



Consumption and use of soybean by the caterpillar *Helicoverpa armigera*

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ABSTRACT: A deep insight into the population dynamics of a pest can be achieved by being acquainted with its nutritional ecology, which further assists in understanding the damage potential and decisions to control mainly in diversified production systems such as those in Brazil. The present research aimed to evaluate the effect of dietary regimens, comprising combinations of accessible organs of soybean plants, at different stages of development, consumption, and utilization of food by the 4th, 5th, and 6th larval instar of *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae). For this purpose, the vegetative and reproductive organs of soybean were brought together in three feeding regimes, followed by making it available to caterpillars. After evaluations, it was found that under laboratory conditions, the development of *H. armigera* 4th to 6th instar caterpillars was better when they were purely fed on leaves than on combinations of vegetative and reproductive parts, such as fresh leaves + old leaves + pods at the beginning of grain development, and old leaves + pods at the beginning of grain development + pods with well-developed grain.

Key words: old-world caterpillar, nutritional indexes, nutritional ecology.

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RESUMO: O conhecimento sobre a ecologia nutricional de uma praga pode auxiliar na compreensão da sua dinâmica populacional, na definição do potencial de danos e nas decisões para controle, principalmente, em sistemas de produção diversificados como os existentes no Brasil. O objetivo desse trabalho foi avaliar o efeito de regimes alimentares, constituídos por combinações de órgãos disponíveis nas plantas de soja, em diferentes estádios de desenvolvimento, no consumo e no aproveitamento do alimento pelas lagartas de 4^o, 5^o e 6^o instares de *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae). Órgãos vegetativos e reprodutivos da soja foram combinados em três regimes alimentares e fornecidos à lagartas. Os resultados demonstram que, em condições de laboratório, na cultura da soja o desenvolvimento de lagartas do 4^o ao 6^o instar de *H. armigera* foi melhor quando se alimentaram com folhas do que com folhas novas + folhas velhas + legumes no início do desenvolvimento do grão e folhas velhas + legumes no início do desenvolvimento do grão + legumes com o grão bem desenvolvido.

Palavras-chave: lagarta do-velho-mundo, índices nutricionais, ecologia nutricional.

INTRODUCTION

Helicoverpa armigera (Lep.: Noctuidae: Heliethinae), during the larval phase, has a polyphagous habit due to which its development depends on the plant species on which it feeds (SUZANA et al., 2015). However, very few articles regarding the host adequacy of the caterpillar, especially the efficiency in the utilization of food sources, concerning both species and plant organs, have been reported until now. In this context; although, ROGERS & BRIER (2010), BORTOLOTTO et al. (2014), have mentioned that *H. armigera* shows preference towards the reproductive

organs in soybean (*Glycine max*), the criteria for this inclination of *H. armigera* is whether nutritional adequacy or merely the choice, is unclear. Therefore, it indicates that insect-plant adaptation strategies are exceptionally complex as well as it is inadequately understood, even upon the involvement of pests and plants of economic importance, at the global level such as *H. armigera* and soybean. Thus, gaining knowledge in the direction of the growth and development of *H. armigera* among different hosts will contribute to the understanding of population dynamics of the pest. The pest failure in some species or plant organs can be attributed, to the presence of secondary phytochemicals

or the absence of primary nutrients essential for growth and development (NASERI et al., 2010). However, the role of these nutrient deficiencies as well as the number of defense compounds (secondary phytochemicals) among various organs of soybean, on which the pest feeds exclusively, at different stages of development, is still unidentified. Further, it has been established by SUZANA et al. 2015 (unpublished data) that in comparison to the leaves of other hosts, leaves of soybean do not provide optimum conditions required for the pest development and growth. In spite of this, *H. armigera* in soybean under natural conditions colonizes successfully, which in turn can be correlated to its capability to opt for more nutritious or less defensive tissues via locomotion. It has been already demonstrated in other pest host plants (PERKIN et al. 2013). Thus, one can elucidate that according to the type of food available throughout the soybean cycle, under natural conditions, the larvae of *H. armigera* is capable of feeding itself on different organs, which are either isolated or not.

The current investigation aims to evaluate repercussions of feeding regimes, constituted by combinations of vegetative and reproductive organs available in soybean plants at different stages of development, in consumption and utilization of food by *H. armigera* caterpillars.

MATERIALS AND METHODS

H. armigera caterpillars grown on an artificial diet (GREENE et al., 1976) were used in the Entomology Laboratory of the Universidade de Passo Fundo (UPF), in an air-conditioned room ($25 \pm 2^\circ\text{C}$, $60 \pm 10\%$ relative humidity and photophase of 12 hours). Further, for feeding the caterpillars, vegetative and reproductive organs of soybean (cv. BMX Ativa RR), grown in 8 liters pot in a greenhouse, were collected.

Three treatments were constituted, by making combinations with the plant organs, for determining the effect of vegetative and reproductive organs of soybean on nutritional indexes of *H. armigera* caterpillars. The experiment was carried out on 260 caterpillars (replicates) for each regimen, in a completely random design. With the intention of characterizing, in the constitution of each regime, the organs that are accessible for the caterpillars in different stages of development of the soybean plants with being subjected to pest attack, by the scale of HANWAY & THOMPSON (1967), the term "feeding regimes" were defined as the real field situations. The three regimes subjected to evaluation are 1) New leaves + old leaves (FN + FV regime),

collected in the V6 - V8 stages, with five to seven fully opened trifoliolate leaves; 2) New leaves + old leaves + pods at the beginning of grain development (FN + FV + LIG regime), which occurs at the R5.1 stage, thereby representing the beginning of grain formation; and 3) Old leaves + pods at the beginning of the development of the grain + pods with the well-developed grain (FV + LIG + LDG regime), which occurs in the stages R5.5 - R6, characterized by legumes in full grain filling stage.

In the beginning, the caterpillars to be employed for the experiments were placed individually in the Petri dishes (4cm² in diameter). Subsequently, only sprouts and tender soybean leaves were made available to them for consuming, thereby simulating a real situation followed by observation of the natural behavior of freshly hatched caterpillars in plants in the early stages of development. The growth of the larvae was monitored, on a daily basis. At the 4th instar stage, the larvae was transferred to individual Petri dishes (9cm² in diameter), thereby supplying the feeding regimes to be estimated. The data regarding the dry mass of the food consumed, weight gained by the insect, and the excreted matter, were obtained through daily observations. The samples collected, were oven dried at 60°C for 48 hours and were subsequently weighed on a precision scale (0.0001 g). For each instar, the gain in the weight was estimated using aliquots of 20 caterpillars. For this purpose, each instar upon entering the 4th, 5th, and 6th instar stage as well as the instar exhibiting the first signs of pre-pupae, were sacrificed. Following are the list of parameters evaluated for 4th, 5th, and 6th instars caterpillars: (i) Relative consumption rate (RCR), (ii) relative metabolic rate (RMR), (iii) relative growth rate (RGR), (iv) feed conversion efficiency (ECI), (v) conversion efficiency of digested food (ECD), (vi) approximate digestibility (AD), and (vii) metabolic cost (CM) according to PARRA (1991). Further, data normality test and covariance analysis, using RCR as co-variable, was carried out on the obtained data with a subsequent comparison of the averages, by the Tukey-Kramer test ($p \leq 0.05$), upon indication of significant differences between the values.

RESULTS AND DISCUSSION

From the experiment, in the 4th instar stage, higher values were obtained for the relative consumption rate (RCR) and the relative metabolic rate (RMR) in the FV + LIG + LDG regimen, while the relative growth rate (RGR) exhibited no difference between the regimens (Table 1). As a result, the

Table 1 - Nutrient indices (mean \pm SEM) of caterpillars in the 4th instar of *Helicoverpa armigera* under the diet regime, in the laboratory (25 \pm 2°C, 60 \pm 10% RH and 12 hours of photophase).

Dietary regimens (n)	RCR (mg/mg/dia)	RMR (mg/mg/dia)	RGR (mg/mg/dia)
FN+FV (84)	6.56 \pm 0.23 c	3.80 \pm 0.19 c	0.81 \pm 0.02 ns
FN+FV+LIG (84)	37.60 \pm 1.40 b	33.86 \pm 1.32 b	0.83 \pm 0.02
FV+LIG+LDG (82)	69.09 \pm 2.21 a	64.89 \pm 2.16 a	0.77 \pm 0.02
CV. (%)	17.94	3.94	12.58

Tukey-Kramer test ($P < 0.05$), not significant (ns). New leaves (FN); old leaves (VF); pods at the beginning of grain development (LIG); pods with well developed grain (LDG), relative consumption rate (RCR); relative metabolic rate (RMR); relative growth rate (RGR); standard error of the mean (EP), and number of repetitions (caterpillars) (n).

utilization of food, as indicated by the efficiency of conversion of ingested (ECI) and digested (ECD) food, were higher in the FN + FV regime, thereby leading to low metabolic cost (CM) (Table 2). Further, the high RMR value, which is the amount of food spent in metabolism, as obtained in the case of FV + LIG + LDG regime, indicates that a large amount of the food consumed was being employed for maintaining the body's activities, resulting in a lower ECD and ECI, thus, demonstrating the lowest weight gain among all regimens. Additionally, in the case of FN + FV + LIG and FV + LIG + LDG regimens, in which pods were being offered, the percentage of ingested food that is effectively assimilated by the insect, as expressed by the term "approximate digestibility (AD)" was reported to be higher. For 5th instar caterpillars, following observations were made, (i) RCR and RMR were found to be higher in the FV + LIG + LDG regimen (Table 3), (ii) RGR, ECI, and ECD were higher in the FN + FV regimen, thereby causing lower CM (Table 4), and (iii) the AD showed no difference between the regimens.

In the case of 6th instar, the experiment resulted in higher values of RCR and RMR in the FV + LIG + LDG regimen with RGR showing no difference between the treatments (Table 5). Similarly, ECI, ECD, and CM also exhibited no difference between the regimens (Table 6). Whereas, the value of AD was on the higher side in the FV + LIG + LDG and FN + FV + LIG regimens. Thus one can elucidate that, statistically, these variables showed no difference; however, a trend was observed regarding greater conversion efficiency in the FN + FV regimen, which in turn validated the results obtained in the 4th and 5th instar stage of the caterpillar.

Additionally, for the three instars evaluated, the highest ECI and ECD values were certified, upon feeding the caterpillars exclusively with leaves (FN + FV regimen). Therefore, in addition to these foods present greater nutritional adequacy, a greater ability of caterpillars regarding utilization of food for growth and development was demonstrated. Hence, the higher conversion ability completely influenced the growth and development

Table 2 - Nutrient indices (mean \pm SEM) of caterpillars in the 4th instar of *Helicoverpa armigera* under the diet regime, in the laboratory (25 \pm 2°C, 60 \pm 10% RH and 12 hours of photophase).

Dietary regimens(n)	ECI (%)	ECD (%)	AD (%)	CM (%)
FN+FV (84)	13.50 \pm 0.60 a	20.66 \pm 1.24 a	69.55 \pm 1.33 b	79.34 \pm 1.24 b
FN+FV+LIG (84)	2.35 \pm 0.07 b	2.57 \pm 0.09 b	92.13 \pm 0.63 a	97.43 \pm 0.09 a
FV+LIG+LDG (82)	1.24 \pm 0.07 c	1.33 \pm 0.08 c	94.73 \pm 0.35 a	98.67 \pm 0.08 a
CV. (%)	19.35	26.80	5.29	4.22

Tukey-Kramer test ($P < 0.05$), not significant (ns). New leaves (FN); old leaves (VF); pods at the beginning of grain development (LIG); pods with well developed grain (LDG), conversion feed conversion efficiency (ECI); conversion efficiency of digested food (ECD); approximate digestibility (AD); metabolic cost (CM); standard error of the mean (EP), and number of repetitions (caterpillars) (n).

Table 3 - Nutrient indices (mean \pm SEM) of caterpillars in the 5th instar of *Helicoverpa armigera* under the diet regime, in the laboratory (25 \pm 2°C, 60 \pm 10% RH and 12 hours of photophase).

Dietary regimens(n)	RCR (mg/mg/dia)	RMR (mg/mg/dia)	RGR (mg/mg/dia)
FN+FV (53)	7.09 \pm 0.47 b	4.32 \pm 0.50 c	0.71 \pm 0.04 a
FN+FV+LIG (52)	11.66 \pm 1.46 b	9.63 \pm 1.41 b	0.60 \pm 0.04 b
FV+LIG+LDG (40)	18.48 \pm 3.09 a	15.11 \pm 2.67 a	0.55 \pm 0.04 b
CV. (%)	43.08	15.77	20.21

Tukey-Kramer test (P<0.05), not significant (ns). New leaves (FN); old leaves (VF); pods at the beginning of grain development (LIG); pods with well developed grain (LDG), relative consumption rate (RCR); relative metabolic rate (RMR); relative growth rate (RGR); standard error of the mean (EP), and number of repetitions (caterpillars) (n).

of caterpillars, thereby producing larger pupae, which in turn exhibits a direct correlation with adult fertility, being essential ecologically for the survival of this insect (DARYAEI et al., 2007). Furthermore, it can make the reproductively adult more competitive as the size influences the copulation success, the fecundity, as well as the dispersion capacity (PARRA et al., 2009). In the FN + FV regimen in the three instars, the observation of the lowest RCR and the highest ECI and ECD has two possible explanations; (a) the first one being justifying it by mentioning that consumption of food in less quantity by the caterpillars leads to slow movement of it through the digestive system, thereby enhancing its chance to undergo complete conversion with further utilization by the insect, (b) the second explanation was based on the improved quality of the food, as a result, the caterpillars consume less food, thus, converting it more efficiently, thereby taking maximum advantage of it without requiring larger amount (SOO HOO & FRAENKEL, 1966).

Apart from above factors, the higher efficiency of conversion in the exclusive regime with soy leaves may also get affected by the new soybean plant tissues as they seem to be more attractive to the pests, mainly due to their more palatability and higher nutritional value (FITT, 1989), thereby presenting higher quality. On the first three instars, caterpillars of *H. virescens* and *H. armigera* feed in the upper region of the plants attacking vegetative parts and, when they reach the fifth instar, they move towards the reproductive parts, including flowers, fruits, and seeds (ROGERS & BRIER, 2010; BORTOLOTTI et al., 2014). However, the movement of the caterpillars to the reproductive parts does not elucidate it to be the highest quality food for their development. In the FV + LIG + LDG regimen, the higher RCR and RMR observed upon evaluation of the three instars, illustrated the inefficient use of energy because the heart of the proposal lies on the lower consumption of energy for metabolism, thereby allowing its higher utility

Table 4 - Nutrient indices (mean \pm SEM) of caterpillars in the 5th instar of *Helicoverpa armigera* under the diet regime, in the laboratory (25 \pm 2°C, 60 \pm 10% RH and 12 hours of photophase).

Dietary regimens(n)	ECI (%)	ECD (%)	AD (%)	CM (%)
FN+FV (53)	12.31 \pm 1.06 a	21.01 \pm 2.04 a	75.50 \pm 8.29 ^{ns}	78.99 \pm 2.04 b
FN+FV+LIG (52)	8.47 \pm 0.93 b	12.21 \pm 1.97 b	81.18 \pm 1.88	87.79 \pm 1.97 a
FV+LIG+LDG (40)	5.29 \pm 0.50 c	7.01 \pm 0.79 c	80.624 \pm 1.64	92.99 \pm 0.79 a
CV. (%)	23.19	31.8	19.52	7.93

Tukey-Kramer test (P<0.05), not significant (ns). New leaves (FN); old leaves (VF); pods at the beginning of grain development (LIG); pods with well developed grain (LDG), conversion feed conversion efficiency (ECI); conversion efficiency of digested food (ECD); approximate digestibility (AD); metabolic cost (CM); standard error of the mean (EP), and number of repetitions (caterpillars) (n).

Table 5 - Nutrient indices (mean \pm SEM) of caterpillars in the 6th instar of *Helicoverpa armigera* under the diet regime, in the laboratory (25 \pm 2°C, 60 \pm 10% RH and 12 hours of photophase).

Dietary regimens(n)	RCR (mg/mg/dia)	RMR (mg/mg/dia)	RGR (mg/mg/dia)
FN+FV (20)	2.22 \pm 0.23 b	0.81 \pm 0.15 c	0.31 \pm 0.02 ^{ns}
FN+ FV + LIG (16)	2.61 \pm 0.34 b	1.23 \pm 0.21 b	0.40 \pm 0.05
FV + LI G+ LDG (9)	4.37 \pm 0.67 a	3.00 \pm 0.67 a	0.34 \pm 0.04
CV. (%)	25.69	20.58	17.66

Tukey-Kramer test ($P < 0.05$), not significant (ns). New leaves (FN); old leaves (VF); pods at the beginning of grain development (LIG); pods with well developed grain (LDG), relative consumption rate (RCR); relative metabolic rate (RMR); relative growth rate (RGR); standard error of the mean (EP), and number of repetitions (caterpillars) (n).

for the growth and development, which in turn will result in gain in the body weight.

In the current investigation, the mean RGR for diets was 0.80, 0.62, and 0.35 for 4th, 5th, and 6th instar, respectively. Conversely, for several soybean cultivars, the average RGR values in the case of *H. armigera* caterpillars was 0.03 in the 3rd and 4th instars and 0.36 in the 5th instar (SOLEIMANNEJAD et al., 2010), which elucidated the consistency of the results for the last instar.

The results obtained in the present study are very likely to be associated with the development of digestive enzymes (proteinases). In the first instars, the caterpillars consume tender leaves and sprouts. However, with the development of the enzymes, they begin to feed on the reproductive structures of soybean, thereby further damaging it (PATANKAR et al., 2001). In the 4th instar, the maximum weight gain was established in the regime in which caterpillars were made accessible to only leaves. Further, it can be explained by stating that as the 4th instar caterpillars are crucial for their establishment, as an adult, the first

and third instar prefers to consume leaves exclusively in order to avoid damaging their mandibles with harder organs (ZALUCKI et al., 2002). Furthermore, the present study illuminated the fluctuation in the existence of *H. armigera* caterpillars between leaves and pods, perhaps due to the feeding tendency of caterpillars on both structures during their growth and development.

On the top of digestive enzymes, the presence of secondary phytochemicals or antibiotic and antioxenotic agents, such as isoflavones, or the absence of primary essential nutrients for the growth and development of this pest, can be the additional factors responsible for lower adequacy of few host plants or organs of these plants (RIBEIRO et al., 2007; NASERI et al., 2009; HEMATI et al., 2012), leading to higher or lower dietary capacity of plant species or organs. Similarly, being already demonstrated in other host plants, the successful colonization of the caterpillar in soybean, under natural conditions, were attributable to its constant locomotion in search of more nutritious or less well-protected tissues (PERKINS et al., 2013).

Table 6 - Nutrient indices (mean \pm SEM) of caterpillars in the 6th instar of *Helicoverpa armigera* under the diet regime, in the laboratory (25 \pm 2°C, 60 \pm 10% RH and 12 hours of photophase).

Dietary regimens(n)	ECI (%)	ECD (%)	AD (%)	CM (%)
FN+FV (20)	17.31 \pm 2.24 ^{ns}	39.29 \pm 4.94 ^{ns}	49.22 \pm 4.28 b	60.71 \pm 4.94 ^{ns}
FN+ FV + LIG (16)	21.11 \pm 4.52	31.86 \pm 5.59	65.05 \pm 4.54 a	68.14 \pm 5.59
FV + LI G+ LDG (9)	9.09 \pm 1.55	14.76 \pm 3.68	72.23 \pm 5.63 a	85.24 \pm 3.68
C.V. (%)	21.29	21.77	16.15	12.71

Tukey-Kramer test ($P < 0.05$), not significant (ns). New leaves (FN); old leaves (VF); pods at the beginning of grain development (LIG); pods with well developed grain (LDG), feed conversion efficiency (ECI); conversion efficiency of digested food (ECD); approximate digestibility (AD); metabolic cost (CM); standard error of the mean (EP), and number of repetitions (caterpillars) (n).

CONCLUSION

Conclusively, in the soybean crop, the development of *H. armigera* 4th to 6th instar caterpillars was better, when these caterpillars were allowed to consume only leaves in comparison to new leaves + old leaves + pods at the beginning development of the grain and old leaves + pods at the beginning development of the grain + pods with well-developed grain.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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