

Development of *Amblyseius largoensis* as biological control agent of the broad mites (*Polyphagotarsonemus latus*)

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ABSTRACT

The broad mites, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) is a cosmopolitan pest of many important crops as potatoes, beans, peppers, and citrus which lives in tropical, subtropical regions and greenhouses worldwide. According to its biological and ethological characteristics its control is complex by traditional methods of plant protection. For this reason, the use of the predatory mites of the Phytoseiidae family is an adequate and efficient management alternative. The present paper comprises the characterization of the potentiality of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) as biological control agent of *P. latus* integrating, for the first time, the predator's biological characteristics; feeding behavior and the numeric and functional responses; development of methodologies for mass rearing of the biological agent and parameters for quality control processes; the study of pest behavior in the protected production of Cuban hybrids of pepper, adapting the sampling methodology and determining the thresholds to estimate the population levels in these conditions. The compatibility of this predator with the principal chemicals and biological products used in the protected production and their effectiveness as biological control agent of *P. latus* in different conditions were evaluated. The results obtained demonstrated that *A. largoensis* is a new biological alternative for the management of broad mite populations in the protected production of peppers, which could be used in other agroecosystems; those that *P. latus* constitutes a problem, prior validation.

Keywords: *Polyphagotarsonemus latus*, *Amblyseius largoensis*, *Capsicum annuum*, biological control

Introduction

The broad mites, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) is a serious cosmopolitan pest in tropical and subtropical regions and in greenhouses worldwide [1]. In Cuba, it is considered as one of the most harmful pest mite, due to their polyphagy and the negative impact in crops of economical interest as: citrus, beans, pepper and potato [2]. In the protected production systems of recent development in the country, it provokes serious affectation in pepper, tomato, cucumber and other horticultural crops [3]. With few exceptions, *P. latus* is controlled exclusively with chemicals acaricides [4]. In Cuba, the strain LBt-13 of *Bacillus thuringiensis* Berliner has been used with favorable results for several years. However, the expected results are not always achieved, because this mite develops on the undersides of leaf surfaces, that is, it goes unnoticed in crops, confusing their symptoms with diseases. This means that applications are not being carried out in an adequate moment.

The use of the biological control, through the phytoseiid mites, constitutes a new form for managing the

populations of *P. latus*, which have the advantages of achieving an efficient control in any site of the leaf; further reducing the import of chemicals products and protecting the natural enemies present in the agroecosystem, contributing to the preservation of the environment [5, 6].

To develop an efficient strategy, the development of a group of researches is required in order to demonstrate the feasibility of using these organisms, which is achieved only carrying out the basic and applied studies, and determining their potentiality as biological control agents.

In answer to the necessity of finding new management strategies of broad mite populations, the present paper aims at determining the most important biological parameters of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), characterizing their feeding behavior, establishing a mass rearing method, describing the populational behavior of *P. latus* in the protected production of pepper and estimating the effectiveness of *A. largoensis* as a biological control agent of *P. latus*.

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Results and discussion

Basic biological studies

An integral study of the most important biological aspects of *A. largoensis* was carried out; the morphological and behavioral characteristics of the predator were described. The duration of the development for both sex and total was determined, the reproductive parameters, including the rate of release, the fertility and daily oviposition rate on *P. latus* when feeding on potato, pepper and citrus were determined [7, 8]. In most of the cases, the biological parameters of *A. largoensis* on broad mite, obtained in this study, are similar or higher than the observed for this species when were fed on other prey or pollen [9] and in some other cases are comparable and more favorable, to those reported for phytoseiid mites, that are considered as good biocontrols of this pest, therefore evidences their potential present in the *A. largoensis* in order to control *P. latus*.

Predator-prey interaction

The study on the basis of feeding behavior and numeric and functional responses of *A. largoensis* on broad mites allowed increasing the knowledge of predator-prey interaction. *A. largoensis* consumed all stages of prey. However, the predator showed preference for consuming immature stages, mainly eggs and active larvae that represent 73% of all consumed.

In addition, the time spent feeding or to consume a prey time is increased while the different development phases of the prey are occurring; as well the *A. largoensis* dedicates the highest proportion of time in active components (search and feeding on the prey) [8, 10]. These results demonstrated the marked ability of *A. largoensis* to find, capture and consume all the stages of prey, very important aspect considering its biological and ethological characteristics of *P. latus*. These elements are useful in order to define the optimal rate to design the future management strategy of *P. latus* with this mite. The knowledge of feeding behavior, the ratio of successful attacks and feeding preferences on several life stages, are studies carried out for the first time in Cuba in order to evaluate the predatory capacity of a natural enemy. From the methodological point of view, they have a great importance since they could serve as a benchmark for similar testings with other biocontrols with the purpose of predicting their potentialities as biological control agents.

A. largoensis showed a type II functional response to prey [11]. The average number of *P. latus* attacked by *A. largoensis* increased with prey density, until reaching a maximum, in which remained stable over time or came to the commonly known stage of 'plateau'. The Holling's model was the best to estimate attack coefficients and handling times. Theoretically, *A. largoensis* could consume one *P. latus* in 5.9 min; an adult female could consume a maximum of 243 broad mites per day.

Starting from the relationship between functional and numerical responses of *A. largoensis*, the predator searching efficiency and the potential of an individual *A. largoensis* female to suppress *P. latus* was calculated at different densities. This value allowed to

know that the predatory mites maintain a satisfactory efficiency until a density of approximately 60 mites. This would suggest that the rate of release could coincide with this density [12]. This result confirms that *A. largoensis* has a marked ability to regulate the *P. latus* population and could affirm that it possesses all the biological characteristics to be selected as an effective biocontrol of this tarsonemid mite.

The results obtained in the experiments of biology, feeding behavior and numeric and functional responses demonstrated that this procedure is adequate and allows to determine the potentiality of *A. largoensis* as biological control agent of broad mites and becomes a methodology that could be used, with similar success, in order to evaluate other natural enemies. This information also offered the scientific indispensable support for a second experimental phase comprising the tests of control in greenhouses and field.

Evaluation of mass rearing methods

All the methods evaluated for mass rearing permitted the increase population of *A. largoensis*. In all the cases the initial population was multiplied several times, which demonstrated that the rear methods used, as the offered food, have had a favorable interaction in the production of the predator. The method of the trays offered the best results in general. Based on these results and the simplicity of the technique, it was the method selected for the massive reproduction of the predator [13]. It is also important that an alternative method for the maintenance of the mother culture was proposed, with a rigorous system of evaluation of the quality and that the pollen of corn could be a supplementary food when the prey are insufficient [14].

With these results a productive system was designed considering two basic subsystems closely related: the maintenance of the mother culture of *A. largoensis*, which will serve to recycle the system and to start the massive production and the massive reproduction properly. The studied systems can be transferred to the Entomophagous and Entomopathogen Reproductive Centers (CREE) of the country, because of the essential equipment to carry out this production is available. It is important to consider that this is the most complete study achieved in the country for the evaluation of mass rearing methods of predatory mites and the first with a generalist species. It contributes to accelerate the introduction of these predators to the productive practice.

This result allowed to provide the necessary individuals to evaluate the compatibility of *A. largoensis* with the principal chemical and biological products used in the protected production, determining that the predator is compatible with the acaricides Dicofol and the strain LBT-13 of *B. thuringiensis*, the insecticide Confidor and the fungicides sulfur, Ridomil and Mesuram; while the insecticides Polo and Corsario were highly toxic to the predator (Table).

This result has a great applicability because it provides to design intervention strategies in order to combine the use of a biological control agent, to regulate the populations of *P. latus*, with the chemical control, in case of being necessary for other pests.

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Incidence of the broad mite on peppers hybrids

The population behavior of *P. latus* was studied on pepper in protected systems during two years, which allowed evidencing the ineffectiveness of the chemical acaricides to maintain the populations of the broad mites below the threshold of economic damage [15]. These results may be considered as possible modification of the methodology pest monitoring in systems protected, with relationship to the form of take out the sample in the house of cultivation, the quantity of leave to extract, which could be reduced in a 35%, as well as the possibility of the use of a presence-absence sampling, on the base of a 16% of leave infested with at less a female of *P. latus*. These results simplify the process of sampling, without losing their efficacy with a significant reduction of time and resource during the process.

The study of the influence of the phenological phases on the damages produced by *P. latus* on two Cuban pepper hybrids allow to know that the vegetative growth and blossoming are the most vulnerable phase in presence of the pest. Therefore, this would be one of the phases that must be protected with greater emphasis to avoid the negative impact of the broad mites damage in the yields. This knowledge will allow a higher production of pepper hybrid seed, which will facilitate the reduction of cost by this concept. These results are the first ones about broad mites-pepper interaction in protected systems and are also indispensable to the design of more effective management strategies of this important pest.

Effectiveness of *A. largoensis* to control *P. latus* in pepper

The study showed the validity of the basic studies carried out indicating that *A. largoensis* had the biological potential to be an effective biological control agent of broad mites. The results of the release of *A. largoensis* for controlling broad mites on peppers in greenhouses are shown in the figure. The Dicofol treatment always caused a rapid decline in broad mite population, but it tended to increase again at the end of the trials. The treatment in which *A. largoensis* was released at two predatory mites per plant (Figure A), the number of broad mite continued increasing for two weeks and by the third sampling after the release, there was a reduction in the number of broad mites. This trend continued until the end of the trial. Nevertheless, from the second sampling, the treatment with *A. largoensis* reduced the broad mite density significantly to the end of the trial ($p < 0.001$, $F = 29.55$, $df = 2$) when compared with the no-predator control. The density of broad mites in the control treatment peaked in the second sampling and it gradually declined thereafter, because the damage of the broad mite feeding effect the vegetative grown and not permit the presence of new leaves.

When four predatory mites were released (Figure B), the broad mite density continued increasing for one more week and, thereafter, it started to decline until the end of the trial. Seven days after the predator had released, the treatment with *A. largoensis* reduced the broad mite density significantly ($p < 0.0001$, $F = 133.7$, $df = 2$) to the fourth sampling when compared with the

Table. Percentage of mortality of *Amblyseius largoensis* females sprayed in the laboratory with field rates of chemical and biological products

| Commercial Name (active ingredient) | Mortality (%) ^a | | | Remarks | Mortality Class |
|--|----------------------------|-------|-------|----------------|-----------------|
| | 24 h | 48 h | 72 h | | |
| Acaricides | | | | | |
| Dicofol CE 18,5 (dicofol) | 0 | 0 | 5 | Harmless | 1 |
| Comoran Supra SC 72 (sulfur) | 14.7 | 14.7 | 14.7 | Harmless | 1 |
| Strain Lbt- 13 (<i>Bacillus thuringiensis</i>) | 18.18 | 18.18 | 27.27 | Slightly toxic | 2 |
| Insecticides | | | | | |
| Polo SC 50 (diafenthiuron) | 40.9 | 72.7 | 86.4 | Toxic | 4 |
| Confidor PS 70 (imidacropid) | 0 | 0 | 5.56 | Harmless | 1 |
| Corsario CE (2.71 cypermethrin + 21.69 diazinon) | 100 | - | - | Toxic | 4 |
| Fungicides | | | | | |
| Ridomil MZ PH 68 (metalaxyl + mancozeb) | 0 | 0 | 0 | Harmless | 1 |
| Mensuram PH 80 (mancozeb) | 0 | 0 | 5.27 | Harmless | 1 |
| Control | | | | | |
| Water | 0 | 0 | 0 | - | - |

^aPercentage of mortality by Class: 1(0-20%); 2 (21-50%); 3 (51-80%); 4 (plus 80%).

no-predator control. In the sampling five and sixth no significant difference between broad mite density in *A. largoensis* treatment and the control was observed. The density of broad mites in the control had a similar behavior to the observed in the previous trial.

When eight predatory mites per plant were released (Figure C), the broad mite density showed a similar behavior to that observed in the second trial, but the reduction of the broad mite density by *A. largoensis* compared with the control treatment was not significant, except to sampling third ($p < 0.002$, $F = 7.80$, $df = 2$) and sixth ($p < 0.0001$, $F = 181.14$, $df = 2$).

The most favorable result was obtained when eight *A. largoensis*/plant were released, regardless of the extremely high density of the broad mite. This is logical because it was the highest release rate. With 2 and 4 predatory mites/plant, the control effect was observed a week later, but the result was the same at the end of the trial. It is important to consider that the release rate used in this study was much lower than the commonly used for other phytoseiid mites [5, 6].

This was in agreement with previous studies on the biological characteristics of *A. largoensis* that showed this mite as a highly effective predator of the broad mite [8, 12].

Similar results were found when *A. largoensis* in release was evaluated in pepper plants, covered microplots and tunnels. In both opportunities, the predator mites were found primarily on leaves located at the middle of the pepper plants. This behavior has also been informed for *Neoseiulus californicus* (Oudemans) in release carried out in order to control *P. latus* pepper [5].

These are the first results that demonstrate the effectiveness of a predatory mite as biocontrol agent of the broad mites for Cuba. *A. largoensis* represents a new perspective for the management of this important pest in the country; particularly in protected systems that need management alternatives for the organisms noxious friendlier with the environment. It is assured

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that people could be benefitted of a healthier product and higher quality.

It is evident the great effectiveness of this predator on their preys, allowing to reach control indexes comparable with other predators that are commonly marked internationally, with much lower release rate [5, 6].

Taking into account the costs generated by the protection of a hectare of protected pepper with acaricides chemicals and the costs of the predatory release for a similar surface, it provides to save between 13.73 to 40.37 CUC.

These results demonstrate the existence of a new biological alternative for the management of the populations of broad mites in the protected production of pepper. The introduction of *A. largoensis* in the agricultural practice will help to reduce the populations of broad mites without the need of applications of chemicals acaricides and consequently an economical and ecological benefit is obtained.

Relevance of the study

The aspects relative to the *A. largoensis* biology on *P. latus*, constitute a contribution to the knowledge of this predatory mites, because this interaction has not been studied before, in Cuba neither worldwide.

The data obtained on feeding behavior of *A. largoensis* as predator of *P. latus* constitute a completely novel result for Cuba and for the world.

The mathematical models in order to estimate the functional responses of a predator are applied for the first time in the country.

The method used to evaluate the potentiality of *A. largoensis* as a biological control agent of *P. latus*, based on basic study of biology, the feeding behavior and the numeric and functional responses, possesses a high methodology value, because it could be adapted for similar studies in other groups of natural predators.

The system for mass rearing of the *A. largoensis* was designed based on the predator's biological knowledge, with several alternatives of food and methodologies of rearing, as well as with a rigorous system of quality for the maintenance of mother culture, being the most complete study carried out in the country.

The compatibility of *A. largoensis* with other pesticides of frequent use in protected production was demonstrated, providing alternatives for the management strategy of this important pest.

The status update of *P. latus* as pest in protected production, the damage and the population fluctuation on pepper hybrid of national production and methodology of monitoring are elements of vital importance on taking decisions for the management of this pest. A new biological alternative for the management of broad mite populations for protected production of pepper is offered, that could be useful in other agroecosystems where *P. latus* is a problem; thus it would provide to diminish broad mite populations without the need of acaricides chemical applications, highly contaminant and expensive, therefore and consequently an economical and ecological benefit is obtained.

A. largoensis constitutes a new line of production for the CREEs of the country, which will contribute to

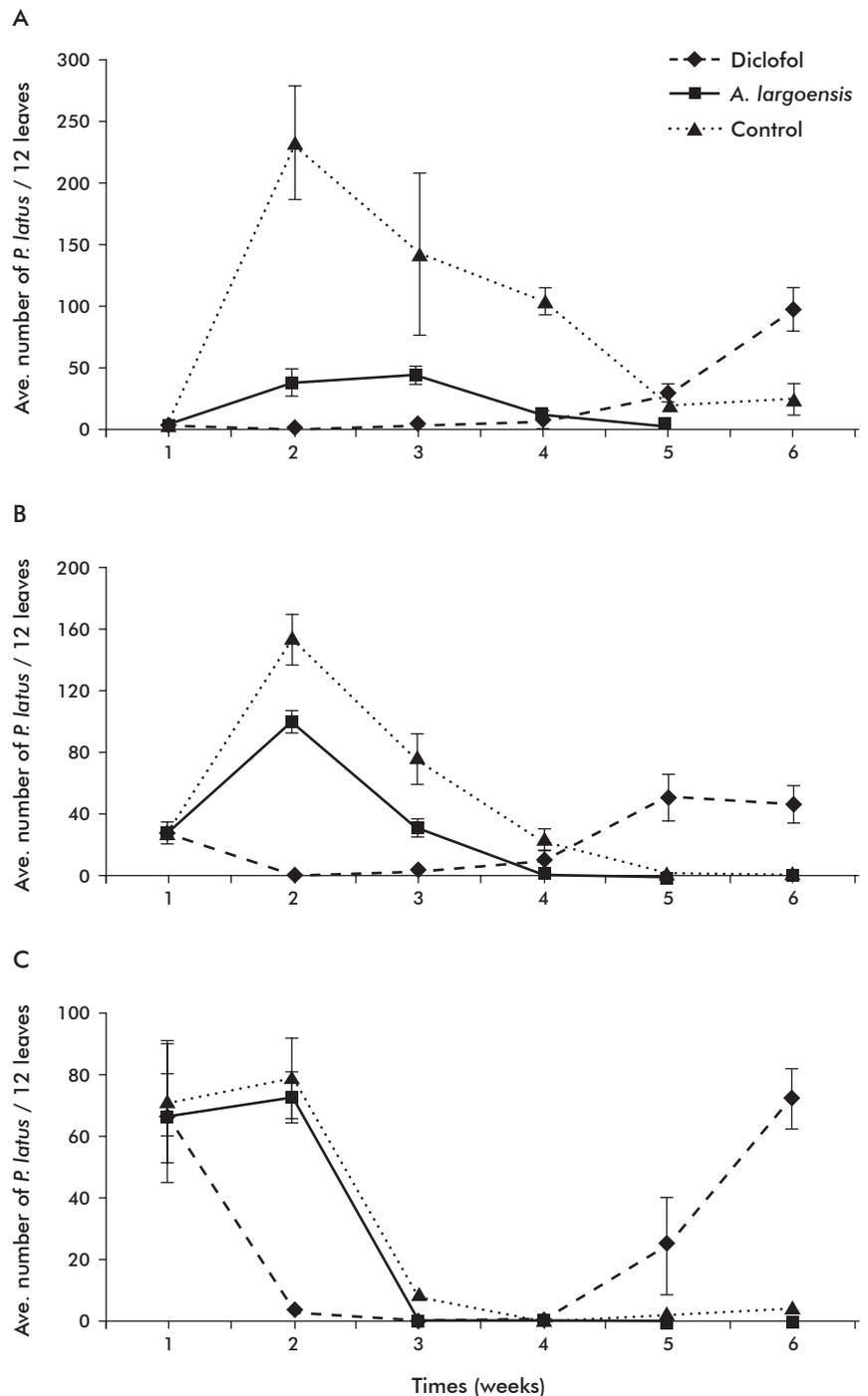


Figure. Population dynamics of *Polyphagotarsonemus latus* on LPD-5 F1 sweet peppers after the release of *Amblyseius largoensis*. Two (A), four (B) and eight (C) predators were assayed per plant in greenhouse. Data points are the means of 12-leaf sample \pm SEM. The release rate was evaluated in independent trials.

the economic rentability of these institutions and offer new opportunities of employment, mainly to women.

Conclusions

The results demonstrated that *A. largoensis* has the biological potential to be an efficient predator of *P. latus*. This affirmation is based on their biological

parameters, the marked ability to find, capture and consume all the stages of *P. latus* and the functional responses type II. There were also evidences of their mass rearing viability are also very important; and the tray method was the most promissory.

On the other hand, the importance of broad mites in the protected production of pepper as pests was corroborated, as well as the unawareness of the phytosanitary personnel on the methodology, monitoring and control of *P. latus*, which suggests the need of preparation in order to apply the established measurements appropriately. The poor efficiency of the chemical control was evidenced to maintain the population levels of *P. latus* below the thresholds of application. This permitted to adjust the methodology of sampling, monitoring and control of *P. latus* for the protected production of pepper, with a reduction of the size of the sample in a 30% and was settled that the sign for the release of *A. largoensis* as a biological control agent of 0.5 broad mites by leaf.

Other important element was demonstrated that *P. latus* produced the highest injuries in pepper hybrids HIRAM F₁ and LPD-5 F₁, in vegetative growth and blossoming stages, reducing height, the leaf area of the plants and the number and weight of the fruits.

Amblyseius largoensis is compatible with the acaricides Dicofol, Sulfur and the strain LBT-13 of *B. thuringiensis*, the insecticide Confidor and the fungicide Ridomil and Mensuram. The insecticides Polo and Corsario were highly toxic to the predator.

The releases of the *Amblyseius largoensis* controlled efficiently the broad mites in greenhouses, covered microplots and tunnel of protected production of pepper, with a release rate between 4-8 predators by plant. These results allowed to confirm that *A. largoensis* is an effective biological control agent of *P. latus*, with a positive economic and environmental repercussion.

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