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Structure, Abundance, and Relative Frequency of Phytophagous Insects, Associated to Beans (*Phaseolus vulgaris* L.) at Two Different Sowing Times

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Abstract

Context: The implementation of a strategy of Integrated Pest Management is based on characterization of pests and crops, and the relationships existing between the two.
Objective: To determine the structure, abundance, and relative frequency of phytophagous insects associated to bean variety Bat-304.
Methods: Research took place on San Miguel Farm, Jaruco, Mayabeque, at two different sowing times: On September 5, 2015 (early), and February 18, 2016 (late). Overall, six samplings were made 15 days after sowing, at a weekly rate. Samples were collected from 15 plants at random, double diagonal, for which a leaf was taken from each level, (higher, middle, lower). The samples taken were analyzed at the Entomology Laboratory of the Faculty of Agronomy, Agrarian University of Havana, for quantification and identification of several species.
Results: The phytophagous insects detected were, Empoasca kraemeri Ross and Moore; Bemisia tabaci Gennadius; Liriomyza trifolii Burgess; Thrips palmi Karny; Diabrotica balteata Leconte and Cerotoma ruficornis Olivier.). T. palmi was very abundant during the two sowing times, whereas L. trifolii was not as abundant in either time. E. kraemeri and B. tabaco were variably abundant at each sowing time.
Conclusions: Phytophagous insects kraemeri; B. tabaci; L. trifolii, and T. palmi, were very abundant at the two sowing times. A greater biological diversity was observed in sowing time 1 (early), whereas dominance was higher during sowing time 2 (late).

Key words: Phaseolus vulgaris, diversity indexes, integrated pest management.

Introduction

Common beans (*Phaseolus vulgaris* L.) is one of the oldest foods ever. It has been an important part of human diet for thousands of years, since it became one of the first domesticated and then cultivated nutritional plants. In Cuba, it is one of the main choices in the menu, being black beans the most common in the national cuisine. It comes originally from the Americas, but it has been intensively cultivated in tropical areas and some temperate regions of the planet (Castillo & González, 2008; Castillo et al., 2016; Lamz-Piedra et al., 2017).

Both in Cuba and abroad, beans are the most commonly consumed legumes, an important source of proteins (22%), vitamins, and minerals (Ca, Cu, Fe, Mg, Mn, Zn) in the diet of countries in the Americas, particularly, in developing countries. The annual production of industrial countries surpasses 21 million metric tons, accounting for more than half of the total production of legumes for consumption internationally (Brigide et al., 2014).

Despite its importance and the fact that it is a traditional crop, its yields have been affected by several pests. Some of the most harmful insects to beans are whitefly, *Bemisia tabaci* Gennadius; leafhopper, *Empoasca kraemeri* Ross and Moore, that causes leaf curl; banded cucumber beetle *Diabrotica balteata* Leconte and *Cerotoma ruficornis* (Oliver.), which produces bores in the leaves, and transmits the yellow speckle viroid and cowpea mosaic; leafminer *Liriomyza trifolii* Burgess; the complex of thysanoptera, and weevils of stored beans, *Acanthoscelides obtectus* Say and *Zabrotes subfaciatus* (Boheman) (Murguido et al., 2002; Martínez et al., 2006; Castillo & González, 2008; Jiménez & Rodríguez, 2014; Castillo et al., 2016).

Generally, these harmful organisms are controlled with the use of chemical pesticides, which also affect the beneficial fauna, including parasitoids, predators, and pollinators, causing significant expenses, and damage to human health and the environment. This leads to pest resistance, favoring the emergence of secondary pests (del Puerto, Suárez & Palacio, 2014).

The implementation of economical pest management strategies that do not affect the environment to increase annual yields, is below the national demands; therefore, the country must earmark high amounts of funds to import beans (BCC, 2014).

The cultural measures are based on long-used agronomic practices, an example of methods implemented to prevent pests. Cultural control is the basis of any Integrated Pest Management strategy, which means learning about pest and crop characteristics, as well as the relations between them (Vázquez, 2008; Centeno, 2016).

Choosing a proper date for sowing is a culturing action that helps decrease population densities of several harmful organisms. Starting before or after the sowing or harvesting dates, pest attacks can be prevented. Sowing can take place when pests are absent during certain periods of the year, or at a time when the most susceptible stage of crops coincides with the time of year when pests are least abundant.

In Cuba, Murguido et al. (2002) upon evaluation of different integrated pest management actions to beans in various provinces, reported the incidence of insect pests attacking this crop at different dates of sowing. However, climate change has caused variations in the behavior of these harmful organisms. Furthermore, on San Miguel Farm, no research has been conducted to determine the structure of the community of phytophagous insects that concur on this particular crop at different sowing times.

Considering the above, the authors suggest to determine the structure, abundance, and relative frequency of the community of phytophagous insects associated to beans, variety Bat-304, at two different sowing dates.

Materials and Methods

This research was done on San Miguel farm, from CCSF Niceto Perez, in the municipality of Jaruco, Mayabeque, at two different sowing times: On September 5, 2015 (early), and February 18, 2016 (late).

The bean variety used was Bat-304, sown on brown carbonate soil, according to Hernández et al. (2014) at a row distance of 0.70 m, and 0.07 m between plants, in either date. The experimental surface was 0.6 ha.

Culturing was performed following the technical standards for the crop, in either date (Faure et al., 2013).

List of phytophagous insects associated to beans at two different sowing times

Random samples from 15 plants were taken at each time to list the phytophagous insects present in the crop 15 days after sowing. Six samplings were performed at a weekly rate, using the double diagonal technique. A leaf from each level of all the plants (higher, mid, lower) was taken, and the microorganisms were observed through a magnifying glass. The samples taken (leaves from each level) were placed in a previously labeled plastic bag with the date of sampling and the plant level. Later, the samples were analyzed at the Entomology Laboratory of the Faculty of Agronomy, Agrarian University of Havana, for quantification and identification of different species.

The adult thrip species were mounted according to the conventional technique of Mound & Marullo (1996), with a NOVEL stereoscopic microscope x1.5 magnification. The keys of Mound & Marullo (1996) and González & Suris (2008) were used for identification. The key made by Zayas (1988) was used for identification of hemiptera. Identification of the leafminer was done using the key made by Alayo & Garcés (1989).

Estimation of abundance and relative frequency of phytophagous insects associated to beans at two different sowing times

To estimate abundance and relative frequency of phytophagous insects associated to beans at two different sowing times, the data from the previous samplings made at two different dates, were used, based on these formula:

Relative abundance

$Ra = ni/N * 100$

where,

Ra = Relative abundance (%)

ni = Number of individuals in the species i

N = Total number of individuals

Relative frequency

$Fr = Mi/Mt * 100$

where,

Fr = Relative frequency of species emergence (%)

N = Total number of samplings of the species i

Mt = Total number of samplings

The evaluation of relative frequency values (RF) was done using the Masson & Bryssnt (1974) scale, which indicates this is a Very Frequent species, if Fi>30; a Frequent species, if 10≥Fi≤30; and Little Frequent species, if Fi < 10. The same criteria was assumed to evaluate relative abundance (RA); Very Abundant, if RA>30; Abundant, if 10≥RA≤30; and Little Abundant, if RA < 10.

Determination of the structure of phytophagous insects associated to beans at two different sowing times

Software DIVERS (Pérez & Sola, 1993) was used to estimate the indexes of biological diversity. According to the aim of the research, the index of species richness was, Specific richness (S), and the structure indexes were, Uniformity (E); Simpson's dominance index (DSp), and the Shannon-Weinner index (H'), all indicating alpha activity (α).

Results and discussion

List of phytophagous insects associated to beans at two different sowing times

Table 1 shows the species of phytophagous insects detected during the collection of samples at two different sowing dates. As could be seen in the two dates of sowing, insect species from four different orders and five families were inserted. The insect order with the highest incidence in terms of different families was hemiptera, with two families (Cicadellidae and Aleyrodidae), which accounted for 40% of all the families detected.

Table 1. Species of phytophagous insects detected on beans at two different sowing times

Species	Order	Family
Empoasca kraemeri Ross & Moore	Hemiptera	Cicadellidae
Bemisia tabaci Gennadius	Hemiptera	Aleyrodidae
Liriomyza trifolii Burgess	Diptera	Agromizidae
Thrips palmi Karny	Thysanoptera	Thripidae
Diabrotica balteata Leconte	Coleoptera	Chrysomelidae
Cerotoma ruficornis Olivier	Coleoptera	Chrysomelidae

The results achieved are similar to Martínez et al., (2006); Castillo & González (2008); Jiménez (2014) and Castillo et al. (2016), who consider that these are the most frequent insects affecting beans in Cuba and other countries.

In that sense, Murguido et al. (2002) in an experiment to design and develop an Integrated Pest Management System of insects that attack beans in five provinces of the country (Havana, Ciego de Avila, Las Tunas, Holguin, and Granma), reported that the most commonly observed pests in all the areas were *B. tabaci* and *E. kraemeri*. Other species, like *L. trifolii;* chrysomelids, and *T. palmi* were observed in Havana, Ciego de Avila, and Holguin.

Estimation of abundance and relative frequency of phytophagous insects associated to beans at two different sowing times

In the early sowing time (1), phytophagous insects (*B. tabaci* and *T. palmi)* were very abundant, whereas *E. kraemeri* was abundant. Leafminer *L. trifolii* was little abundant. In the late sowing time (2) *E. kraemeri* and *T.palmi* were very abundant. The white fly was abundant in the second sowing time (late). Leafminer *L. trifolii* was little abundant in the two dates (Table 2).

Table 2. Relative abundance of phytophagous insects associated to beans at two different sowing times (%)

Species	Time 1	Time 2
E. kraemeri	22.13 (A)	39.64 (VA)
B. tabaci	36.88 (VA)	16.87 (A)
L. trifolii	3.82 (LA)	1.66 (LA)
T. palmi	37.1 (VA)	41.80 (VA)

Legend: VA: very abundant; A: abundant; LA: little abundant

T. palmi was found very abundant in the two sowing times. In relation to that result, CNSV (2003); Martínez et al. (2006); Vázquez (2008); EPPO (2014) ; CABI (2016) and SENASICA-DGSV (2016) noted that this thysanoptera is a polyphagous species, with reports of at least 200 host species, largely affecting important crops, such as potato, (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), pumpkin (*Cucurbita pepo* L.), cucumber (*Cucumis sativus* L.), beans (*Phaseolus vulgaris* L.), sesame (*Sesamum indicum* L.), tobacco (*Nicotiana tabacum L.*), and purslane (*Portulaca oleracea* Linnaeus). Its importance as a crop pest can be defined in two directions: as a phytophagous insect whose damage consists in scratching the surface tissues of the plant to extract the sap from the leaves, flowers, or fruits, changing the color of foliage into silver-tan; when the intensity of the attack is high, it takes a burned appearance. As a tospovirus, this insect transmits the tomato spotted wilt virus (TSWV), which has not been detected in Cuba (Riley, 2011 cited by Barba & Suris, 2015). Because of its high and fast reproductive capacity, the large variety of wild and cultivated host plants, the warm climate, and living habits that favor reproduction, the large populations originated cause significantly important damage, thus affecting normal plant development, and the quality of harvest (Salas and Cermeli, 1995 cited by Murguido et al., 2002).

Elizondo et al. (2003), in an effort to respond to various questions arisen after the appearance of *Thrips palmi* Karny in Cuba, established a tracking system and field evaluations to detect the presence of the pest in crops, wild plants, and weeds. The confirmation of their diagnostic was made at the Central Laboratory of Plant Quarantine, where a massive infestation was produced in potato and beans, followed by pepper, in the 2000-2001 harvest.

Furthermore, significant damages caused by this insect were observed in garden vegetables in different parts of the world. In Mexico, for instance, there were reports of 5-80% damage in watermelon, and 50-90% in eggplant, and cucumber (OIRSA, 2009 and CABI, 2016). Additionally, this pest was included in the alert list of the European and Mediterranean Organization for Plant Protection, according to OEPP/EPPO (2006), and the System of Phytosanitary Alert of the American Organization for Plant Protection. Accordingly, significant economic losses could be produced to export crops like melon, watermelon, zucchini, and cucumber, due to a ban set on these greens in the international market (NAPPO, 2004).

Research done by Seal et al. (2013) between 1991 and 1994, on research lots and commercial fields at the Center for Tropical Research and Education, in Miami Dade County, Florida, the US, revealed that *T. palmi* was abundant in green beans (*Vigna unguiculata* subsp. *sesquipedalis*), and eggplant (*Solanum melongena* L.).

Whitefly was abundant during the two sowing times, which was similar to *T. palmi* at sowing time 1 (early); it was very abundant and abundant during sowing time 2 (late). The outcome was significant, considering that this aleyrodes is presently the most devastating pest found in tropical and subtropical countries, due to its capacity to transmit viral diseases to different plants, with more than 60 viruses in several crops. Besides from direct damage when feeding, and because of honeydew secretion, product quality is reduced (Perring, 2001 cited by Mansaray & Sundufu, 2009).

Bemisia tabaci is, without a doubt, the most significant species among whiteflies, since it attacks more than 200 crops, transmits more than 150 viruses (Geminivirus), and has the capacity to develop very aggressive genotypes, capable of causing enormous losses by reducing yields, affect crop quality, and increase production costs. The relation between *B. tabaci* and Geminiviruses is persistent-circulative, which means that the acquired viruses circulate in their interior to the salivary glands, injecting saliva when they feed from a healthy tomato plant (Morales et al., 2006 cited by Jiménez & Chavarría 2011; Herrera-Váquez & Cortés, 2015).

Martínez et al. (2006) noted that *B. tabaci* is a very proliferous pest that affects crops like eggplant (*Solanum melongena* L.); sweet potato (*Ipomoea batatas* L.); cabbage (*Brassica oleracea* L.); beans (*Phaseolus vulgaris* L.); papaya (*Carica papaya* L.); lettuce (*Lactuca sativa* L.); peanut (*Arachis hypogaea* L..); watermelon (*Cucumis melo* L.); turnip (*Brassica rapa* subsp. rapa); potato (*Solanum tuberosum* L.); cucumber (*Cucumis sativus* L.); pepper (*Capsicum annuum* L.); okra (*Abelmoschus esculentus* L.); raddish (*Raphanus raphanistrum* L.); soybean (*Glycine max* L.), and tobacco (*Nicotiana tabacum*). Some of their host plants are amaranthus (*Amaranthus* spp.); common mallow (*Malva* sp.), and purslane (*Portulaca oleracea* Linnaeus) (Herrera-Vásquez & Cortes, 2015; Herrera-Váquez et al. (2016).

Murguido et al. (2002) on evaluation of different integrated pest management actions to beans in several provinces of the country, found that in all the areas, the most commonly occurring pests were whitefly and grasshopper.

In contrast to the results achieved in this study, research done in Guatemala, Hilje (2001) cited by Casados (2005), explained that the nymphs of *B. tabaci* were more abundant in curcubitaceae and solanaceae, in comparison to other crops.

E. kraemeri was another phytophagous insect spotted at the two sowing times during the study. It was abundant and very abundant in sowing times 1 and 2, respectively, which coincides with Vázquez (2008), who noted that in annual crops, *Empoasca* is very frequent and abundant, especially all kinds of beans. The author adds that sowing in the optimal season for every region is one of the most effective tactics, largely contributing to the reduction of the effects of this insect.

Martínez et al. (2006) and Miranda et al. (2016) stated that this cecadellidae is spread throughout the country, and it can attack at any phenological phase of the crop. Its incidence leads to considerable yield reductions, and sometimes, to total losses. It affects various species of beans (*Phaseolus vulgaris* L.); soybean (*Glycine* max L.); papaya (*Carica papaya* L.), and potato (*Solanum tuberosum* L.), in addition to a large number of host weeds. Although considered a transmitter of the virus, in Cuba this type of damage is not evident. Crops are harmed by both the nymph and adult, sucking the sap from leaves and stems. The plants attacked show yellowing at the edges of the first single leaves. In more developed plants, harm is characterized by leaf curling, plant squatting, and distortion of cones and pods. Under intense attacks, the plants may produce a more intense green, and necrosis on the leaf veins (Gómez et al., 2009 cited by Sánchez et al., 2016).

During the two sowing times, *L. trifolii* was little abundant, though very frequent, which coincides with the reports made by Vázquez (2008), who stated that part of the life cycle of this dipterous insect is efficiently regulated by several of its natural predators (parasitoids, predators, an even fungi and bacteria, among others). Experience in subtropical conditions has help demonstrate that the biorregulators of this insect are very efficient; therefore, they are well preserved within an Integrated Pest Management Program, since populations are kept at acceptable levels of harmful activity, without having to apply chemical pesticides. The capacity of these populations to quickly acquire resistance to various pesticides has been thoroughly documented in the literature.

Accordingly, CNSV (2003); Martínez et al. (2006) have stated that in recent years *L. trifolii* has begun to exert an intense activity on beans, being present in all the regions of the country, with high populations, especially in areas adjacent to tomato and potato. In some cases, it causes considerable harm, particularly when the population of their natural enemies is reduced.

All the species of phytophagous insects detected during this research at the two times of sowing, were very frequent (Table 3), which corresponds to Murguido et al. (2002); Martínez et al. (2007); Castillo & González (2008); Jiménez (2014), and Castillo et al. (2016), who consider that these are the most frequent insects affecting beans in Cuba and other countries.

Table 3. Relative frequency of phytophagous insects associated to beans at two different sowing times (%)

Species	Time 1	Time 2
E. kraemeri	100 (MF)	100 (MF)
B. tabaci	83.33 (VF)	100 (MF)
L. trifolli	83.33 (VF)	83.33 (VF)
T. palmi	100 (MF)	100 (MF)

Legend: VF- Very frequent; F- Frequent; LF- Little frequent

E. kraemeri was very frequent at the two sowing times. According to this result, Gómez et al. (2009); Hernández et al. (2013) noted that this insect generally emerges from the vegetative stage of the seedling, and increases its population throughout the whole cycle of bean. Martínez et al. (2006) said that *E. kraemeri* attacks at any phenological phase of the crop, causing considerable yield reductions, and sometimes total losses. Lighter color bean varieties are more prone to *E. kraemeri* attacks than darker bean varieties due to the secondary metabolites, like saponins, phenols, and tannins present in these plants.

During all the evaluation periods, the presence of *B. tabaci* was detected in the crop at the two sowing times, which corresponded to the reports made by CNSV (2014), that the whitefly is seen on the crop since the very first days of sowing, and until flowering. This is a significant aspect, considering that Martínez et al. (2006); Holguín-Peña et al. (2010), who deemed this insect as very polyphagous, and causes harm during its larval and adult stages, by suctioning the sap from leaves. In Cuba, it is the only vector for the Golden Mosaic of beans (BGMV).

Concerning *L. trifolii*, a species observed in all the samples taken in the two sowing times, authors like Martínez et al. (2006) and CNSV (2003), stated that infestations by this miner start at the beginning of the crop's cycle, and its presence is permanently observed in fields and crops due to its wide range of host plants.

Thysanoptera species *T. palmi* was very abundant and very frequent during the two sowing times. Accordingly, studies conducted in Cuba demonstrated the existence of a close relation among insect attacks, crop development, and yield losses, which is known as critical period. Particularly in beans, the critical period goes from germination throughout pod formation; in potatoes, it comprises the lapse between sprouting and 60 days; and in eggplant, from blossoming to the emergence of fruits (Murguido et al., 2001 cited by Vázquez, 2003).

The creation of new integrated management programs for *T. palmi* has led to decreases in population, which can be maintained without major losses; however, this phytophagous insect can be very harmful when efficient management tactics are not implemented. Assessment of the economic effects caused by this pest in some countries of the Americas revealed losses between 50 and 90% when the populations are high (Murguido et al., 2001).

Determination of the structure of the phytophagous insect community associated to beans at two different sowing times

The indexes of biological diversity at the two sowing times are whown in table 4, in terms of richness of species, which was the same at the two sowing times (6 species of phytophagous insects); however, the number of individuals was higher during sowing time 2 (late). Uniformity or equitability (E) of every species detected was greater in sowing time 1 (early), which means that abundance of species was more uniform than sowing time 2 (late). Consequently, the Shannon (H') index tended to be higher in sowing time 1 (early), which means that the biological diversity was greater then, due to more uniformity in relation to abundance of each species detected. Hence, there must be a greater balance among all the species, compared to sowing time 2 (late). This outcome has high practical significance, according to Bellon & Penvern (2014); Gliessman (2015), who stated that a greater biological diversity allows ecosystems to withstand environmental changes, making it less vulnerable and more resilient, because the status of the system depends on interspecies relations, and the extinction of any of them is less critical to the stability of the whole set than in in less diverse ecosystems which are marked by dominance.

Table 4. Indexes of biological diversity in the two sowing times

Indexes	Time 1	Time 2
Richness of species (S)	6	6
Uniformity (E)	0.86154	0.79347
Simpson index (DSp)	0.32272	0.36010
Shannon (H') index	1.19435	1.09999

Evaluation of biological diversity is essential in terms of preservation and management, many species are threatened as a result of human actions, which requires a more rational use of natural resources. The remarkable complexity of biodiversity does not offer a unique parameter that can be used as a response; hence several indicators are used for analysis (Chamizo, Socarrás & Rivalta, 2012).

The dominance index of Simpson (DSp) showed its highest during sowing time 2 (late) which implied greater dominance and, therefore, the existence of two species (*E. kraemeri* and *T. palmi*), prevailed over the rest of the species.+++

Measuring biological diversity makes sense if the objective is to bring knowledge to the ecological theory, set parameters that help make decisions or issue recommendations to preserve taxas or threatened areas, or monitor the effect of environmental disturbances. Measuring relative abundance of all species allows to identify the most sensible ones due to their little representativeness in the community, which are more sensitive to environmental and or anthropic variations. Besides, it facilitates the identification of changes in diversity, considering the number of species, distribution of species abundance or dominance, which raises a red flag concerning impoverishing process (Magurran, 1988).

Biodiversity reflects the number, variety, and variability of live organisms. The concept also includes the way this diversity changes from place to place, and in time. Indicators like the number of species of certain areas may help follow up several aspects of biodiversity; therefore, it is considered the basis of multiple strategies used to face production and sustainability of farming systems. Moreover, it indicates the deterioration degree (Pujol, 2007 cited by Ramírez & Chang, 2017).

According to Vargas (2011), biodiversity guarantees health and balance of the biosphere. The diverse elements that make it up are true functional units that provide and ensure many of the basic services to human life. Diversity favors the differentiation of habitats, increases the opportunities of species coexistence and interaction, and generally, it carries along greater efficiency of resource use.

Conclusions

The phytophagous insects associated to beans at two different sowing times were, *Empoasca kraemeri* Ross and Moore; *Bemisia tabaci* Gennadius; *Liriomyza trifolii* Burgess; *Thrips palmi* Karny; *Diabrotica balteata* Leconte and *Cerotoma ruficornis* Olivier.

T. palmi was very abundant during the two sowing times, whereas L. trifolii was little abundant in either time. E. kraemeri and B. tabaco were variably abundant in each sowing time.

Phytophagous insects (*E. kraemeri*; *B. tabaci*; *L. trifolii* and *T. palmi*) were very abundant in the two sowing times.

A greater biological diversity was observed in sowing time 1 (early), whereas dominance was higher during sowing time 2 (late).

Author contribution

Neisy Castillo Reyes: research conception, design, and planning. Result analysis, interpretation, and redaction of the scientific paper.

Anicel Delgado Alvarez: data gathering and processing, redaction of the paper.

Luis Mirabal Acosta: assistance in data collection.

Carlos González Muñoz: identification of trips species, and critical review of the document. Conflicts of interest.

Conflicts of interest

Not declared.

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