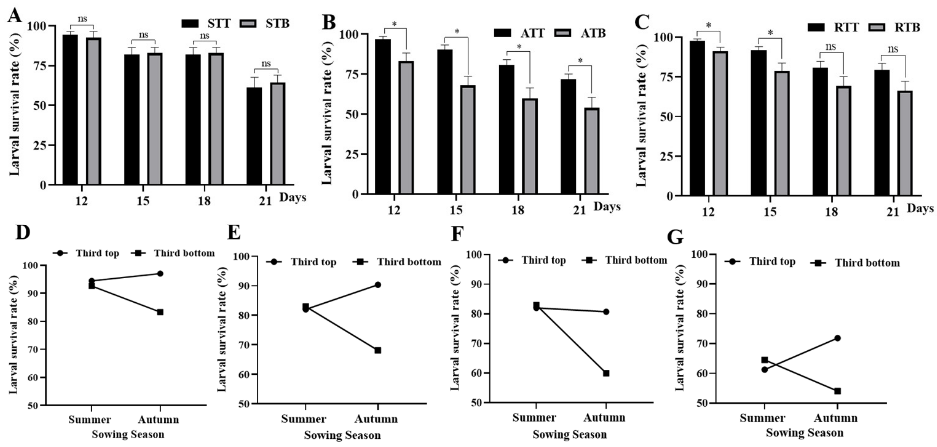

Figure 6. Trends in the larval survival rates between leaf positions, and the effects of the interactions of leaf position and sowing season on the larval survival rates. (**A**–**C**) Trends in survival rates of

different-day-old larvae at different leaf positions; (**A**) at soybean V6 stage in summer sowing; (**B**) at soybean V6 stage in autumn sowing; (**C**) at soybean R3 stage in autumn sowing. (**D**,**G**) Effects of leaf position and sowing season interactions on larval survival rates; (**D**) 12-day-old larvae; (**E**) 15-day-old larvae; (**F**) 18-day-old larvae; and (**G**) 21-day-old larvae. STT, STB: in summer sowing soybean V6 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively; ATT, ATB: in autumn sowing soybean V6 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively; RTT, RTB: in autumn sowing soybean R3 stage, the eggs inoculated at the third-top and the third-bottom leaf positions, respectively. ns and *, which stand for not significant difference and represents significance at 0.05 probability level, respectively.

4. Discussion

4.1. Effect of Sowing Season on the Larval Growth of C. bilineata tsingtauica

The growth of CBT larvae is impacted by two factors related to the sowing season. Firstly, the effect of the season on soybean growth has a direct impact [42], while soybean growth has an indirect impact on larvae growth. Secondly, the different environmental conditions of each sowing season directly affect larvae growth [43,44]. In the present study, the 21-day-old larval weight was significantly higher in summer than in autumn. During autumn sowing, the lower temperature and reduced sunshine duration had a significant impact on the plants, resulting in a faster entry into the reproductive growth stage [45]. The rainfall in summer and autumn was different, which can affect the plant's resistance to insects. It has been shown that lower watering levels increase resistance to cotton bollworm in tomato plants [46]. The light intensity in summer and autumn was different, and it is an important factor affecting maize lodging at high plant density [47]. The high density and low light intensity of summer-sown soybeans can lead to plant elongation and even lodging [48], which affects larval survival. Therefore, adjusting the period to inoculate CBT eggs appropriately and the reasonable number of eggs and plant density according to the characteristics of different sowing seasons is necessary [36]. And then, it is possible to achieve dynamic equilibrium between soybean plant and CBT larvae growth in CBT rearing.

Temperature may have a direct effect on the growth and development of CBT larvae. The developmental rate and feeding capacity of 3rd to 5th instar CBT larvae were greatest at (28 ± 1) °C [49]. The weight of the larvae harvested under autumn sowing conditions was lighter than that in the spring sowing group [36], which is consistent with the present study. We deduce that the larval growth rate decrease was caused by low temperatures in autumn possibly. Later tests need to investigate the entire larval growth period to compare the final larval weights of summer and autumn sowing to account for differences between sowing seasons.

4.2. Effect of Soybean Growth Stages on the Larval Growth of C. bilineata tsingtauica

Plants have different insect resistance at different growth stages. Feeding third instar *Spodoptera litura* larvae with leaves of different soybean varieties at the R2, R4, and R6 stages, then the maximal weight gain of *S. litura* larvae that were feeding on the R2-stage leaves was found [50]. Similar results were also observed in the orientation preference experiment of *Megacopta cribraria* (F.) (Heteroptera: Plataspidae); adults preferred the R1 stage of the soybean at four growth stages (V2, V4, R1, and R5) [51]. The soybean leaves at different growth stages for rearing larvae have a significant impact on the growth of CBT larvae [36]. In the present study, under autumn sowing condition, the weight of larvae whose eggs were inoculated in the V6 stage was significantly higher than those in the R3 stage by 33.9%. The previous research results were similar to this study, under spring sowing condition; the weight of the larvae whose eggs were inoculated in the V6 stage was significantly higher than that in the R3 stage [36].

The difference in larval weight at different growth stages of soybeans may also be related to the nutrient content of the leaves. The cumulative concentrations of monosaccharides and organic acids, used for energy production, in the vegetative stage of soybeans were 2.5 and 1.7 times greater than those found in samples from the reproductive stage, respectively [52].

4.3. Effect of Leaf Position on the Larval Growth of *C. bilineata tsingtauica*

The growth of CBT larvae was also significantly affected by leaf positions. The present study found that the weight of CBT larvae fed with upper leaves was higher than that fed with lower leaves. Similar to our results, *Soybean looper* larvae feeding on the second- or third-closest fully expanded leaves at the top of the plant had a higher larval growth rate than those feeding on the fourth-closest and lower leaves [53].

The differences in weight of CBT larvae feeding on different leaf positions vary depending on the sowing season and soybeans' growth stage. In our study, larval weight was significantly affected by different leaf positions during summer sowing, whereas this effect was significantly reduced for autumn sowing. At the V6 stage, the difference in the weight of larvae feeding on different leaf positions during summer sowing was 40.9%, while only 25.5% did for autumn sowing. This might be related to a temperature decrease in autumn sowing (Figure 2A), leading to a slow larval growth rate [54]. In autumn sowing, the difference in the weight of the larvae fed at different leaf positions in the V6 stage was more significant than in the R3 stage. This might be related to the lower nutrient content of leaves in the R3 stage [52].

The survival rate of CBT larvae was highly significantly affected by the sowing season and leaf position. The larval growth and survivorship of rice leaf rollers were also affected by the position of the leaves consumed [31]. Higher persistence levels of entomopathogenic fungi and a lower larval survival rate was caused by lower temperatures, higher humidity, less UV light, and poorer ventilation around the lower leaves of the soybeans [55,56]. The survival rate of larvae feeding on the third-bottom leaf of the V6 soybeans with autumn sowing was significantly reduced in our study. This result was consistent with the above conclusions. In addition, we extrapolated that a lower larval survival rate might also be related to the lower position of the leaves which are near the ground especially in autumn. Accordingly, subsequent experiments need to analyze the nutritional value of leaves at different leaf positions.

4.4. Effect of Soybean Varieties on the Larval Growth of *C. bilineata tsingtauica*

Different soybean varieties have different resistances to insects [57], and feeding on insect-resistant soybean varieties can result in larvae growth slowing down or even diapause [58]. Insects have a feeding preference for soybean varieties, for example, CBT larvae significantly prefer the soybean variety Ruidou-1 over Guandou-3 and 'September cold' [59]. In the present study, compared to Lamar, NN89-29 and NN1138-2 were clearly more suitable for increasing CBT larval weight. Therefore, we reasonably infer that selecting adaptable soybean varieties plays a crucial role in CBT rearing.

4.5. Inspiration on the Evaluation of Soybean Varieties with Adaptability to *C. bilineata tsingtauica*

An accurate and easy-to-operate variety evaluation method is of great significance for CBT rearing. The bagging isolation feeding method utilized in this study can serve as a means to screen for soybean adaptability to CBT larvae. Compared to using small net houses in the previous study [36], bagging isolation feeding allows for easier control of larval number and ensures result accuracy. Moreover, this method also reduces the cost and expenses associated with the experiments. In addition, by comparing the results of various rearing-factor combinations, we believe that bagging isolation feeding on the top-third leaf at the V6 stage during summer sowing is the relatively best combination for use. Using the weight of 21-day-old larvae as an indicator can accurately and efficiently evaluate the adaptability of soybean varieties to CBT. In the future, we will combine soybean genotypes with various rearing factors to further screen for soybean varieties' adaptability for CBT.

5. Conclusions

In larvae, the correlation between the two indicators of weight and survival rate turned out to be small. The larval weight was more repeatable than the larval survival rate. Among all larval weights we investigated, the 21-day-old larvae's weight had high levels of stability and repeatability, making it suitable as an evaluation indicator for screening soybean adaptability to CBT larvae. Highly susceptible-soybean varieties, NN89-29 and NN1138-2, showed adaptability to CBT larvae, while the insect-resistant soybean variety, Lamar, showed no adaptability to CBT larvae. In the V6 stage, the weight of the larvae fed with NN89-29 and NN1138-2 showed a significant difference between its different leaf positions. The weight of larvae feeding on the third-bottom leaf was significantly lower than that on the third-top leaf. And, this difference gradually increased with the increase of larvae's age, while this change was not significant in autumn sowing. In autumn sowing, compared to the V6 stage, the weight of larvae fed in the R3 stage decreased, the difference of larval weight between leaf positions in the R3 stage further decreased, and the survival rate of larvae fed at lower leaf positions in the V6 stage was the lowest.

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References

1. Gerland, P.; Raftery, A.; Sevcikova, H.; Li, N.; Gu, D.; Spoorenberg, T.; Alkema, L.; Fosdick, B.; Chumn, J.; Lalic, N.; et al. World population stabilization unlikely this century. *Science* **2014**, *346*, 234–237. [CrossRef] [PubMed]
2. Henchion, M.; Hayes, M.; Mullen, A.M.; Fenelon, M.; Tiwari, B. Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. *Foods* **2017**, *6*, 53. [CrossRef]
3. Huis, A.V.; Oonincx, D.G.A.B. The environmental sustainability of insects as food and feed. A review. *Agron. Sustain. Dev.* **2017**, *37*, 43. [CrossRef]
4. Pliantiangtam, N.; Chundang, P.; Kovitvadhi, A. Growth performance, waste reduction efficiency and nutritional composition of black soldier fly (*Hermetia illucens*) larvae and prepupae reared on coconut endosperm and soybean curd residue with or without supplementation. *Insects* **2021**, *12*, 682. [CrossRef]
5. Halloran, A.; Roos, N.; Eilenberg, J.; Cerutti, A.; Bruun, S. Life cycle assessment of edible insects for food protein: A review. *Agron. Sustain. Dev.* **2016**, *36*, 57. [CrossRef] [PubMed]
6. Hong, J.; Han, T.; Kim, Y.Y. Mealworm (*Tenebrio molitor* Larvae) as an alternative protein source for monogastric animal: A review. *Animals* **2020**, *10*, 2068. [CrossRef]
7. Hawkey, K.J.; Lopez-Viso, C.; Brameld, J.M.; Parr, T.; Salter, A.M. Insects: A potential source of protein and other nutrients for feed and food. *Annu. Rev. Anim. Biosci.* **2021**, *9*, 333–354. [CrossRef] [PubMed]
8. Finke, M.D. Complete nutrient content of four species of feeder insects. *Zoo Biol.* **2013**, *32*, 27–36. [CrossRef]
9. Zhang, S.; Qiu, K.; Tan, Z.; Zhu, F. Research progress of insect vitamin. *HuaZhong Insect Res.* **2020**, *16*, 126–131.
10. Rumpold, B.A.; Schluter, O.K. Nutritional composition and safety aspects of edible insects. *Mol. Nutr. Food Res.* **2013**, *57*, 802–823. [CrossRef]
11. Nakane, W.; Nakamura, H.; Nakazato, T.; Kaminaga, N.; Nakano, M.; Sakamoto, T.; Nishiko, M.; Bono, H.; Ogiwara, I.; Kitano, Y.; et al. Construction of TUATinsecta database that integrated plant and insect database for screening phytophagous insect metabolic products with medicinal potential. *Sci. Rep.* **2020**, *10*, 17509. [CrossRef] [PubMed]

12. Alvarez, D.; Wilkinson, K.A.; Treilhou, M.; Tene, N.; Castillo, D.; Sauvain, M. Prospecting peptides isolated from black soldier fly (Diptera: Stratiomyidae) with antimicrobial activity against *Helicobacter pylori* (Campylobacterales: Helicobacteraceae). *J. Insect Sci.* **2019**, *19*, 17. [CrossRef] [PubMed]
13. Tian, H.; Zhang, Y.M. Advances of integrated utilization on resource insect *Clanis bilineata tsingtauica*. *Guizhou Agric. Sci.* **2009**, *37*, 111–113.
14. Zou, S.W. *History of Chinese Entomology*; Science Press: Beijing, China, 1981; pp. 1–186.
15. Sun, Z.; Pan, J.; Wang, K.; Li, Q.; Guo, M.; Zhang, G.; Chen, F. Current status and prospects of rearing *Clanisb ilineata tsingtauica* Mell. *Agric. Dev. Equip.* **2019**, *5*, 64–67.
16. Wu, S.; Meng, X.; Chen, S. The analysis and evaluation of main nutrient components for *Herse bilineata tsingtauica*. *J. Huaihai Inst. Tech.* **2000**, *9*, 58–61.
17. Yan, M.; Lu, C.; Zhang, G.; Li, H.; Nie, X.; Zheng, D.; Zheng, X. Research advance in the exploitation and utilization of *Clanisbilineata tsingtauica* Mell. *J. Anhui Agric. Sci.* **2008**, *36*, 874–876.
18. Gao, Y.; Zhao, Y.; Xu, M.; Shi, S. Soybean hawkmoth (*Clanis bilineata tsingtauica*) as food ingredients: A review. *CyTA J. Food* **2021**, *19*, 341–348. [CrossRef]
19. Gao, Y.; Zhao, Y.; Xu, M.; Shi, S. *Clanis bilineata tsingtauica*: A sustainable edible insect resource. *Sustainability* **2021**, *13*, 12533. [CrossRef]
20. Wang, X.; Chen, C.; Zhao, H.; Zhang, Y. Rearing *Clanis bilineata* in soybean field and its profit. *Entomol. Know.* **2002**, *39*, 30–33.
21. Wu, S.; Xia, Z. Hight-yield culture techniques for *Clanis bilineata* Walker and its benefits analysis. *J. Anhui Agric. Sci.* **2008**, *36*, 14135–14136.
22. Freitas, M.M.; Souza, B.H.S.; Nogueira, L.; Bello, M.M.D.; Junior, A.L.B. Soybean defense induction to *Spodoptera cosmioides* herbivory is dependent on plant genotype and leaf position. *Arth. Plant Interact.* **2018**, *12*, 85–96. [CrossRef]
23. Howe, G.A.; Jander, G. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* **2008**, *59*, 41–66. [CrossRef]
24. Gomez, J.D.; Pinheiro, V.J.M.; Silva, J.C.; Romero, J.V.; Merino-Cabrera, Y.M.; Coutinho, F.S.; Lourencao, A.L.; Serrao, J.E.; Vital, C.E.; Fontes, E.P.B.; et al. Leaf metabolic profiles of two soybean genotypes differentially affect the survival and the digestibility of *Anticarsia gemmatalis* caterpillars. *Plant Physiol. Biochem.* **2020**, *155*, 196–212. [CrossRef]
25. Cui, Z.; Gai, J.; Ji, D.; Ren, Z. Evaluation of soybean germplasm for resistance to leaf-feeding insects. *Soybean Sci.* **1997**, *16*, 93–102.
26. Yang, Y.; Xing, G.; Gai, J. Evaluation of antibiosis to common cutworm (*Spodoptera litura*) and screening for resistance sources among wild soybeans (*Glycine soja*) in China. *Soybean Sci.* **2016**, *35*, 448–454.
27. Li, J.; Zhang, F.; Xing, G.; Gai, J. Influence of different defoliation rates at different growth stages to agronomic and quality traits of soybean cultivar NN99-6. *Soybean Sci.* **2018**, *37*, 715–722.
28. Malabarba, J.; Meents, A.K.; Reichelt, M.; Scholz, S.S.; Peiter, E.; Rachowka, J.; Konopka-Postupolska, D.; Wilkins, K.A.; Davies, J.M.; Oelmuller, R.; et al. ANNEXIN1 mediates calcium-dependent systemic defense in Arabidopsis plants upon herbivory and wounding. *New Phytol.* **2021**, *231*, 243–254. [CrossRef] [PubMed]
29. Song, G. The Spatio-Tamporal Expression Difference of Bt Gene and Its Regulation in Different Leaf Postions of Bt Bollworm Resistant Cootton. Master's Thesis, Northwest Sic-Tech University of Agriculture and Forestry, Yangling, China, 2003.
30. Simmons, A.M.; Godfrey, L.D.; Yeargan, K.V. Ovipositional sites of the Potato Leafhopper (Homoptera: Cicadellidae) on vegetative stage soybean plants. *Environ. Entomol.* **1985**, *14*, 165–169. [CrossRef]
31. Miyashita, T.; Kawanishi, K. Effects of rice leaf position and growth stage on larval development and survivorship of the rice leaf roller *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Jpn. J. Appl. Entomol. Zool.* **2003**, *47*, 85–90. [CrossRef]
32. Rogge, S.A.; Meyhofer, R. Leaf age is important for assessment of resistance in chrysanthemum against *Frankliniella occidentalis*. *J. Plant Dis. Prot.* **2021**, *128*, 511–525. [CrossRef]
33. Zhou, X.; Carter, N. Effects of temperature, feeding position and crop growth stage on the population dynamics of the rose grain aphid, *Metopolophium-dirhodum* (Hemiptera, Aphididae). *Ann. Appl. Biol.* **1992**, *121*, 27–37. [CrossRef]
34. Terry, I.; Bradley, J.R., Jr.; van Duyn, J.W. Survival and development of *Heliothis zea* (Lepidoptera: Noctuidae) larvae on selected soybean growth stages. *Environ. Entomol.* **1987**, *16*, 441–445. [CrossRef]
35. Xu, R.; Wang, C.; Li, W.; Zhang, L.; Xing, H. Advances in identification of the resistance of soybean to pests. *Soybean Sci.* **2007**, *26*, 771–774.
36. Liu, X.; Yan, Y.; Liu, N.; Xu, Y.; Jiang, H.; Ye, Z.; Wang, H.; Gai, J.; Xing, G. Evaluation of rearing factors affecting *Clanis bilineata tsingtauica* Mell larvae fed by susceptible soybean variety NN89-29 in spring and autumn sowing. *Insects* **2023**, *14*, 32. [CrossRef] [PubMed]
37. Villanuena, R.R.; Araneda, M.E.; Vela, M.; Seijo, J.C. Selecting stocking density in different climatic seasons: A decision theory approach to intensive aquaculture. *Aquaculture* **2013**, *384*, 25–34. [CrossRef]
38. Guo, M.; Liao, H.; Deng, P.; Li, D.; Li, J.; Zhang, J.; Pan, J.; Chen, F. Effects of soybean varieties (lines) and planting density on survival and development of *Clanis bilineata tsingtauica* Mell larvae. *J. Environ. Entomol.* **2020**, *42*, 1401–1408.
39. Xu, Y.; Ye, Z.; Xie, Z.; Zhang, D.; Liu, X.; Yan, Y.; Sun, L.; Chen, F.; Gai, J.; Xing, G. Comparison of trapping effects of different traps and monitoring the occurrence dynamics of *Spodoptera litura* in soybean fields of Dangtu, Anhui Province, China. *Agrononmy* **2023**, *13*, 47. [CrossRef]
40. Xing, G.; Liu, K.; Gai, J. A high-throughput phenotyping procedure for evaluation of antixenosis against common cutworm at early seedling stage in soybean. *Plant Methods* **2017**, *13*, 66. [CrossRef]

41. Fehr, W.R.; Caviness, C.E.; Burmood, D.T.; Pennington, J.S. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Sci.* **1971**, *11*, 929–931. [CrossRef]
42. Fordonski, G.; Okorski, A.; Olszewski, A.; Dabrowska, J.; Pszczolkowska, A. The effect of sowing date on the growth and yield of soybeans cultivated in north-eastern poland. *Agriculture* **2023**, *13*, 2199. [CrossRef]
43. Ludwig, D.; Anderson, J.M. Effects of different humidities, at various temperatures, on the early development of four saturniid moths (*Platysamia cecropia linnaeus*, *Telea polyphemus cramer*, *Samia walkeri* felder and felder, and *Callosamia promethea drury*), and on the weights and water contents of their larvae. *Ecol. Soc. Am.* **1942**, *23*, 259–274.
44. Eberle, S.; Schaden, L.M.; Tintner, J.; Stauffer, C.; Schebeck, M. Effect of temperature and photoperiod on development, survival, and growth rate of mealworms, *Tenebrio molitor*. *Insects* **2022**, *13*, 321. [CrossRef] [PubMed]
45. Nico, M.; Miralles, D.J.; Kantolic, A.G. Natural post-flowering photoperiod and photoperiod sensitivity: Roles in yield-determining processes in soybean. *Field Crop. Res.* **2019**, *231*, 141–152. [CrossRef]
46. Lin, P.A.; Paudel, S.; Afzal, A.; Shedd, N.L.; Felton, G.W. Changes in tolerance and resistance of a plant to insect herbivores under variable water availability. *Environ. Exp. Bot.* **2021**, *183*, 104334. [CrossRef]
47. Xue, J.; Gou, L.; Zhao, Y.; Yao, M.; Yao, H.; Tian, J.; Zhang, W. Effects of light intensity within the canopy on maize lodging. *Field Crop. Res.* **2016**, *188*, 133–141. [CrossRef]
48. Shan, F.; Sun, K.; Gong, S.; Wang, C.; Ma, C.; Zhang, R.; Yan, C. Effects of shading on the internode critical for soybean (*Glycine max*) lodging. *Agronomy* **2022**, *12*, 492. [CrossRef]
49. Feng, Y.; Ma, G.; Jin, Q.; Lv, B.; Peng, Z.; Jin, T.; Wen, H. Effect of temperature on developmental duration and feeding amount of *Clanis bilineata tsingtauica*. *Chin. J. Trop. Crop.* **2014**, *35*, 2442–2444.
50. Hu, Z.; Xu, X.; Pan, L.; Li, M.; Zeng, J.; Muhammad, K.R.; Xing, G.; Gai, J. Resistance analyses of soybean organs to common cutworm (*Spodoptera litura*) at different reproductive stages. *Soybean Sci.* **2020**, *39*, 932–939.
51. Yang, L.; Hu, X.P.; Golec, J.R.; Zeng, X. Effects of legume species and plant growth stage on attraction, fecundity, and development of the Kudzu Bug (Heteroptera: Plataspidae). *J. Econ. Entomol.* **2018**, *111*, 2217–2224. [CrossRef]
52. Park, Y.J.; Lee, J.S.; Park, S.; Kim, Y.J.; Mani, V.; Lee, K.; Kwon, S.J.; Park, S.U.; Kim, J.K. Metabolite changes in soybean (*Glycine max*) leaves during the entire growth period. *ACS Omega* **2023**, *8*, 41718–41727. [CrossRef]
53. Reynolds, G.W.; Smith, C.M. Effect of leaf position, leaf wounding and plant age of two soybean genotypes on soybean looper (Lepidoptera: Noctuidae) growth. *Environ. Entomol.* **1985**, *14*, 475–478. [CrossRef]
54. Power, N.A.; Ganjisaffar, F.; Perring, T.M. Effect of temperature on the survival and developmental rate of immature *Ooencyrtus mirus* (Hymenoptera: Encyrtidae). *J. Econ. Entomol.* **2020**, *113*, 1675–1684. [CrossRef]
55. Wang, L.; Yi, J.; Zhu, S.; Li, B.; Chen, Y.; Shen, W.; Wang, W. Identification of a single-nucleocapsid baculovirus isolated from *Clanis bilineata tsingtauica* (Lepidoptera: Sphingidae). *Arch. Virol.* **2008**, *153*, 1557–1561. [CrossRef]
56. Souza, T.D.; Fernandes, F.O.; Sanches, A.C.; Nascimenyo, J.D.; Pinto, A.A.; Rpolanczyk, R.A. Relation between *Helicoverpa armigera* (Hubner) (Lepidoptera/Noctuidae) mortality and entomopathogenic fungi persistence in soybean leaflets. *Egypt. J. Biol. Pest Control* **2022**, *32*, 10. [CrossRef]
57. Adie, M.M.; Krisnawati, A.; Baliadi, Y. Evaluation for soybean resistance to armyworm *Spodoptera litura* (Lepidoptera: Noctuidae). *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *484*, 012020. [CrossRef]
58. Smith, C.M.; Gilman, D.F. Comparative resistance of multiple insect-resistant soybean genotypes to the soybean looper. *J. Econ. Entomol.* **1981**, *74*, 400–403. [CrossRef]
59. Qian, L.; Chen, B.; Gui, F.; Qin, Y.; Deng, P.; Liao, H. Nutritional and feeding adaptability of *Clanis bilineata tsingtauica* larvae to different cultivars of soybean, *(Glycine max)*. *Foods* **2023**, *12*, 1721. [CrossRef] [PubMed]

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