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Research Article

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PREDATORY CAPACITY OF *HIPPODAMIA CONVERGENS* OF APHID COMPLEX IN EARLY PHENOLOGICAL STAGES OF ALFALFA CROP

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Abstract

Herbivorous insects in pastures of alfalfa(*Medicagosativa*L.), influence growth and crop plant health. Noone really knows phytophagous interaction with the predatorand the host.*Aphiscraccivora*Koch, *Aphis fabae*Scop. and *Aphis gossypii*Glover. are conducting phytophagyinalfalfa. The phonological stages were studied, Mv: mean vegetative and Lv: Vegetative late. Different ranges of significance in the study of the cumulative number of total budsper plant regardless of the phenological stage. The average range was 3.26±0.28to19.4 ± 0.21. The height of the plant has linear trend. In Mv was: y = *0.1936x+10.58* and Lv: y = *0.2784x+12.888.* The best rates of effective predation occur red at eight hours of evaluation, regardless of the phenological stagefor treatments four and five adult predators. The dry matter recorded, different ranges of statistical significance of: 11.56 ±1.54 to 23.48 ±0.92. This predatory coccinellidae has high capacity of the complex of phytophagous alfalfaaphids in different phenological stages and evaluated under the climatic conditions of CADET.

Key Words: Ethology, Herbivores,Natural enemies, Pest, Plant Physiology

INTRODUCTION

The herbivorous insects attack the most vigorous parts of the plants, those of fast growth and abundant nutrients (Price, 1991; Foottit and William, 1993); for this reason, the variations in the availability of the resource provided by the plants, in terms of quantity and quality per phenological stage, will decisively affect the herbivorous populations. The development of herbivorous insects is directly related to the availability of resources and, in turn, is a measure of the survival of juvenile stages, their growth and their subsequent fertility (Thompson, 1988).

Grasslands provide their high content of nutrients necessary for the production of milk, meat, wool and other products (Amaguaña *et al.,* 2009). Alfalfa (*Medicago sativa* L.) is important forage for animal feed mainly in the vegetative state, in particular for dairy cattle, as their nutritional qualities favor a good physiological development of the animal. In the Sierra Centro Norte del Ecuador, milk production is based on pastures established with forage species, such as alfalfa (Paladines, 2007). Alfalfa goes through ten stages of maturity according to the physiological development of the stems. The ideal temperature for the cultivation of alfalfa is 14 °C, this fabaceous has an average yield of 8.83 t ha^{-1} $year^{-1}$, often with three average cuts (Albán, 1992).

Among other factors, its production is threatened throughout its cycle by the attacks of some phytophagous that fundamentally affect the forage, reducing its yield and shortening its useful life, even producing the death of the plant if other factors are combined; although it depends on the behavior of the different cultivars (Summers and Gilchrist, 1991). The affluent population in alfalfa depends on several factors, height, age of the plant, humidity, presence or absence of direct solar radiation, temperature, wind speed and rainfall (Buntin and Isenhour, 1989); in addition, the spontaneous existence of natural enemies such as predators, parasitoids and entomopathogens (García *et al.,* 1994, Rodríguez and Arredondo, 2007). Its population increases in autumn and spring (in countries of four seasons), although there are variations depending on the different species (Valenciano *et al.,* 1997), mainly due to the influence of temperature on its population (Summers *et al.,* 1984; Harper *et al.,* 1993). Aphids are a cosmopolitan pest that feeds on several hosts. Legume lesions caused by aphids, initially induce severe malformation of the leaves of recent emergence (Comas and Pons, 2000). Predators are very important in the regulation of aphids, which contribute to control their population (Valenciano and Paravano, 2002). The coccinellids are an important family of Coleoptera from the point of view of biological control agents, since most species are predators, both adult and larvae, of aphids that are considered pests in crops of interest. Valenciano *et al.,* (1997), mention that coccinellids are the main aphid predators since they consume a high number of prey; For this reason, they are the most successful predators in the biological fight against agricultural pests (Milne and Bishop, 1987, Núñez *et al.,* 1992). There are reports in Ecuador of *Hippodamia convergens* Guér. by 1957 they were found in various crops as aphid controllers and since then they are considered important control agents, although at first they were mistaken as a dangerous plague (Merino and Vásquez, 1962). Numerous studies have shown that populations of insect predators and parasitoids can be directly influenced by their host; relatively few of these studies have come to identity the subsequent effects on herbivores and herbivory levels (Van Der Meijden and Klinkhamer, 2000). What need to be done are experiments that separate the effects of the amount of resources generated in phytomass and the natural enemies of herbivore populations with their respective herbivory levels.

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In Ecuador there is scarce information about the use of coccinellids as effective and efficient biological controllers of aphids regulated by the phenology of the crop, for which reason the present work of investigation has been determined to determine the influence of the complex of aphids and the predatory in the capacity of *H. convergens* in relation to several of the physiological functions of the plant in two early phenological stages of alfalfa cultivation.

MATERIALS AND METHODS

The present investigation was carried out in the La Tola Experimental Teaching Academic Field (CADET) of the Central University of Ecuador, located in Tumbaco, Pichincha at 2475 masl, with annual average temperature records of 17°C and precipitation of 860 mm.

The factors under study were, Phenological stages: e1 = vegetative medium, e2 = late vegetative and number of adult predators: a1 = 0 coccinellids per pot, a2 = 1 coccinellids per pot, a3 = 2 coccinellids per pot, a4 = 3 coccinellids per pot, a5 = 4 coccinellids per pot, a6 = 5 coccinelids per pot, the same ones that constituted the treatments (Table 1).

Table 1 Treatments in the evaluation of the influence of the aphid's complex and the predatory capacity of *Hippodamia convergens* in early phenological stages of alfalfa crop

TREAT.	DESCRIPTION
t1 (E1 A0)	Phenological stage 1 + aphids + 0 coccinellids
t2 (E1 A1)	Phenological stage 1 + aphids + 1 coccinellids
t3 (E1 A2)	Phenological stage 1 + aphids + 2 coccinellids
t4 (E1 A3)	Phenological stage 1 + aphids + 3coccinellids
t5 (E1 A4)	Phenological stage 1 + aphids + 4 coccinellids
t6 (E1 A5)	Phenological stage 1 + aphids + 5 coccinellids
t7 (E2 A0)	Phenological stage 2 + aphids + 0 coccinellids
t8 (E2 A1)	Phenological stage 2 + aphids + 1 coccinellids
t9 (E2 A2)	Phenological stage 2 + aphids + 2 coccinellids
t10 (E2 A3)	Phenological stage 2 + aphids + 3 coccinellids
t11 (E2 A4)	Phenological stage 2 + aphids + 4 coccinellids
t12 (E2 A5)	Phenological stage 2 + aphids + 5 coccinellids

The National variety was used as a plant material and two plants were placed in pots cover that contained a substrate of black earth, sand and gravel. We used recently emerged alfalfa plants, developed in plastic covers covered individually with tulle fabric.

In total, sixty experimental units: Thirty in the middle vegetative stage - 20 days (Mv), containing five pots for each treatment, each considered as observations; in the same way for the late vegetative stage - 20 days (Lv); The distribution of the treatments in the experimental site was randomized.

All treatments were handled in a similar way in terms of their initial fertilization and irrigation frequency. Three days after the transplant in the phenological stage Mv, adult aphids were infested; similarly in the same period of time the aphids were infested in the phenological stage Lv.

Collection, identification and rearing of aphids – coccinellids

The field arthropods were collected, estimating the phenological phases under study. Adult aphids were obtained from alfalfa cultivars present in the CADET. The equipment used for this activity was a vacuum suction device D-vac (Southwood and Henderson, 2000), two-way, with which no damage was caused to the mouth apparatus of the aphids; In the same way, the collection of adult coccinellids was done manually with the help of a flexible bristle brush and Petri® dishes, which contained moistened paper towel inside. These were taken from the same cultivars in which the aphids were collected.

For the identification of aphids, a sample of 100 individuals was taken per species (morphological differences) to perform a correct identification based on software by Favret and Miller (2012); regarding identification coccinellidae predator, it was made by morphological characterization - Taxonomic, wherein an amount of 50 individuals as part of the sample was taken following the taxonomic keys Larson (2013).

Simultaneously with the development of the experiments, predator breeding material and its prey based on guidelines from Lenteren (2003) was maintained. In the case of aphids, the initial collection, placed in pots alfalfa National variety, covered with tulle fabric, making cuts of the pasture every 15 days to stimulate the generation of apical meristems, main site of phytophagy of aphids, this colony remained constant, as a food source of the coccinellidae in all the states of life that performs predation.

Infestation of aphids and release of coccinellids

After 24 hours in which the population of adult aphids was kept in quarantine, three aphids were infested per plant, six per pot. To avoid the escape of the aphids, to the implementation of the experiment, a tulle fabric was installed, which enclosed the plants and allowed the accounting and evaluation of the variables with respect to the aphids before and after the release of the coccinellids. For the subsequent infestations of the late phenological stage, the aphids and coccinellids were independently reared in entomological cages simulating the banker plants system.

Twenty days after the infestation of the aphids in the experimental pots, the coccinellids were released according to the specification of the treatments; One day before proceeding to the liberation of the predators they were fasted. In a similar way we proceeded in time and form for the phenological stage Lv. The criteria for the release of adult predators was based on the Population Density Table of Aphids in Alfalfa Cultivars (Table 2), proposed by Pons and Lloveras (1999). For each of the bioassays, the possible natural mortality was corrected.

Table 2 Table of Population density of Aphids in alfalfa crops. Scale of abundance.

Class	Number of aphids per stem
0	0
1	1 – 4
2	5 - 10
3	11 - 34
4	35 – 100
5	101 – 300
6	301 – 900

Source: Pons y Lloveras (1999)

Number of shoots and height of the plant per phenological stage

For the registration of this variable, it was counted every five days after the partial application of the treatments (infestation of aphids) in the different experimental units in each studied phenological stage, with the purpose of knowing really how much the aphophytic phytophagy influences (adults and progeny) in the physiology of the plant. It was considered bud by plant when it was with the presence of protoderm and leaf primordia detached from its main axis.

In the same way, the height of the plants was evaluated every five days, in each of the phenological stages studied. This variable was recorded in centimeters from the ground to the maximum growth point. It was measured taking into account the erect part of the highest terminal bud.

Efficiency in predation capacity

Before the release of adult predators, aphids (adults - nymphs) per plant were counted; and the respective treatments were applied, based on the Scale of Abundance, Class 4 (Pons and Lloveras, 1999). This data served as a starting point to establish the average number of aphids per plant, regardless of the state of development of the same. The values of predation were recorded every four hours for the period of one day, in the two phenological stages evaluated.

The effectiveness of the predation capacity of *H. convergens* was evaluated according to the potential of the predator, influenced by the number of predators per unit area (host - phenological stage). The number of prey consumed every four hours during a day was established. With the above information, the percentage of total consumption per coccinellids adults was determined.

Percentage of Dry Matter

At the end of the evaluation by phenological stage of cultivation, the cutting of the plants was carried out, from the ground level, to determine the percentage of dry matter. These cuts of plant material were individually weighed for each treatment and then placed in paper bags of known weight. The samples were dried in a forced air circulation oven (Unitherm Drying Oven, S & T Engineering Company, Birmingham, England) at 65 °C, for 48 hours; After this, the samples were weighed at room temperature (Petruzzi *et al.,* 2005) and the percentage of dry matter was calculated.

Statistical analysis

The results obtained were subjected to the analysis of variance (ANOVA), to evaluate the effect of the treatment on the response variables, under a completely random design with a factorial arrangement of 2 x 6. All the statistical procedures, including tests for normality and homoscedasticity, were carried out using the INFOSTAT® statistical program. In the case, when significant differences were found, the Tukey multiple significance range test was performed *(P <0.05)*.

The route between the height distributions of the plant over time was evaluated by regression analysis; the models were selected based on the best R^2 values.

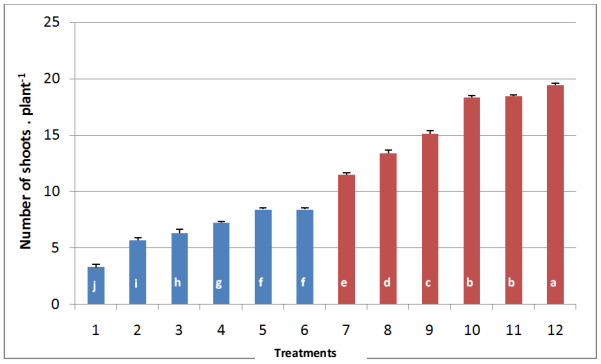
RESULTS AND DISCUSSION

In relation to directed sampling of aphids on with specific host alfalfa, and once run the taxonomic keys determined by the software of Favret and Miller (2012), it was establishment that within the complex of interacting aphids in this agro ecosystem between the months of March to August the species are found: *Aphis craccivora* Koch, *Aphis fabae* Scop. and *Aphis gossypii* Glover; in similar population densities. As for the coccinellidae, according to the taxonomic keys of Larson (2013), it was confirmed that it was *Hippodamia convergens* Guér.

Number of shoots and height of the plant

As cited, Murphy and Briske, (1992), the morphology of plant development is translated into the definition of their architectural organization, palatability, accessibility to herbivores, and their ability to grow after defoliation; Hence, the results of this research indicate that the treatments significantly affected the number of shoots per plant ($F_{11.59}$ - 2972.73;P<0.0001), with the generation of ten levels of statistical significance in the phenological stages studied, the range in the treatments under study were on average 3.26 $\pm$ 0.28 to 19.4 $\pm$ 0.21. They were established for stage Mv, from 3.26 to 8.4, finding different levels of statistical significance for each treatment except for those who had four and five adult coccinellids, in which statistically they are similar in the responses of the number of shoots per plant (Figure 1).

There is a definite tendency to increase this variable as the number of adult predator's increases. The reasons for this increase in sprouting are due to a greater availability per plant of natural enemies, which results in greater bud viability due to the herbivory effect of aphids. Several studies mention that to achieve an adequate management of alfalfa, the characteristics of its growth must necessarily be known and its mechanism of reserves in the roots, crown and buds must be understood, which will allow keeping alive and vigorous plants throughout the years (Hidalgo, 2010). The meristematic growth can also continue from the buds of the stems themselves when stubble is left very high. This regrowth from secondary stems is generally less vigorous and tends to detach from old stems more easily (Muslera and Ratera, 1991).



Averages with different letters indicate significant difference according to the Tukey test (P< 0.05)

Figure 1 Mean ($\pm$SD) number of shoots per plant of the treatments under study in the evaluation of the influence of the aphid complex and the predatory capacity of *Hippodamia convergens* in early phenological stages of alfalfa culture.

In the phenological stage Lv, similar distribution was presented that in the Mv. The range of results was between 11.46 $\pm$ 0.20 to 19.40 $\pm$ 0.21, but, unlike in this stage, the stabilization of the highest responses for the number of outbreaks was from having three adult predators, in which it is similar range of statistical significance; this would be interpreted as the quickest action to the effect of gregarious persistence of the herbivorous insects at the stage when the plants are smaller (Neuenschwander *et al.,* 2003).

The shoot generation responses have a logarithmic tendency with the consequent stabilization of the incremental

intervention of predators, indistinctly of the phenological stage (Figure 2). The reduction of shoots consequently decreases the total leaf area, as cited by Mazorra *et al.,* 2002, that the affections to the foliar surface in citrus fruits imply decreases in fruit production, since it is necessary to have a foliar area of 2.3 m^2 to produce one kg of orange.

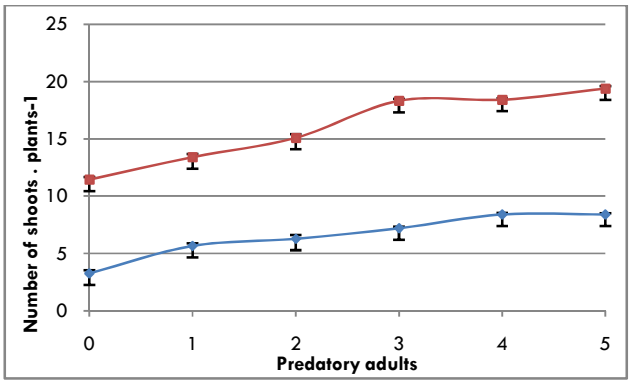


Figure 2 Distribution of the number of shoots per plant of the treatments of the study in the evaluation of the influence of the aphid complex and the predatory capacity of *Hippodamia convergens* in early phenological stages of alfalfa culture. Mv (blue line) and Lv (red line).

Considering that hemipteran had the opportunity to regulate the sap phytophagy of the outbreaks, because they were offered at will, it could be inferred that the difference in number of outbreaks demonstrated in both phenological stages, is given by the amount of predators and in addition it could be mentioned that the presence of the different concentrations of secondary metabolites by age of the leaves, influence in the herbivory; these compounds in a general sense are elaborated by plants to prevent predation by insects, fungi and viruses (Van Soest, 1994). The plant generates secondary compounds and concentrates them in their different plant tissues, according to a defense strategy depending on how important the tissue is for the plant in that physiological state (Klingler et al., 2005). Whether the plant is consumed or not depends to a greater or lesser extent on the concentration of the defensive toxin of the plant (Harborne, 1999); in this way, higher concentrations of these compounds are found in buds, buds, very young leaves, reproductive and dispersal organs (Ramos *et al.,* 1998).

Regarding the height of the plant, the distribution of the responses over time is different to that registered with the number of outbreaks, as can be seen in (Figure 3), which presents an adjustment of all treatments to a linear trend. For stage Mv, the equation was, ***y = 0.1936x + 10.58*** and for Lv of, ***y = 0.2784x + 12.888***. With these equations, taking into account the biotic factors can be estimated the height of the plant in the different phenological stages studied, with the influence of the number of prey and predators used.

Growth and development define the energy and nutritional requirements that the assimilation system has to provide, basically through photosynthesis. In a given environment, the climatic and phenological characteristics of the alfalfa plant depend on adverse biotic factors, this factor, especially dependent on the stages of crop implementation, is of considerable importance due to its difficult modification once the pasture is established (Buxton and Fales, 1994).

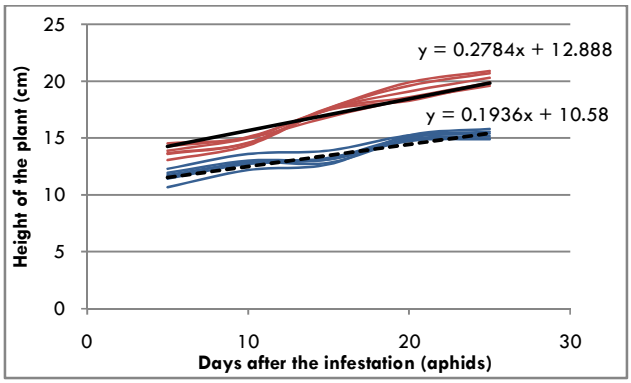


Figure 3 Dynamics of growth curves in terms of the plant height of the treatments under study in the evaluation of the influence of the aphid complex and the predatory capacity of *Hippodamia convergens* in early phenological stages of alfalfa culture. Linear trends Mv (segmented black line) and Lv (solid black line).

It must be borne in mind that alfalfa contains few phytoestrogens, except when it suffers from foliar affections. The attack of pathogenic aphids or fungi can cause this fabaceous plant to produce phytoalexins of the estrogenic coumestans type, including Cumestrol, Satinol and 4'-methoxy Cumestrol (Bickoff et al., 1969). Environmental effects, such as humidity and age of the plant, decrease Cumestrol concentrations; while the amount of fertilizer or temperature increases it. A concentration of Cumestrol as low as 0.005% DM, induces the uterine enlargement of cattle if the diet is provided for more than 180 hours (Saloniemi *et al.,* 1995).

The change in the structure of the vegetation determined by the age of the plant can alter the behavior of the parasitoids and predators; consequently, the effects on herbivorous insects usually change (Faeth, 1994, Turlings *et al.,* 1995).

Efficacy of predation capacity

It was determined that for each plant within the experimental observation unit after twenty days of infestation, the average number of aphids was 95 $\pm$ 5 individuals, indistinctly from the phenological stage of the crop. The development time of the average cycle of the aphids in the environmental conditions studied and on the specific host, was 21 days, in the same way the average number of nymphs reproduced per female was 33.

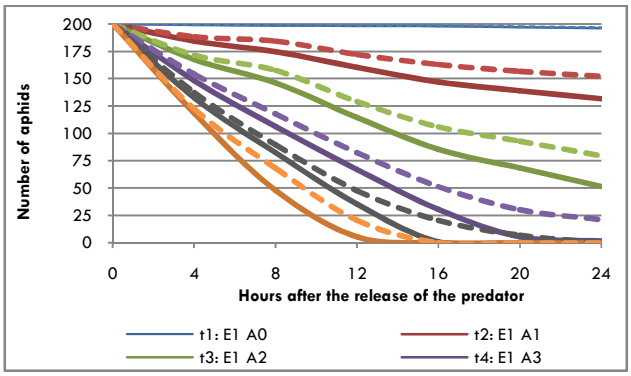
After performing the corrected mortality in the treatments, it was observed that in those that did not release the predators (E1 A0 and E2 A0), the mortality can be attributed by the effect of biotic or abiotic factors that are not coccinellids.

As we can see in (Figure 4), the effective treatments by effective predation rate (mortality over 50%) at eight hours of evaluation, regardless of the phenological stage studied were for those who had four and five adults.

Between 16 and 20 hours after the release of the predators, all the treatments reached mortalities higher than 50% except for those in which a predator was released (E1 A1 and E2 A1); this for the two phenological stages.

The trend in response expression for the predation capacity of *H. convergens* over time is constant. The total daily consumption of the dams exposed for the treatments was only presented in stage Mv for 3, 4 and 5 adult predators; while in stage Lv for 4 and 5 adult predators, this difference can be attributed to the dispersal capacity of the prey on the alfalfa host, consequently, the greater the distribution area of

the plant architecture, the greater the search distance to achieve consumption by depredation. Many authors have highlighted the importance of natural enemies in the control of densities of herbivorous insects (Cornell and Hawkins, 1995; Hawkins *et al.,* 1997; Cornell *et al.,* 1998) and the hypothesis that they naturally regulate populations of herbivores, and are limited by trophic levels.



Similarly, it is constant that the greater the number of predators, the consumption rate in time is greater (Cardoso and Lázzari, 2003), simulating a linear trend expressed by the capacity of predation and the specificity of aggregation of the prey; similar responses showed the studies of Valenciano *et al.,* (1997), which affirm that coccinellids are the main biological control agents of agricultural pests in Argentina with 87.2% followed by syrphids, fungi and viruses.

Hunter (2001) points out that the abundance of herbivores depends to a greater extent on the effect of natural enemies than on the available sources of food, determined by the incidence of new plant tissue.

Percentage of Dry Matter

The DM content, expressed as a percentage, of the treatments under study was directly proportional to the number of predators; the treatments significantly affected the DM content ($F_{11.59}$ - 268.40, P<0.0001); however, due to the fact that this study was carried out with early phenological stages, the average percentages of DM in several treatments were presented at the same level of statistical significance, hence the DM value has been used up to now as the parameter with which results of production and evaluation of the forage resource are expressed (Roguet *et al.,* 1998). This response is affected by the value of green matter, which depends on the moisture content of the forage and varies with the species, the phenological stageand phytosanitary conditions and to a lesser extent with the season (Barnes and Gordon, 1972).

Eight levels of statistical significance were established for this variable, with MS contents ranging from 11.56 $\pm$ 1.54 to 23.48 $\pm$ 0.92 (**Table 3**). The largest responses were presented in stage Lv.

Comparing for each phenological stage with the same number of predators, we can see that in stage Mv the treatments of 3, 4 and 5 coccinelids have similar statistical significance, as well as those of 2, 3 and 4. These responses give us think that the DM content in this phenological stage is not influenced by the number of predators, starting two adult predators.

Table 3 Percentages of dry matter from the treatments under study in the evaluation of the influence of the aphid complex and the predatory capacity of *Hippodamia convergens* in early phenological stages of alfalfa culture.

TREAT.	PERCENTAGE OF DRY MATTER
t1 (E1 A0)	11.56 h
t2 (E1 A1)	13.48 g
t3 (E1 A2)	15.30 f
t4 (E1 A3)	16.09 ef
t5 (E1 A4)	16.19 ef
t6 (E1 A5)	17.00 e
t7 (E2 A0)	17.03 e
t8 (E2 A1)	18.27 d
t9 (E2 A2)	19.09 d
t10 (E2 A3)	20.19 c
t11 (E2 A4)	21.22 b
t12 (E2 A5)	23.48 a
LSD = 0.9801	**

Averages with different letters indicate significant difference according to the Tukey test (P< 0.05)

On the other hand, in stage Lv, if different levels of statistical significance were presented for all treatments, except for those with 1 and 2 predators, these were presented in the same statistical range. The DM content in the Lv stage without predator was 17.03%, unlike the treatment that has 5 predators with 23.48%, without a doubt the effect that is indirectly obtained with the predators is seen to reach higher DM content, but it is necessary consider that as the cut of the alfalfa is postponed until late phenological stages the yield per hectare increases linearly due mainly to the increase in the weight of the stem fraction, but this is associated with a decrease in the leaf / stem ratio and changes in chemical composition that determine a lower nutritional value (Sheaffer *et al.,* 1988).

CONCLUSIONS

The results of this research suggest that the physiological parameters studied are strongly influenced by the density of natural enemies, that is, it must be taken into account that the minimization of the presence of herbivores must occur during periods of greater growth activity vegetative. The lowest level of reserves of the plant usually occurs around two to three weeks after defoliation, so in these phases it would not be advisable to graze, cut and keep in mind the management of herbivorous insects, which are taken into account to estimate the amount of them present in the new generation shoots by specificity in the phenological phase.

Insects represent one of the main limitations of the yield potential and longevity of the alfalfa crop. The foliar quality of plants (increases with a higher density of photons) and the radiation efficiency (increases with the increment in the red / far red ratio), photoperiod (increases with the increase of natural photoperiod), water availability, mineral nutrition and temperature. Integrated pest management as a crop protection approach offers the best prospects for an efficient and economical suppression of insect pests, while contributing to greater protection of the environment, man and wildlife. Therefore, to develop integrated management strategies for aphids in alfalfa, one must take into account the population dynamics of the aphids and their relationship with the phenological stage of the crop.

It has been shown that *H. convergens* have high predatory capacity of the phytophagous aphids complex of alfalfa,

under the climatic conditions of CADET, depending on the months evaluated. More research related to the subject is required, since there are many interactions that would help us to understand tritrophic systems (plant - insect pest - control agent).

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