EFFECTS OF DIFFERENT HOST-PLANT COMPONENTS ON BIOLOGICAL INDICES IN *Spodoptera frugiperda*

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SUMMARY

Spodoptera frugiperda *(J. E. Smith, 1797) (Lepidoptera: Noctuidae) is an invasive pest that is ravaging crops in many provinces of China. In order to specifically control this invasive pest, understanding of the relationship between the insect and hosts is necessary. In this study, we have compared the biological indices of S. frugiperda by feeding it with five different host-plants (*Zea mays, Triticum aestivum, Digitaria sanguinalis, Glycine max *and* Eleusine indica*) under laboratory conditions. The biological indices of* S. frugiperda *feeding on* Z. mays *were the best. The pupa weight and fecundity of* S. frugiperda *fed with* G. max *and* E. indica *were significantly lower than those fed with other hosts. The host total phenol content was negatively correlated with the growth and development of* S. frugiperda*, while the C/N ratio was positively correlated. When fed on different plants, the biological indices of* S. frugiperda *were different, but the insect completed its life cycle in any host. Therefore,* S. frugiperda *also has a tendency to feed on other hosts, especially plants with high C/N content, when* Z. mays *is insufficient.*

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the most important pests that have appeared in Asian countries. In the 1980s, researchers discovered two *S. frugiperda* races associated with corn or rice, respectively (Pashley, 1986). Corn-strains prefer corn, sorghum and cotton, while rice strains prefer rice and wild grasses (Hay-Roe *et al*., 2011; Nagoshi *et al*., 2017). *Spodoptera frugiperda* was first discovered on Chinese corn in January 2019. Over time, the *S. frugiperda* had spread to many provinces, threatening corn crop yields of China (Guo *et al*., 2018; Zhang *et al*., 2019). The damage caused by this insect is extensive. Although there is a big difference in crop-planting structure between north and south in China, it can still cause great loss and harm (even no grain harvest) due to the wide range of food sources in larval stage and strong migratory ability in adult stage (Sena *et al*., 2003; Lima *et al*., 2010; Jing *et al*., 2020). In addition, crop hosts, some weeds such as *Veronica polita*, *Euphorbiah helioscopial*, *E. indica*, *Digitaria sanguinalis* are also used as hosts by *S. frugiperda* (Wang *et al*., 2020; Yao *et al*., 2020; Fang *et al*., 2020). Therefore, there are potential risks to the next crop due to the ability of noctuid transferring to other hosts around when the preferred or primary host (corn) is not abundant (Guo *et al*., 2021).

Under suitable environmental conditions, the growth and development rate, reproduction rate and survival rate of insects are dependent of the host (types and quantity of nutrients). Balanced and abundant nutrients are conducive to the growth, development and reproduction of insects (Awmack and Leather, 2002; Nosil *et al*., 2002). Carbohydrate, amino acids, and protein were reported as important in the growth and development of phytophagous insects. Carbohydrate can provide the energy needed for insect growth and development (Beenakkers, 1969). The amino acid and protein of the host plants have a main effect on the larval feeding preference and larval growth, while sugar was only stimulated slightly (Hedin *et al*., 1981; Hedin *et al*., 1990). Besides the host plants also contain secondary metabolites. It is known that secondary metabolites (phenols and flavonoids) found in plants can be considered as a defensive mechanism against insect pests (Abir, 2021; Salminen, 2002). The treatment of compounds in host plants after feeding can reflect the adaptability of insects to these hosts. However, researches on *S. frugiperda* mainly focuses on prevention and control (Tambo *et al*., 2019), or genomic and genetic differences studies (Nagoshi and Meagher, 2016; Gouin *et al*., 2017; Stuhl *et al*., 2008). In China, research about the characteristics of *S. frugiperda* on different hosts are reported rarely, l*et al*one effects studies between the physical and chemical properties of host and the characteristics of *S. frugiperda*. The shortage of studies in this area seriously limits the

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EFECTOS DE DISTINTOS COMPONENTES DE LA PLANTA HUÉSPED SOBRE LOS ÍNDICES BIOLÓGICOS EN *Spodoptera frugiperda*

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RESUMEN

Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera: Noctuidae) *es una plaga invasora que está devastando los cultivos en muchas provincias de China. Para controlar esta plaga invasora, es necesario comprender la relación existente entre el insecto y los hospedadores. En este estudio, se compararon los índices biológicos de* S. frugiperda *alimentándola con cinco plantas hospedantes diferentes* (Zea mays, Triticum aestivum, Digitaria sanguinalis, Glycine max *y* Eleusine indica) *en condiciones de laboratorio. Los índices biológicos de* S. frugiperda *alimentada con* Z. mays *fueron los mejores. El peso de la pupa y la fecundidad de* S. frugiperda *alimentada con* G. max *y* E. indica *fueron significativamente menores que en aquellos alimentados con las otras tres plantas huéspedes. El contenido de fenoles totales de la planta huésped se correlacionó negativamente con el crecimiento y desarrollo de* S. frugiperda*, mientras que la relación C/N se correlacionó positivamente. Los índices biológicos de* S. frugiperda *fueron distintos al alimentarse con las diferentes plantas, aún así el insecto completó su ciclo de vida en cualquiera de los huéspedes. Por lo tanto,* S. frugiperda *tiende a alimentarse de otros hospedantes, especialmente de plantas con alto contenido de C/N, cuando no es suficiente la cantidad de* Z. mays *disponible para alimentarse.*

EFEITOS DE DIFERENTES COMPONENTES DA PLANTA HOSPEDEIRA NOS ÍNDICES BIOLÓGICOS EM *Spodoptera frugiperda*

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RESUMO

Spodoptera frugiperda *(J. E. Smith, 1797) (Lepidoptera: Noctuidae) é uma praga invasora que está devastando os cultivos em muitas províncias da China. Para controlar essa praga invasora é necessário entender a relação existente entre o inseto e seus hospedeiros. Neste estudo, os índices biológicos de* S. frugiperda *foram comparados alimentando-a com cinco plantas hospedeiras diferentes (*Zea mays, Triticum aestivum, Digitaria sanguinalis, Glycine max *e* Eleusine indica*) em condições de laboratório. Os índices biológicos de* S. frugiperda *alimentada con* Z. mays *foram os melhores. O peso da pupa e a fecundidade de* S. frugiperda*, alimentada com* G. max *e* E. indica*, foram significativamente menores do que aquelas alimentadas com as outras três plantas hospedeiras. O teor de fenóis totais da planta hospedeira correlacionou-se negativamente com o crescimento e desenvolvimento de* S. frugiperda*, enquanto que a relação C/N correlacionou-se positivamente. Os índices biológicos de* S. frugiperda *foram diferentes quando alimentada com as diferentes plantas, mesmo o inseto tendo completado seu ciclo de vida em qualquer um dos hospedeiros. Portanto, quando não é suficiente a quantidade de* Z. mays *disponível,* S. frugiperda *procura se alimentar de outros hospedeiros, especialmente de plantas com alto teor de C/N.*

determination of damage scope caused by *S. frugiperda* and the prediction of host species that provide the supplies of insect population accumulation.

In this study, five hosts associated with Chinese cornfields were carefully selected based on weed growth abundance in the field or crops grown around them. We assessed the growth development of immature stages on various host plants and the subsequent adult performance. We determined the composition of different host plants, insect herbivores and their host-plants correlation analysis were also investigated.

Materials and Methods

Insects and plant material

Spodoptera frugiperda was collected from Sunji cornfield in Huagang Town, Feixi County, Hefei City, Anhui Province in June 2019. Insects used in experiments had been fed on artificial diet for more than ten generations. Larvae were reared under controlled conditions at 25°C ±1 °C, 70% ±5% relative humidity (RH), and a photoperiod of 16L : 8D (Light : Dark). Egg masses laid by females were gathered and deposited in a plastic box (2cm x 15cm x 8cm). Then newly hatched larvae from the egg masses were collected for experimental use.

Zea mays, *Triticum aestivum*, *Glycine max*, *Digitaria sanguinalis* and *Eleusine indica* were all planted in the agricultural garden of Anhui Agricultural University in Hefei, Anhui Province, China. Leaves were cut from the plants and used throughout the experiments. The environmental conditions of 25 ±1°C, 70 ±5% relative humidity (RH).

Effects of different host plants on the biological characteristics of Spodoptera frugiperda

For each different host plant, 120 newly hatched larvae with the same body weight were selected and to feed and randomly divided into three groups. The larvae were placed in a 21cm × 15cm × 8cm sealed plastic box, the lid of the box was punctured with insect needles, and the bottom of the box was padded with filter paper. During the larval stage, fresh hosts of the same weight were replaced regularly every day, and feces were cleaned promptly, while the developmental period and survival rate of larvae were recorded. Fifteen pupae were randomly selected and weighed on the second day after pupation. The survival time and survival rate of all pupae were recorded. Fifteen pairs of *S. frugiperda* adults were selected and reared in a 70cm × 70cm × 70cm cage with 10% (w/v)

aqueous honey. Eggs were collected and counted daily. Environmental control conditions: 25 ±1°C, 70 ±5% relative humidity (RH), 16L: 8D photoperiod. The environmental controlled conditions of 25 ±1°C, 70 ±5% relative humidity (RH), and a photoperiod of 16L : 8D.

Determination of nutrients and secondary substances in different host plants

Soluble sugar: 100mg grated host tissue was added to 10mL ethanol in a volumetric flask, then the volumetric flask was placed in 80 ~ 85°C water bath and stirring for 30min. Afterwards, the solution was cooled down and centrifuged for 1min at 5000r/min. Then the supernatant was transferred to a beaker. The beaker was placed in 85°C water bath to evaporate the remanent ethanol, and then it was adjusted to 50mL with distilled water. Ultimately, the content of soluble sugar in the extract was determined by anthrone colorimetry.

Protein: 2g sample, 0.g copper sulfate, 6 potassium sulphate, and 20mL sulphuric acid were added into a dry 500mL Kjeldahl flasks in turn. After shaking gently, the Kjeldahl flasks was slanted at 45-degree angle on asbestos net with a small funnel placed at mouth of the bottle. Then the bottle was carefully heated. When the content was completely carbonized (no foam was produced), the heat should be further increased to keep the liquid boiling slightly until the liquid changed to be clear blue-green. After another continuous heating for 0.h ~ 1h, the Kjeldahl flasks was removed out and cooled to room temperature. Then the solution was transferred to a 100mL volumetric flask, meanwhile, a small amount of distilled water was used to wash the Kjeldahl flasks several times with the lotion transferred into the 100mL volumetric flask, too. Finally, distilled water was added to the scale line and the solution was mixed thoroughly. The protein content was determined by Automatic Kjeldahl nitrogen analyzer.

Total amino acids: mL hydrochloric acid ($6mol \cdot L^{-1}$) was added into homogenate (2g) and the well mixed solution was added to 10mL by hydrochloric acid in a hydrolysate tube, 3 drops phenol were added into the solution and the hydrolysate tube was put into refrigerant for 3 ~ 5 minutes. The tube was then connected with the suction pipe of a vacuum pump for vacuumizing, afterwards, it was filled in with nitrogen (repeating for 3 times) for sealing. The sealed hydrolytic bottle was put into an electric blast incubator at 110°C ±1°C for 22h and it was taken out and cooled to room temperature after heating. The mixed solution was filtered, and was transferred into a 50mL volumetric flask to bring to volume by distilled water, 1mL liquid from the volumetric flask was dried and steamed in a 15mL test tube, and then it was dissolved by 2mL sodium citrate buffer solution. After being passed through a 0.22μm filter membrane, the solution was transferred to an amino acid automatic analyzer (JJG1046-2011) injection bottle for accurate determination.

C/N ratio: 0.1g host sample, 0.1g silver sulfate powder, 5mL potassium dichromate standard solution ($0.8\ mol \cdot L^{-1}$) and 5mL concentrated sulfuric acid were added into a test tube. The C/N ratio results were obtained through digestion and titration procedures.

Water: 10g leaves of each host were weighed and dried in a drying box at 85°C. After drying, the mass of the corresponding dried leaves was weighed by an electronic balance to calculate the water content of the leaves.

Total flavonoids: 1g sample and 30mL anhydrous ethanol were added into a 100mL conical flask for extraction in an ultrasonic cleaner (1h). After cooling to room temperature, the solution was filtered to a 50mL volumetric flask and bought to volume by anhydrous ethanol. Sample absorbance at 420nm was measured, and the obtained standard curve was used to calculate the total flavonoid contents.

Tannin: 2g homogenized sample and 80mL distilled water were added into a 100mL volumetric flask in boiling water to extract tannin for 30min. After being cooled to room temperature, brought it to marked volume by distilled water. 2mL solution was sucked into a centrifuge tube for centrifugation at 8000 r/min (4min). 1mL supernatant (after centrifugation), 5mL water, 1mL mixture solution of sodium tungstate and sodium molybdate, and 3mL sodium carbonate were mixed for color reaction (2 hours). After this step, sample absorbance was measured at 765 nm and the obtained standard curve was used to calculate the tannin content.

Total phenol: 0.5g sample was milled to slurry with 3 mL 95% ethanol and filtered, then the filtrate was transferred to a 25mL volumetric flask and brought to volume by 95% ethanol. 2mL sample solution and 2mL folin were mixed and shaked for 3 minutes in 10mL test tube, then 2mL 10% sodium carbonate was added. After vibrating and standing (1 hour), the mixed solution was measured by colourimetric absorbance at 700nm and the total phenol content was calculated from the standard curve.

All the above samples were fresh host leaves, and all determinations were carried out in triplicate.

Data analysis

Excel 2003 was used to conduct statistics on the original data. SPSS 23.0 was used to analyze the contents of different host components, nutritional indices and biological indices of *S. frugiperda* feeding on different host plants by one-way analysis of variance (one-way ANOVA) and the means were separated tby Tukey's test (P <0.05). The relationship between chemical components of different hosts and growth indicators of *S. frugiperda* was also analyzed by SPSS 23.0 with Pearson correlation coefficient.

Results

Biological characteristics of Spodoptera frugiperda *feeding on different host plants*

As shown in Table I, the survival rate of early- larval instars (1 to 3) and older- larval instars (4 to 6) of *S. frugiperda* fed on *Z. mays*, 99.17 (df=4, F=23.071) and 81.52% (df=4, F=55.025), respectively, was higher than in other hosts, while larvae fed on *G. max* have the lowest survival rate (85.83% and 64.06%) (df=4, F=23.071, F=55.025). The developmental durations of older-instar larval fed on *E. indica* (10.43 days, df=4, F=23.290) is the longest, while early-instars larval fed on *Z. mays* have the shortest developmental time (6.22 days, df=4, F=33.728).

The pupal weight of *S. frugiperda* fed on *Z.mays* in larval stage is heaviest, up to 296.67mg, followed by larval fed on *T. aestivum* (285.33mg, df=4, F=22.746). larval fed on *G. max* has the lowest pupal weight (257mg, df=4, F=22.746). Egg-production amount (representation of fecundity) of *S. frugiperda* fed on *Z.mays* in the larval stage has the largest number, as much as 1308 eggs (df=4, F=106.047), while the minimum number (994 eggs, df=4, F=106.047) of *S. frugiperda* fed on *E. indica* in larval stage.

Nutrient composition and secondary substance content of different host plants

Some differences in the nutrient contents and the secondary substances among different host-plants (all P values < 0.05). The soluble sugar (Figure 1a), protein (Figure 1b), total amino acids

TABLE I
SURVIVAL RATES, DEVELOPMENTAL DURATIONS, PUPA WEIGHT AND FECUNDITY OF *Spodoptera Frugiperda* ON *Zea Mays, Triticum Aestivum, Glycine Max, Digitaria Sanguinalis* AND *Eleusine Indica*

Indicators	*Zea mays* (mean ± SE)	*Triticum aestivum* (mean ± SE)	*Digitaria sanguinalis* (mean ± SE)	*Glycine max* (mean ± SE)	*Eleusine indica* (mean ± SE)	df	F
Survival rate of early-instars larval / %	99.17 ± 0.83 a	93.33 ± 0.83 b	92.50 ± 1.44 b	85.83 ± 0.83 c	93.33 ± 0.83 b	4	23.071
Survival rate of older-instar larval / %	81.52 ± 0.77 a	77.69 ± 0.69 b	73.88 ± 0.59 c	64.06 ± 1.19 d	72.31 ± 1.04 c	4	55.025
Development period of early-instars larval / days	6.22 ± 0.44 d	6.29 ± 0.01 d	6.53 ± 0.05 c	6.68 ± 0.06 b	6.84 ± 0.05 a	4	33.728
Development period of older-instar larval /days	9.20 ± 0.17 c	9.62 ± 0.05 bc	9.80 ± 0.03 b	10.40 ± 0.12 a	10.43 ± 0.16 a	4	23.290
Pupa weight / mg	296.67 ± 2.96 a	285.33 ± 3.18 b	273.33 ± 1.20 c	257.00 ± 5.03 d	259.67 ± 4.10 d	4	22.746
Fecundity / eggs	1308.00 ± 6.35 a	1204.67 ± 9.24 b	1119.33 ± 8.99 c	1059.33 ± 26.97 d	994.00 ± 19.40 d	4	106.047

Early−instar larval: larva of instar 1 to 3; older−instar larval: larva of instar 4 to 6. Fecundity: egg-production amount. The data in the table are mean ± SE, different lowercase letters following data in the same row indicate significant difference ($P < 0.05$).

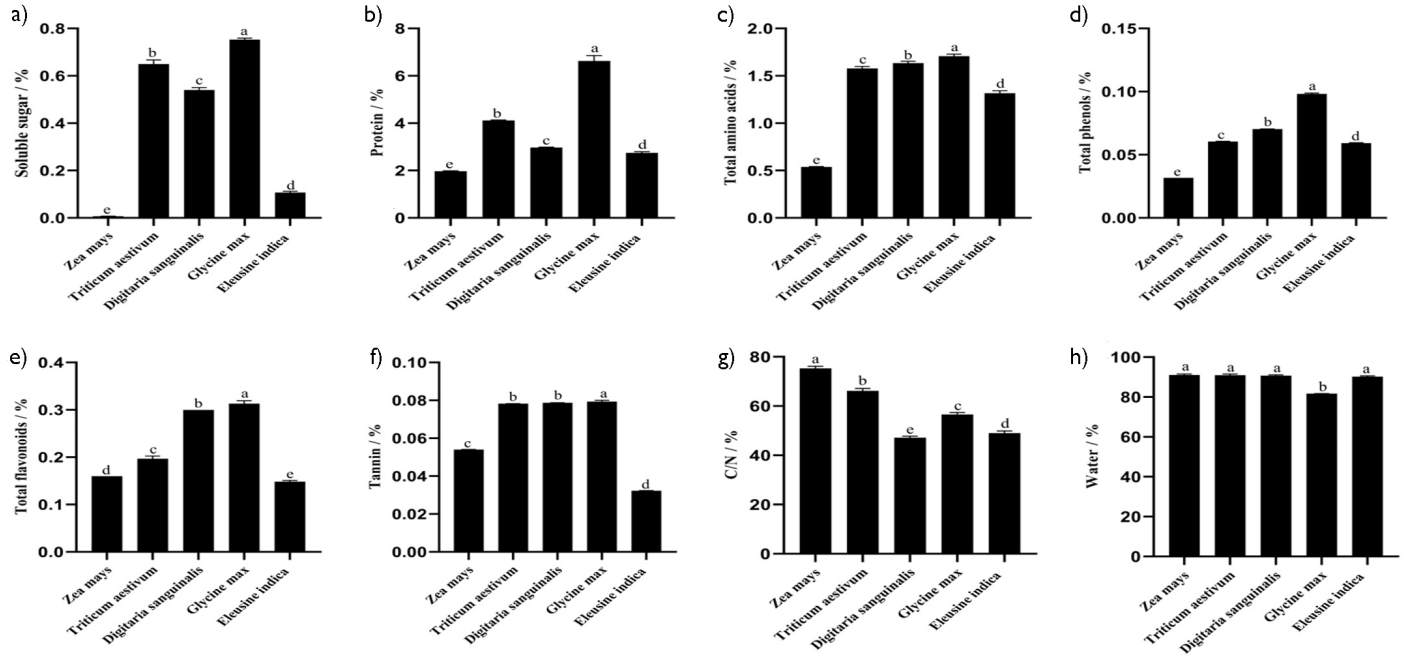

Figure 1. Mean ± SE numbers of phytochemical content of different hosts (*Zea mays*, *Triticum aestivum*, *Glycine max*, *Digitaria sanguinalis* and *Eleusine indica*) a) Soluble sugar; b) Protein; c) Total amino acids; d) Total phenols; e) Total flavonoids; f) Tannin; g) C/N y h) Water. Different lowercase letters indicate means are significantly different at $P < 0.05$.

(Figure 1c), total phenol (Figure 1d) contents of *Z. mays* are lowest, while those of *G. max* are highest. The total flavonoid (Figure 1e), tannin (Figure 1f) contents of *E. indica* are lowest, while those of *G. max* are highest. As shown in Figure 1g, the C/N content is higher in *Z. mays* than other hosts, while it is much lower in *D. sanguinalis*. However, the water contents for all hosts display no significant differences (Figure 1h).

Correlation analysis between host plant and Spodoptera frugiperda

As shown in Table II, the survival rate of early-instars larval and older-instar larval are mainly affected by the protein (R =−0.862 and R =−0.793) (all *P* values <0.01), total amino acids (R −0.773 and R =−0.651) (all *P* values <0.01), total phenols (R =−0.943 and R =−0.916) (all *P* values <0.01), Total flavonoids (R =−0.706 and R =−0.643) (all P values < 0.01), C/N (R =0.516 and R =0.605) (all *P* values < 0.05), and water (R 0.801 and R = 0.847) (all *P* values < 0.01) in the host plants. In addition, soluble sugar content has a significant effect on the survival rate of early-instars larval (R =−0.634, *P* < 0.05) but the survival rate of older-instar larval is not affected by it obviously. The developmental period of larval (early-instars and older-instar) is negatively correlated with C/N (R =−0.795 and R =−0.701) (all *P* values < 0.01) but positively correlated with total phenol content (R =0.560 and R =0.707) (*P* <0.05 and *P* < 0.01, respectively). Pupa weight is negatively correlated with protein (R =−0.531, *P* < 0.05), total amino acid (R =−0.605, *P* < 0.05) and total phenol (R =−0.758, *P* <0.01); and it is positively correlated with C/N (R =0.776, *P* <

TABLE II
CORRELATION BETWEEN ANALYSIS FROM THE PHYTOCHEMICAL CONTENT OF DIFFERENT HOSTS AND BIOLOGICAL INDICATORS OF *Spodoptera Frugiperda* AFTER FEEDING ON DIFFERENT HOSTS USING PEARSON CORRELATION COEFFICIENT

Indicators	Soluble sugar	Protein	Total amino acids	Total phenols	Total flavonoids	Tannin	C/N	Water
Survival rate of early-instars larval / %	−0.634*	−0.862**	−0.773**	−0.943**	−0.706**	−0.440	0.516*	0.801**
Survival rate of older-instar larval / %	−0.457	−0.793**	−0.651**	−0.916**	−0.643**	−0.217	0.605*	0.847**
Development period of early-instars larval / days	−0.023	0.289	0.438	0.560*	0.190	−0.350	−0.795**	−0.413
Development period of older-instar larval / days	−0.272	0.522*	0.559*	0.707**	0.281	−0.165	−0.701**	−0.578*
Pupa weight / mg	−0.235	−0.531*	−0.605*	−0.758**	−0.400	0.080	0.776**	0.589
Fecundity / eggs	−0.003	−0.217	−0.570*	−0.531	−0.149	0.318	0.887**	0.230

Early−instar larval : larva of instar 1 to 3; older−instar larval: larva of instar 4 to 6. Fecundity: egg-production amount. The data in the table are correlation coefficient: *=significant at $P < 0.05$, **= significant at $P < 0.01$.

0.01). The fecundity is mainly negatively correlated with the total amino acid content (R =−570, $P < 0.05$) of the host, and positively correlated with C/N (R =0.887, $P < 0.01$).

Discussion

Based on these results, it can be concluded that biological characteristics of *S. frugiperda* fed on different host plants in larval stage had significant difference. As the polyphagous insects, *Spodoptera frugiperda* will produce different adaptability for every host plant when feeding on different hosts (Naseri *et al*., 2009; Razmjou *et al*., 2014; Guo *et al*., 2021). For the most species of insects, developmental stage of larval, pupa weight, and number of eggs laid by female can be used to judge the best host plants for insects as a criteria (Greenberg *et al*., 2002; Xu *et al*., 2010). In this study, compared with other hosts, *S. frugiperda* had the highest survival rate and the shortest developmental cycle after feeding on *Z. mays*. In addition, the development period of the older-instar larval fed on *G. max* and *E. indica* were relatively longer, and the pupal weight and fecundity were lower than fed on other hosts. Thus, combining the effects of fed on five hosts in this study on the growth and development of *S. frugiperda*, *Z. mays* were optimal host and *E. indica* is the least suitable host for *S. frugiperda*.

There are different nutrient and secondary substance content with different hosts (Wilson *et al*., 2019). In general, the insects fed on high-quality hosts have shorter duration of the whole biological cycle, higher growth rate (Awmack and Leather, 2002; Vellau *et al*., 2013; Cunha *et al*., 2008). According to the correlation analysis of the host phytochemical content with the biological indices of *S. frugiperda*, we find that C/N plays an active role in the growth of *S. frugiperda*. This is consistent with the conclusion obtained by Holopainen (2002). Phenolic substances, one of the main chemical defense substances in the host plants (Awmack and Leather, 2002; Steinbauer, 2018), have a significant negative correlation to the growth and development indices of *S. frugiperda*. Besides, the total flavonoid content affects the survival rate of the early-instars and older-instar larval of *S. frugiperda*. There is no significant correlation between the tannin content and the entire growth and development of *S. frugiperda*. In other related studies, it has been found that the protein and amino acid content of plants can promote the growth and development of insects (Dai *et al*., 2020), but the results obtained in this study are contrary to the results of Dai *et al.* (2020). This might be caused by other factors in the feeding process. There is a complex dynamic relationship between the content of phytochemicals and the herbivorous insects (Steinbauer, 2018). The nutrient content and secondary metabolite content of the host plant will change the feeding behavior and feeding response of insects. Therefore, cannot be done to make a broad generalization of the effect of a single host phytochemical on herbivorous insects, and the quality of the host plant is also affected by the physical properties (hardness, surface hair density and shape), more research is needed to help us understand these assumptions.

In this study, although the adaptability to *D. sanguinalis*, *G. max* and *E. indica* is not as good as that to *Z. mays* and *T. aestivum*, *S. frugiperda* can still complete its life cycle after feeding on them. Therefore, it is necessary to pay attention not only to crops, but also to the species and quantity of the surrounding weeds in the control process of *S. frugiperda*. Besides, it is found in this study that the higher C/N content, the more favorable effect for *S. frugiperda*. It has also been reported that the level of fertilization will affect the C/N expression in plants in other related literatures (Ibrahim *et al*., 2011; Deng *et al*., 2020). Therefore, the control of *S. frugiperda* can also be carried out by adjusting the amount of fertilizer.

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