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Research Article

Estimation of alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae) economic damage based on the larval laboratory feeding rate

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Abstract. The alfalfa weevil (AW), *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), is a major pest of alfalfa *Medicago sativa* L. (Fabaceae). Both adults and larvae feed on alfalfa foliage, damage and reduce hay yield and quality, especially in spring. Larval feeding rate was estimated based on alfalfa leaflet consumption under laboratory conditions (18 ± 2 °C, 60–70% RH). Adults did not consume after emergence, apparently due to diapause. The first instar larvae of AW (n=150) were removed immediately after hatching in the laboratory and were confined with alfalfa leaves. The leaf weights (g) were measured prior to and each 24h after larval feeding. The larvae fed for 33-35 days in the laboratory and underwent four instars. The results revealed that average larval consumption is 8.178±0.153 g leaf tissue during the whole larval stages. Our estimates show that insecticide treatment will cost 6,700,000-14,700,000 IRR/ha depending on market values. One kg alfalfa feed appreciates 40000 IRR. Hence, 167.5-367.5 kg of feed corresponds to control costs. If all larvae survive to pupate (whole lifetime consumption), 2.0-4.5 larvae m^{-2} are tolerable and need no action. However, if a total mortality of 25, 50, or 75% occurs with a constant rate, tolerable densities will increase to (2.5-5.4), (3.2-7.0), and (4.8-10.6) m^{-2}, respectively. Assuming that an insecticide kills 90- 99% of the pest, 7.9-17.4 to 21.1-46.3 larvae m^{-2} will cause no loss if farmers intervene on time.

Keywords: Control costs, Intervention, Leaf consumption, Tolerable densities, Yield value

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Introduction

Alfalfa (*Medicago sativa* L.) is the most important cultivated forage crop worldwide. It improves soil structure and provides a good quality feed (rich protein and digestive fiber content) (Chandel *et al.*, 2021). It also provides good refuge for either beneficial or non-pest insects non-related to alfalfa. Alfalfa weevil (AW), *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) is the most serious insect pest of alfalfa worldwide (Giles, 1992). The alfalfa weevil has four larval instars through a single annual generation in northwest of Iran. The AW primarily feed on the alfalfa early season since germination until the first cut and continue to feed on the after-cut growth shortly before beginning its annual dormancy phase (Khanjani., 1986; Haddadi *et al.*, 2014; Karimzadeh *et al.*, 2016; Dalir *et al.*, 2024).The female weevils insert their eggs in clusters of 5–20 in stems and often hatch within 1–2 weeks. Both adults and larvae feed on alfalfa leaves, however, adult feeding rarely causes significant yield loss. The main injury is made by larvae (Pellissier *et al.*, 2017). Larval damage accounts for 25-40% of the first harvest yield early spring, in Spain (Levi-Mourao *et al.*, 2022). The most destructive instars are the third and fourth instar larvae that can remove considerable biomass, by consuming leaf tissue, that it may in turn delay crop maturity. Heavily infested fields show a white appearance due to skeletonizing or whole consumption of leaflets (DeGooyer, 1993). The IPM program of AW in United States includes cultural, biological, and chemical control measures (Flanders *et al.*, 2000; Pellissier *et al.*, 2017). Early harvesting and insecticide applications may reduce AW populations (Herreid, 2023; McClure *et al.*, 2023). The time of Insecticide application needs a decision-making criterion, namely economic threshold (ET). The concept of ET is closely related to economic injury level (EIL). The calculation of ET is more difficult than EIL, because it can be affected by environmental factors. The EIL is a more accessible goal to calculate although it is also subjected to uncertainty considering changes in market values, insecticide efficiency, etc. EIL concept is essential for IPM programs, because it indicates which levels of pests can be tolerated and which ones cannot (Pedigo *et al.*, 1986; Higley and Pedigo, 1993; Higley, 1993, Godfrey *et al.*, 2004; Riley *et al.*, 2008; Chason *et al.*, 2015. & Rim *et al.*, 2020).

A common way to calculate the EIL of a pest was described by Pedigo *et al.* (1986). Riechelderfer (1977) explained an applied algorithm to estimate it by field experiments in which different densities of the pest are present. The field experiments are labor-intensive and costly. They require vast information about the relationship between insect injury at different population levels and damage at different times of the season. Koehler and Pimentel (1973) measured the feeding rate of AW on alfalfa in the laboratory and extended it through modeling field condition to estimate the EIL.They found that larval feeding accounts for 50% weight loss in alfalfa plants after 10 days. The whole AW larval stage feeding averaged 7.34 mg dry weight /larva. Every gram of dry weight of alfalfa foliage converted to 1.59 g fresh weight of insect tissue. Since the compensatory capacity of alfalfa plants vary depending on environmental conditions and plant variety, the estimated EIL-values must be adjusted. In a damage simulation study, Peterson *et al.* (1993) studied the response of alfalfa plants to AW injury at the early bud stage and the results were used for developing economic injury level for AW. The results showed that the simulated injury did not affect stem density before the first cut or during the second growing cycle. Dry weight of the first cut were used to calculate the EIL for the third and fourth instar larvae of AW in the early bud stage of alfalfa.

As far as we are aware, there is no further research investigating the laboratory assessment of larval feeding of *Hypera postica* and the calculation of EIL. The present study estimated the consumption rate of AW larval stage (based on dry weight) on alfalfa leaves under laboratory conditions. The monetary calculations then were carried out. The consumed hay per capita was calculated and used against the spraying costs per unit area to estimate the EIL.

Materials and methods

Measure of leaf tissue consumption during larval development

Plants infested with AW were collected from an experimental alfalfa field at the Faculty of Agriculture, University of Tabriz during early spring 2023. Plants were collected when the stem height reached about 50 to 60 cm. The harvested stands were then transferred to the Laboratory of Insect Ecology at the University of Tabriz. Alfalfa stems bearing AW eggs were picked and placed in plastic boxes (20 × 15 ×10 cm, L × W × H). The eggs were left to hatch. The first larval instar of AW (n=150) was removed immediately after hatching and confined with a sufficient supply of alfalfa leaves. A plastic container (12 × 10 × 10 cm) was used to maintain individual larva. The containers were held at laboratory conditions (18 ± 2 °C, 60–70% RH and natural day). Alfalfa leaves were replaced daily from unsprayed fields. The weight (g) of the leaves was measured just prior to and after feeding for 24h by a digital balance (AND digital balance, GF-600, 610g/0.001 g, Japan). The larvae fed for 33-35 days in the laboratory and underwent four instars. A linear regression analysis was conducted to establish the relation between daily leaf consumption (g) and age of larva (d). Overall leaf consumption of a single larva was calculated by summing daily average consumption over whole developmental time.

EIL calculations

The EIL value was calculated using the equation established by (Pedigo *et al.*, 1998; 2021):

$$\text{EIL} = C/VIDK \qquad \text{(Equation 1)}$$

where EIL is the least density of larvae which will cause economic injury if all larvae survive to pupate,

C is the cost of an insecticide application (or other alternative measure such as inundative release of natural enemies) (IRR per ha), V is the market value of hay (IRR per Kg), I is injury (leaf area reduction) per an individual larva surviving to pupate, D is damage (yield loss) (g) per unit injury which is one for the forage crop, because the weight of the eaten tissue corresponds to the leaf area consumed, and finally, K is mortality rate of the pest following insecticide treatment.

The components of the above equation were calculated as below:

C: Insecticides for AW management can be used against adults and/or larvae by considering the status of pollinators and natural enemies by a regular scouting. The organophosphates, carbamates, or pyrethroids (e.g. Malathion, cypermethrin, fenvalerate and Dursban) are used separately or combined pairwise for controlling the AW population. (Wright *et al.*, 2015). The cost of insecticide application including market value of insecticide, labor, fuel, and rental of spraying tools was obtained from farmers and the 2023 annual reports of East Azerbaijan Agricultural and Natural Resources Research and Training Center (Noorbakhsh, 2023). Each application costs 6,700,000 to 14,700,000 IRR per ha.

V: The market value was estimated at 40,000 IRR per kg of the forage crop in 2023, based on the same report, and information obtained from farmers.

I and D: It was assumed that all larvae will survive to pupate in control fields. The amount of injury corresponding to the control costs was calculated once by including K=1 for control.

K: Because survival of larvae following field spraying is not known, different levels of larval mortality were considered at 25, 50, 75, 90 and 99%. Constant mortality rates were assumed during the whole developmental time from hatching to pupation.

Daily survival was obtained by an exponential decreasing pattern (Barclay, 2005):

$$N=N_0.e^{rt} \quad \text{(Equation 2)}$$

where N = current (daily) survival, N_0 = initial number of larvae on hatch (considered 1 in per capita scale), t=time 0, 1, 2 … 35d of development, and r was declining rate of population which was determined by trial and error so that N reached to 0.75, 0.5, .25, 0.1 and 0.01 of the initial number (N_0) by considering 25, 50, 75, 90 and 99% mortality, respectively. It was assumed that the survived larva may consume as control larvae. We explained an algorithm for decision making in actual situations which a farmer may encounter based on the past damage and the expected future damage of the post-spraying survived individuals.

Data analysis

Daily and cumulative feeding amounts among stages were analyzed using One-way ANOVA and means were compared by Least Significant Difference (LSD) (α=0.05). The relationship between consumed tissue weight (g) and age of larvae (d) was analyzed using linear regression (SAS ver. 9.3; SAS Institute, 2005).

Results

Developmental time and injury

The four larval instars and pupal stage of *Hypera postica* (Fig. 1) were completed in 3, 9, 6, 15 and 7~10 d, respectively. The development of immature stages was completed in 33- 35 days under laboratory conditions at 18±2 °C. Early-stage (first and second instar) larvae chewed small holes in the leaves, the third and fourth larval instars caused majority of the damage by skeletonizing leaflets or defoliation (Fig. 2). The two instars were completed in 18-21 days (Fig. 2). The emerged adults barely ate and survived for 7-10 days under laboratory conditions.

Consumption rate

The daily and cumulative consumption rates were significantly different among larval stages (F=225.43, df=3, 116, P<0.0001, and F=497.57, df=3, 116, P<0.0001 for daily and cumulative consumption, respectively). The fourth larval instar had the highest daily consumption rate followed by the third instar one. Total consumption of the 4th larval instar was four times that of the third instar, due to a longer developmental time of the fourth instar larva. (Table 1). Daily consumption had an increasing trend within each stage, with variability within feeding duration (*F*= 256.19, df= 1, 13, P<0.0001; Fig. 3). The results indicated that larvae can consume

8.178±0.153 g dry leaf tissue per capita during whole larval stages, 69.3% from which realized during the fourth instar, 29.4% during the second and third instars combined, and only 1.3% during the first instar.

Monetary measures

Our estimates show that insecticide treatment will cost 6,700,000-14,700,000 IRR/ha depending on market values. One kg alfalfa-feed at first hay-cutting (harvest) values at 40,000 IRR. An estimated 167.5-367.5 kg of feed corresponds to control costs (6,700 k IRR/40k IRR.kg^{-1} =167.5 and 14,700 k IRR/40k IRR.kg^{-1} =367.5 kg). If all larvae survive to pupate (whole life time consumption), 2.0-4.5 larvae m^{-2} (20,000-45,000 ha^{-1}) are tolerable and need no action. Each larva consumes 8.178 g which corresponds to 167.5 kg per 20,482 and 367.5 kg per 44,938 larvae. However, if a total mortality of 25, 50 or 75% occur with a constant rate, tolerable densities will increase to (2.5-5.4), (3.2-7.0) and (4.8-10.6) m^{-2}, respectively. Assuming that an insecticide kills 90- 99% of the pest, 7.9-17.4 to 21.1-46.3 larvae m^{-2} will cause no loss if farmers intervene on time (Table 2).

Discussion

Our results revealed that the main injury (about 70%) by AW occurs during the 4th larval instar. These findings are similar to those of Ordaz-Silva *et al.* (2020), and Beauzay *et al.* (2013). The consumption was negligible during the first instar, thereafter increased linearly by age/instar. The emerged adults fed little if any (Ronald *et al.*, 2014). Results also revealed that whole consumption rate of AW is around 8.178 g of dry leaf tissue per larva which is higher than 7.34 mg reported by Koehler and Pimentel (1973). The difference in whole consumption rate between the two studies refers to AW populations, physical conditions of the experiments, variety of the alfalfa, soil fertilization, etc. For example, development in a warmer region can be accelerated, some populations of insects grow more rapidly than the others (Stilwell *et al.*, 2010) or feeding on a richer diet (for example higher quality plant or plant growing in more fertile soil) also can accelerate development and then intake (El-Refaie *et al.*, 2024).

Fig. 1. Different stages of alfalfa weevil, *Hypera postica,* 1) larva, 2) cocoon, 3) adult (Original)

Fig. 2. Foliage damage by *Hypera postica* larvae on alfalfa leaflets (original)

Table 1. Mean (±SE) daily, stage-specific and cumulative rate of larval consumption (g dry weight) of *Hypera postica* in laboratory conditions

+	Consumption rate				
	Daily	Stage specific		Cumulative	
		Duration (d)	Consumed tissue (g)	Duration (d)	Consumed tissue(g)
1st	0.0352 ±0.0038 d	3	0.1055±0.0113 d	3	0.1055±0.0113
2nd	0.1210±0.0043 c	9	1.0890±0.0384 c	12	1.1945±0.0515
3rd	0.2198±0.0076 b	6	1.3186±0.0453 b	18	2.5131±0.1189
4th	0.3777±0.0118 a	15	5.6648±0.1587 a	33	8.1779±0.1530

For each column, values with the same latter next to them are not significantly different from each other

Monetary measures damage showed that AW larva is capable of creating economic loss in densities above two larvae m^{-2} providing that all larvae survive to pupate. Harrington *et al.* (2021) reported that 1-3 large larvae (3rd and 4th instars) often cause a significant yield loss justifying AW control measures. These numbers agree with our estimation of EIL. Minor differences may be due to variation in costs, hay's price, growing condition (soil fertility, weather, etc.). Alizadeh *et al.* (2017) and Roshandel (2016) reported that a population density above 50 larvae m^{-2} will destroy alfalfa plants completely. Seiter (2018) reported that the economic threshold of alfalfa weevil was 3 larvae per stem with 25% of leaf tips damaged. This is not directly comparable to our estimate due to different method of evaluation. However, if we assume that 15 plants m^{-2} are present (Dalir, 2024 reported the density of alfalfa plants has been 4.2 per 0.25 m^{-2} or 16.8 m^{-2} at the same period and the same place), and each one has only one main stem, then a density as large as 45 larvae will cause an economic damage. This may be expected from our data only if mortality rate as high as 99% occurs (see Table 2).

A major part of differences between these two studies may be due to costs and incomes. For example, if a pest control measure is more expensive in their region, growers may adopt to tolerate heavier damages and higher number of larvae. Blodgett *et al.* (2017) also reported 4.3 - 7.5 AW larvae per 0.1 m^2 or 43-75 m^{-2} as the EIL that partially agrees with those of Seiter (2018). Tooker (2023) estimated economic thresholds based on the larvae per 30-stem sampling estimation. An estimated hay price of $180/ton, insecticide application at $14/acre, and plants are 48 centimeters tall, will put economic threshold at 58 AW larvae/30 plants. Given that 30 plants can occuppy two-meter square (Dalir, 2024); This ET can be converted to 29 larvae m^{-2}. If we consider a 50% larval mortality, their EIL estimate will be 4-fold of our estimate. This difference can be explained by 4-fold higher control costs (labor plus inputs) in this study compared to the current one.

Decision algorithm

Obviously, occurrence of mortality in different times of a growing season prevents maximum consumption. Some larvae die before completing their development. Early-stage mortality certainly had a larger impact, for example those larvae that die before the third instar, consume 15% of that of a larva that wholly developed. Suppose we monitored AW population in a field, and our sample consisted of 7, 4, and 3 first to third instar larvae m^{-2}. Overall, 14 larvae are present and whole consumption of these larvae will certainly cause an economic damage but suppose again that we decided to spray a dose of an insecticide which kills 100% of all stages. Assuming that those larvae in our sample are at the end of their stage development and they are going to molt soon, at least, 98.7, 85.4 and 69.3% of their feeding remained or 1.3, 14.6 and 30.7% happened (see Table 1).

Table 2. Density of larvae (m^{-2}) that causes yield losses as large as 167.5-367.5 kg ha^{-1} provided that 25 to 99% mortality occur exponentially during whole developmental time, compared to control

Larval mortality (%)	**Number of larvae (m^{-2})**
0	2.0-4.5
25	2.5-5.4
50	3.2-7.0
75	4.8-10.6
90	7.9-17.4
99	21.1-46.3

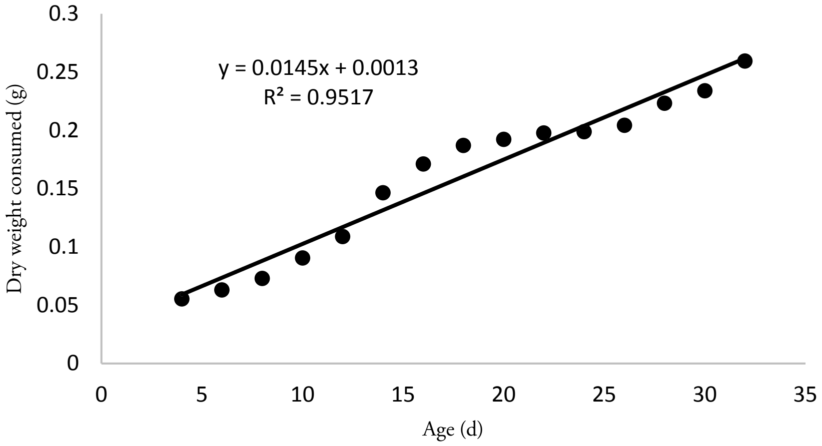

Fig 3. Per capita daily consumption rate of *Hypera postica* in laboratory conditions

Thus, 1.3×7 + 14.6×4 + 30.7×3 = 9.1 + 58.4 + 92.1 = 159.6% or equivalent to 1.6 wholly consuming larval damage will happen. This is well below the range of our EIL estimate, and now we must consider future feeding to decide if intervention is justified. If no mortality occurs, intervention is necessary because 14 larvae are well above the EIL level of 4.5 (Table 2, the first row). Suppose immediately after sampling we sprayed the field. If our spraying kills 100% of all stages, then no action is required in the remaining of the season. But suppose again our spraying kills 90, 80 and 70% of the above-mentioned stages, respectively, then 0.7, 0.8 and 0.9 m^{-2} 1st to 3rd instar larvae will survive after spraying and will continue their damage. This 2.4 larvae m^{-2} will consume 0.7× (100-1.3) % + 0.8× (100-14.6) % + 0.9× (100-30.7) % = 199.8% or equal to 2.0 larvae m^{-2}. Considering before spraying consumption equivalency of 1.6 larvae, the overall damage will be equal to 1.6+2.0=3.6 m^{-2} of wholly consuming larvae which will exceed EIL if you choose the cheapest option, but not for the most expensive one. These calculations are given that neither mortality nor recruitment occur after spraying (adults do not survive to continue egg laying). However, the future trend of population may determine if intervention will be necessary in the future. Therefore, scouting must be continued for determining the future trend of population. In subsequent samples, we need to calculate both past and future damage as delineated above, and intervention will be justified if the sum of the two damages predicts an economic damage.

These estimations suggest that, based on the past damage plus expected future consumption rate of the survived AW larvae, along with a regular scouting program, an upcoming EIL can be predicted. In this regard, expected mortality of different stages also is required. Here, a simple formula like eq.3 is suggested:

$$ED=\Sigma N_i.D_i+\Sigma N_i.S_i.(1-D_i) \quad \text{(Equation 3)}$$

Where ED=expected damage (past + future), N_i is the number of *i'th* stage (i = subscript denoting stage), D is the ratio of cumulative damage happened until the end of the *i'th* stage, S_i = stage-specific survival rate of a spraying action. Whenever ED > EIL, then action is justified. This equation will be subject to some sources of bias:

1) The past damage (first summation) will be overestimated and future damage (the second summation), underestimated if the stage consumption assumed to be completed upon sampling because all consumption of the observed stage is accounted for the past, while it may be divided between future and past. This may lead to a conservative estimation of ED, because the number of individuals survived after spraying is very lower than those present before spraying, although lower estimate of the future consumption rate can somewhat balance our estimate.

2) One can account all damage of the observed stage for future and hence underestimate past and overestimate future damage in eq.3

3) The ED in eq. 3 do not consider pre-sampling mortality (belonging to the first sum). In practice it is likely that a part of larvae perished before sampling. These larvae may be responsible for a partial damage that could not be accounted.

4) It is assumed that all individuals in the sample will survive to complete their development and hence, all the future damage is given will happen (represented by the second sum). In practice some individuals may die before completing development and all the future damage may not occur.

5) No recruitment is considered in eq.3.

Some above-mentioned sources of bias may balance each other, as they are in oppposite directions. For example, bias sources 3 and 5 underestimate, while the source 4 overestimate the damage. To avoide bias of the source 1 or 2, the ED can be adjusted as average of the two estimates. In above example, past and future damages were estimated to be equal to 1.6, and 2.0 larvae, respectively, and overall, equivalent to 3.6 wholly developing larval damage is expected which obtained by the first method. Instead, if we follow the second method the past and future damages will be estimated to be 0.49 and 2.25, summing to 2.74 (because the seven 1st instar larvae are assumed have not begun feeding yet, the four 2nd instar larvae consumed only 1.3% per capita i.e. equal to a 1st instar larvae and finally the third instars consumed 14.6%, i.e. equal to cumulative consumption rate of the 1st and 2nd instar larvae combined, with the 3rd instar consumption rate not included because it is assumed that 3rd instar larvae in the sample are newly molted and did not feed yet. Hence, the pre-sampling damage is 7×0 + 4×1.3 + 3×14.6 = 49.0% or equal to 0.49 larvae, and that of the future is 0.7× (100-0) + 0.8× (100-1.3) + 0.9× (100-14.6) = 225.8% or equal to 2.25 larvae, therefore, the ED by the second method is 0.49 + 2.25 = 2.74. The average of the two estimates is 3.17 which is still in the range of EIL estimate of 2.0-4.5. Finally, after-spraying scouting can reflect the trend of population and take recruitment into account to avoid the bias delineated as source 5. These results and analyses should be proofed and validated by complementary field studies.

Author's Contributions

Mahsa Ghahremani: collected the data, conducted the research, wrote the first draft and revised the article; **Ali Safavi**: supervised, provided the fund, and read and corrected the draft; **Shahzad Iranipour**: conceptualized, provided the material, designed the experiments, analyzed the data, wrote the decision algorithm, read and corrected the original draft and responded to reviewers' querries.

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Data Availability Statement

All data supporting the findings of this study are available within the paper.

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Ethics Approval

Insects were used in this study. All applicable international, national, and institutional guide lines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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تخمین میزان زیان اقتصادی سرخرطومی‌یونجه، *Hypera postica* (Coleoptera: Curculionidae) بر پایه نرخ تغذیه آزمایشگاهی

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چکیده: سرخرطومی یونجه *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) ، یکی از آفات اصلی یونجه *Medicago sativa* L. (Fabaceae) است. حشرات بالغ و لاروهای این آفت از شاخ و برگ یونجه تغذیه می‌کنند، به گونه‌ای که در اوایل فصل باعث آسیب رساندن و کاهش عملکرد و کیفیت یونجه می‌شوند. در این مطالعه، میزان تغذیه لاروها روی برگ یونجه در آزمایشگاه برآورد شد. حشرات کامل بعد از ظهور ظاهراً به دلیل دیاپوز تغذیه نکردند. لاروهای سن اول (n=150) بلافاصله پس از تفریخ در آزمایشگاه با برگ‌های یونجه محصور شدند. وزن برگها (گرم) درست قبل و بعد از تغذیه لارو به مدت ۲۴ ساعت اندازه‌گیری شد. لاروها به مدت ۳۳–۳۵ روز در آزمایشگاه تغذیه و چهار سن لاروی را سپری کردند. نتایج نشان داد که هر لارو می‌تواند ۸/۱۷۸±۰/۱۵۳ گرم بافت برگ را در تمام مراحل لاروی مصرف کند. برآوردهای ما نشان داد که چنانچه هزینه حشره‌کش‌ها ۶۷۰۰۰۰۰–۱۴۷۰۰۰۰۰ ریال در هکتار بسته به ارزش بازار و یک کیلوگرم خوراک یونجه نیز معادل ۴۰۰۰۰ ریال باشد، ۱۶۷/۵–۳۶۷/۵ کیلوگرم محصول معادل هزینه‌های کنترل است. چنانچه همه لاروها زنده بمانند تا شفیره شوند (مصرف کل زندگی)، ۴/۲–۵/۰ لارو در مترمربع قابل تحمل و نیازی به اقدام ندارند. با این حال، اگر کل مرگ و میر ۲۵، ۵۰ یا ۷۵ درصد با نرخ ثابت رخ دهد، تراکم قابل تحمل به‌ترتیب به (۴/۵–۵/۲)، (۳/۲– ۷/۰) و (۶/۸–۱۰/۴) در متر مربع افزایش می‌یابد. با فرض اینکه یک حشره‌کش ۹۰ تا ۹۹ درصد آفت را از بین ببرد، ۷/۹–۱۷/۴ تا ۲۱/۱–۴۶/۳ لارو در هر متر مربع در صورت مداخله به موقع کشاورز ضرری در پی نخواهد داشت.

کلمات کلیدی: سرخرطومی یونجه، هزینه‌های کنترل، مداخله، تغذیه برگ، تراکم قابل تحمل، ارزش عملکرد

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