Scientific Paper

Potential insect pests of *Leucaena leucocephala* (Lam.) de Wit in the seed production stage in Cuba

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Abstract

Objective: To define the potentiality of two phytophagous insects to become pests of *Leucaena leucocephala* (Lam.) de Wit during its reproductive phenophase, in Cuba.

Materials and Methods: Two productive systems with *Leucaena leucocephala* (Lam.) de Wit cv. Peru and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Likoni were evaluated. The characterization of the lesions produced by the insect on the inflorescences was carried out during one year. Meanwhile, the characterization of the insect lesions on the pods and seeds, and that of the associated bacterial symptoms, took place during three years. The link between insect and symptoms in those organs was determined with a regression analysis through the program SPSS®. The relation between number of insects and pods and seeds infected by the bacterium per season was conducted for two years, and it was tested by a proportion comparison test. To evaluate the proportion between the quantity of insects and infested organs, a regression was made; while the estimation of the losses caused by both insects was through rule of three.

Results: The micromoth found on the inflorescences was *Ithome lassula*, which affected more during the dry season. The organisms found in the pods and seeds were the insect *Loxa viridis*, and the bacterium *Pectobacterium* carotovorum subsp. odoriferum, which had a close relation with the reproductive phenophase of *L. leucocephala*. These organisms also have higher presence in the dry season.

Conclusions: Associated to the reproductive organs of *L. leucocephala*, the micromoth *I. lassula*, due to its direct affectation on the inflorescence, and the pentatomid *L. viridis* on the pods and seeds, were detected with potentiality to become pests.

Keywords: *Leucaena leucocephala*, inflorescences, pods, seeds, potential pests

Introduction

Leucaena leucocephala (Lam.) de Wit is one of the most important exotic plant species in animal husbandry agroecosystems in the region of Latin America and the Caribbean, because it is a plant that should be considered against soil erosion, degradation and compaction and the subsequent loss of the productive capacity of the land. Hence it is considered an essential plan t resource for recovering the stability, fertility and agricultural potential of the land (Calle *et al*., 2011). It is also a multipurpose tree, which contributes green manure to the soil, and from which timber is obtained Brewbaker *et al*., 2016.

In sustainable tropical animal husbandry, its use in cattle constitutes a challenge to produce profitably in an environment of conservation of natural resources, where diverse organisms coexist in healthy ecosystems (Cortez-Egremy et al., 2016; Murgueitio-Restrepo *et al*., 2016). Its inclusion in silvopastoral systems also stands out, as alternative to systems with grasses exclusively, in order to reduce greenhouse gas emissions, specifically of CH_4, which is originated in the ruminal fermentation of digestible fiber (Molina *et al*., 2016; Morales-Velasco *et al*., 2016). It has been proven that with the systems with trees the productive increase of the animals and of the environmental services that are generated in the ecosystem, is achieved (Chará *et al*., 2019).

Another one of the most outstanding attributes of *L. leucocephala* is that this forage is capable of producing up to 1 t or more of total seed (TS) ha^{-1}. Specifically, cultivar Peru produces between 450 and 610 kg of TS ha^{-1} without pruning; while when pruning is performed in June, every two years, it can produce from 315 to 588 kg TS ha^{-1} (Milera y Sánchez, 2017). However, its seed production is affected by different phytophagous insects and pathogens, which prevent it from reaching these yields (Ahmed *et al*., 2016).

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In this group of insects, those belonging to the orders Lepidoptera and Hemiptera, of the families Cosmopterigidae and Pentatomidae, in each case, stand out, which affect the inflorescences and pods, respectively. The insects of the family Cosmopterigidae are included in the more than 130 species that existed in the neotropical region, according to reports of a review by Landry (2001). Those of the family Pentatomidae, just like the former, are considered phytophagous pests, of high economic importance in the forage legumes of that region (Panizzi and Grazia, 2015; Grazia *et al.*, 2015). The objective of this work was to define the potentiality of two phytophagous insects to become pests of *L. leucocephala*, during its reproductive phenophase, in Cuba.

Materials and Methods

Location of the experimental areas. The experimental areas were established at the Pastures and Forages Research Station Indio Hatuey (EEPFIH), located in Matanzas province, in the western region of Cuba.

Evaluated experimental areas and their main characteristics. A silvopastoral system (SPS) (paddock) was evaluated, aimed at bull fattening and established with the association of the commercial varieties *L. leucocephala* cv. Peru (leucaena) and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Likoni (Guinea grass likoni), approved in Cuba by MINAG (2017). In addition, another system was studied, with similar species composition, but aimed at beef and seed production (SPSDP). They were both located on a lixiviated ferralitic red soil Hernández-Jiménez *et al*., 2015, with1,3 ha of surface and density of 396 trees ha^{-1} (516 trees in each area) and six years of exploitation.

Main elements of phytosanitary management. No inorganic or organic fertilization, irrigation or chemical or biological pesticides were applied.

Method for insect sampling (collection). The transparent nylon bag was used.

Method for insect evaluation. The method of the envelope in five spots of the paddock was applied.

Procedure for characterizing the lesions of the phytophagous insect associated to inflorescences. In the SPS, during one year, in the full flowering stage (more than 70 % of the plantation flowered), sampling was carried out five times for three weeks (two in the first and second week, and one in the third) in April and August, which correspond to the dry season (DS) and rainy season (RS), respectively. It was performed at the sampling spots formed by one or two trees, depending on the quantity of flowers they had.

Of the climate elements, the accumulated rainfall in both months was recorded: 19,1 mm in April and 112 mm in August. The data were from the meteorological station Indio Hatuey.

At each sampling time, in the four cardinal points of the plant, five buds and five inflorescences with peduncle were taken. The samples were placed separately in the transparent nylon bags. They were closed with an elasticized string to prevent the escape of the insects in the transference from the experimental field to the plant protection laboratory of the EEPFIH.

In the laboratory, with a Zeiss stereoscopic microscope, the 100 inflorescences and the 100 buds of each sampling moment were observed, in order to count the number of eggs, larvae, pupae and adults of the insect. From the total sample of inflorescences, 30 with visible affectations were randomly taken, to quantify, describe the lesions, and determine the number of eggs, larvae, pupae and adults of the insect per individual flower, as well as the quantity of damaged flowers.

Procedure for characterizing the lesions of the phytophagous insect associated with the pods and seeds, and their relation to a bacterial disease. During three years, in the SPS, 5 % of the *L. leucocephala* cv. Peru plants were evaluated, in order to collect pods with green, forming and mature seeds (at the moment of their emergence in each case) as well as the insects present. The collection was carried out every fifteen days, for which nylon bags were used. The insects and the lesions they caused per pod were quantified. In addition, such lesions were described.

The bacterial symptoms present in the collected pods, associated with the lesions produced by the insects, were quantified and described, in order to corroborate the reports in studies conducted by Delgado *et al.* (1989) and Alonso *et al.* (1993). In addition, the number of seeds infected by the bacterium was counted. Afterwards, the bacterial species was isolated and identified in the plant bacteriology laboratory of the National Agricultural Health Center (CENSA, for its initials in Spanish) through morphological, physiological and biochemical tests.

To describe the relation between the insect and the presence of bacterial symptoms in pods and seeds, the regression analysis was applied through the statistical program SPSS®, version 15.0 for Windows®.

As selection norm of the best-fit model, the criteria enunciated by Guerra *et al.* (2003) were taken into consideration.

Procedure to determine the relation between the number of insects and the pods and seeds infected by the bacteria in each season. In the SPS-DP, during two years, four flowering-fructification peaks of *L. leucocephala* cv. Peru were evaluated (specifically at moments close to harvest). In year 1, the first evaluated peak corresponded to the period May-July; the second, to October-November. The third and fourth peaks, both corresponding to year 2, were framed in April-June and September-November, respectively. The first and third peaks corresponded to the rainy season, and the second and fourth, to the dry season. Table 1 shows the data of climate elements that were compiled.

In the above-mentioned months, every seven days, five *L. leucocephala* plants were randomly sampled (with the method that was reported). From them, five racemes of pods with seeds in formation or harvest status were harvested (approximately 25 % of the pods a tree can produce), with symptoms or without them. They were placed in transparent nylon bags, closed with an elasticized string, to be transferred along with the insects to the plant protection laboratory of the EEPFIH, for their observation in a Zeiss stereoscopic microscope.

In the laboratory the number of insects, total and bacterium-infected legumes, as well as of seeds also infected by such microorganism, was quantified. A proportion comparison test was performed on the data, using the Proportion Comparison System (1998), version 2.1., created at CENSA (1998), to determine in which flowering-fructification peak of each season the present of insects and development of the bacterial disease were favored more. In addition, the above-described procedure was used for isolating and identifying the bacterium, in order to corroborate the correspondence with the found species. To explain the relation between quantity of insects (nymphs and adults) and pods and seeds infected by the bacterium, a regression analysis was also carried out, with the same statistical program and selection criterion used in the above-explained experiment.

Estimation of seed loss due to the insect in the inflorescences, and because of the presence of the associated bacterium with insect of the pods. An intermediate point (average) was taken as assumption in the data about the botanical characteristics of the *L. leucocephala* plant, provided by Hudges (1998). That is: an inflorescence produces eight pods, and a legume has 13 seeds, 1 kg has 15 000 seeds, and their seeding dose is 2 kg of seed ha^{-1}. Besides, it was considered that one hectare with 500 trees produces 200 kg of seed. The quantity of sampled inflorescences and pods in the SPS in the above-described experiments was also taken into consideration. The calculation was made through rule of three, from the sampled inflorescences and pods, and according to the above-cited assumption.

Table 1. Climate elements during the four evaluated flowering-fructification peaks.

Year	Month	Air temperature, ºC			Relative humidity, %			Rainfall, mm
		Maximum	Minimum	Mean	Maximum	Minimum	Mean	
1	May	31,5	16,4	24	96	45	74	25,8
	June	32,1	21,9	26,3	98	60	86	349,6
	July	33,9	21,5	26,8	97	51	81	86,1
	October	31,3	18,1	24,6	97	56	83	51,6
	November	30,7	18,5	24,1	97	56	82	46,2
2	April	30,4	15,6	22,8	96	46	76	40,3
	May	31,8	18,6	24,6	96	52	79	120,2
	June	32,9	21,6	26,5	97	55	83	256,0
	September	33,9	19,8	26,3	97	52	83	281,3
	October	33,5	20,7	25,6	97	59	85	216,9
	November	30,1	18,4	23,9	97	59	84	17,5

Source: Meteorological station Indio Hatuey

Results and Discussion

When evaluating the inflorescences, the presence of the micromoth *Ithome lassula* Hodges (Lepidoptera: Cosmopterigidae) was confirmed since the budding phase and in the inflorescence as such. This insect produced lesions that were manifested as circular perforations on the basis of the individual flowers. Generally, one was observed on each floret, although occasionally two were recorded. These observations are similar to the ones reported by Alonso *et al*. (2015).

Each larva can feed from one and up to seven individual flowers at once, because at the same time it perforates the basis of several flowerlets and consumes the different reproductive structures. In addition, it can even devour the capitulum. If it feeds on the buds, they do not open, and thus the formation of the inflorescence is prevented. Hence all these lesions as a whole cause that the pods and, subsequently, the corresponding seeds, are not formed. The individual flowers turn dark brown in color with regards to the healthy ones, and larvae create a capsule formed by silk threads and small parts of the flowerlets they carry with them until the moment of pupating. In general, two larvae were found as average per inflorescence (table 2) and, sometimes, up to 12, which exceeds the value of 7-9, referred by Elder (2008) in studies related to this insect species, author who also worked with the legume evaluated in this research.

The data in table 2 show increase in the number of larvae and lesions, from the bud to the inflorescence phase, in both flowering peaks. Such increase was higher during the April evaluations with regards to the August ones, which is related to the small size of the eggs (0,5 mm). They are inserted in the buds during the first moments of development of the capitulum and the newborn larvae (less than 5 mm), which hinders their easy detection (they were not observed in any of the cases). According to the report by Elder (2008), it is not until the bud opens that a higher number of larvae (with the increase of their size up to 5 mm of length) and their lesions can be observed.

The accumulated rainfall during August was higher with regards to April. This could have influenced the decrease of larvae because of the direct effect of rain, or due to a higher possibility of producing rot of the inflorescences due to the high humidity, contamination by the insect excreta and growth of saprophytic fungi. Thus, this higher affectation by *I. lassula*, on the inflorescences during the flowering peak in April, dry season, is highly important, because it is in such stage when the seed production of cultivar Peru is higher (Matías, 1999). In addition, it is important to state that the presence of this cosmet moth affects the inflorescences and, thus, seed production in cultivars Cunningham, Ipil Ipil and CNIA 250 of *L. leucocephala* (Alonso, 2009), as well as in the species *Leucaena diversifolia* (Schltdl.) Benth., and *Leucaena* spp. (Azani *et al*., 2017).

It was determined that the lesion caused by the insect *Loxa viridis* (Palisot de Beauvois) (Hemiptera: Pentatomidae) consists in small circle-shaped perforations, which measure 2 mm of diameter, produced with the stylet of the insect on the pod surface, fundamentally on the zone where the seed is forming (Alonso, 2009). At the beginning, a slight swelling of the pod epidermis appears, due to sap suction by the pentatomid, which in its end part leaves a drop of saliva secretion. Afterwards, it is observed that the tissue is necropsied, and gives the impression of a black dot. In each pod a variable number of holes is found.

This pentatomid is also found in the other three existing commercial varieties of *L. leucocephala* (Cunningham, Ipil Ipil and CNIA 250) in Cuba (Alonso, 2009).

Regarding the symptoms of the bacterial disease, its appearance is observed in young pods, with small brownish-yellow ulcers, initially, from which exudations with sticky aspect emanate. As the disease advances, the ulcers turn dark brown

Table 2. Total and average number of *I. lassula* lesions and larvae in *L. leucocephala* buds and inflorescences.

Month	Flowering phase	Larvae		Lesions	
		Number	Approximate means	Number	Approximate means
April	Bud	275	1	236	1
	Inflorescence	798	2	467	1
August	Bud	203	1	160	1
	Inflorescence	376	1	260	1

and increase in number and size, until producing similar affectations on the forming seeds, which finally prevent their maturation. And if they mature, they rot due to the presence of bacterial exudations, which is in correspondence with the report by Delgado *et al*. (1989). In Cuba these symptoms were informed by Alonso (2009), who identified the bacterial species *Pectobacterium carotovorum* subsp. odoriferum (Enterobacteriales, *Enterobacteriaceae*).

As shown in table 4, in the three years of evaluation, in spite of the low detection of *L. viridis* individuals, when quantifying the lesions it was observed that the number of holes tended to increase in the pods with forming seeds with regards to those with green seeds. And this occurs because they have higher quantity of available sap, which causes the avidity of the insects for them to increase, besides the fact that the holes of the initial state are accumulated. Meanwhile, in the pods in harvest status (with mature seeds), the lesions decreased because of the hardening reached due to maturation, which confers them a leathery consistency, and also as a consequence of the color change from coffee to brown, which masks the lesion, criterion that coincides with the report by Hudges (1998).

The result of the regression analysis to determine the relation between number of holes made by the insects and seeds infected by the bacterium, as well as the relation between the infected pods and the number of seeds that were affected by such microorganism, indicated better fit with the cubic equations. In figure 1, the curve shows that initially there were negligible values of holes in the pods with green seeds, which is due to the fact that they are not much preferred by the insect. However, when they reached the formation status, the number of seeds infected by the bacterium increased suddenly. This is not only caused by the accumulation of holes until the maturation stage, but as expression of the disease development, which shows the transmitter character of the pentatomid referred by Alonso *et al.,* (1993). A similar behavior occurred between the pods and infected seeds, because as the infected pod matured, a higher number of seeds affected by the bacterium was produced (figure 2).

No close relation was found between the number of holes and the infected pods, which could have been due to the fact that, although the insect makes several holes on the same pod, some of these lesions are not located on the zone of the forming seeds.

According to the result shown in table 4, it was proven that in the DS a significant increase occurred in the percentage of pods infected by *P. carotovorum* subsp. odoriferum, with regards to the RS, because the number of *L. viridis* individuals was also higher. This confirms that the insect was favored by a lower accumulated value of rainfall, which also increases the possibility of bacterium transmission, in spite of the lower number of pods in the sampled plants in that flowering-fructifying peak. This effect was observed equally on the number of seeds affected by the bacteria in the DS (4 188), which was approximately twice the infected seeds as in the RS (1 962).

In spite of the low number of collected *L. viridis* individuals, their presence in the field was shown by the lesions that remained on the pods, which also propitiated that bacterium-caused putrefactions occurred, which by affecting the seeds cause a decrease of their agricultural value.

Figures 3 and 4 show the relation between number of insects, pods and seeds infected by the bacterium, as a result of the regression analysis, the cubic equations showed the best fit (R^2 = 0,90 and 0,91). It was observed that initially declination occurred in the curve, which could have been given by

Table 3. Quantity of insects and lesions by *L. viridis* in the *L. leucocephala* cv. Peru pods.

Indicator		Year		
		1	2	3
Pods with green seeds	Number of insects	2	0	0
	Number of lesions	59	9	5
Pods with forming seeds	Number of insects	1	0	3
	Number of lesions	198	26	41
Pods with mature seeds	Number of insects	3	0	2
	Number of lesions	87	8	3

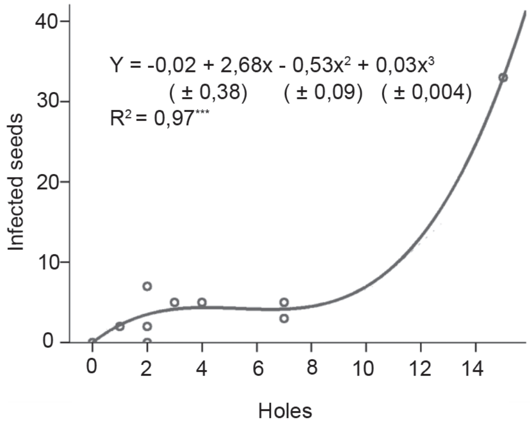

Figure 1. Relation between holes caused by *L. viridis* and seeds infected by *P. carotovorum* subsp. odoriferum.

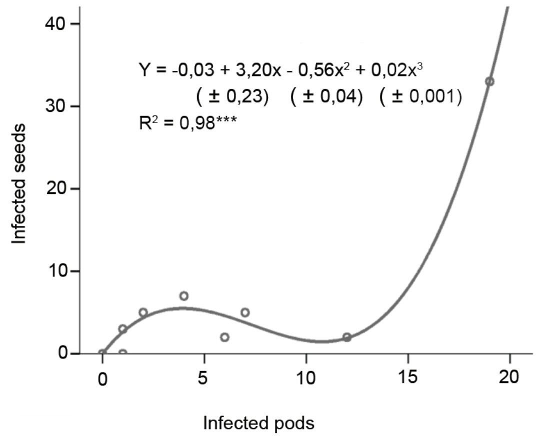

Figure 2. Relation betwwen pods and seeds infected by *P. carotovorum* subsp. odoriferum.

the presence of a generation that ends its life cycle, as well as by a considerable number of egg laying in this period. This implies that when hatching a large number of nymphs appears on the pods with green seeds, in which the lesions by the insects and the symptoms caused by the bacterium are less evident. Meanwhile, when these nymphs reach the adult status, they cause a higher number of lesions as the pods have forming seeds, explaining the increase of the affectation by the bacterium on such organs. Finally, another decline of the curve occurs, which indicates that the affectation by the bacterium decreases. This is due to the fact that, with the maturation process, the lesions by the insects are less possible due to the above-mentioned leathery aspect of the pods and seed hardening.

Although in the available updated literature no criterion was found about the above-described results, Lenné (1991) since detecting the presence of pentatomid insects, associated to bacteria in *L. leucocephala* pods and seeds, stated that such behavior was likely, given the direct link between both organisms. In Brazil, Panizzi and Rossi (1991) also indicated the affectation by the insect species *Loxa deducta* (Walker) (Hemiptera: Pentatomidae) in *L. leucocephala* and soybean.

Segarra-Carmona *et al.* (2016) stated that, in Mexico, *L. viridis* has as host the legume *Senna papillosa* (Britton & Rose). This plant can be used as feed component, as windbreak, and in agroforestry systems to provide shade to the coffee tree, according to Sánchez-Bernes and Moya-Caderón (2018).

When estimating the damage that *I. lassula* can cause on the 1 600 sampled inflorescences (buds and opened inflorescences) in both periods, a loss of 8 kg of seed would occur, by preventing the formation of 8 984 pods and 116 972 seeds, which represents 4 ha without being planted. In terms of production, in one hectare with 500 trees, 71 % of the seed production could be damaged, because 20 500 inflorescences would be affected, for which the formation of 164 000 pods would be prevented, and 142 kg of seed from the possible 200 kg to be obtained would be lost, according to the reference for this surface unit, without using external inputs. This number could be higher, if it is considered that Partridge (1988) stated that cultivar Peru can produce, approximately, 100 kg of seed ha^{-1} when no irrigation is used.

From the affectations caused by the micromoth *I. lassula*, it is valid to consider it as crop pest of *L. leucocephala* in Cuba, if it is considered that the affectation percentage (71 %) would justify taking phytosanitary measures, because according to Elder (2008) the control is performed when in the inflorescences the damage caused by the larvae of this species, is higher than 10 %. Thus, the results reaffirm this author's criterion with regards to the damage this insect can cause on the studied legume, when stating that reductions have been recorded of up to 10 times the quantity of pods that can be formed. This, in turn, reduces similarly seed production.

The importance of the species *I. lassula* also lies on the fact that species from this genus (*Ithome*) have been reported in other countries, in which they attack other legumes of similar importance for silvopastoral systems, such as *Acacia* and *Prosopis* (Gallaher

Table 4. Number of pentatomids and their relation to the infected pods during the flowering-fructification peaks per period.

Flowering-fructification peaks	Rainy season	Dry season
Number of *L. viridis*	33	120
Total pods	8 514	5 278
Infected pods[1]	698	901
Infected pods[1], %	8,19*	17,07*
SE ±	0,003	0,004

*p ≤ 0,05
[1]Pods infected by *P. carotovorum* subsp. odoriferum

and Merlin, 2010). This implies that, if it is present in other tree legumes, the damage they cause can be remarkable, with negative impact for the animal husbandry of the country. As reported by Elder (2008), in Australia, *I. lassula*, is considered abundant and frequent, and of interest for *L. leucocephala* seed farmers, because only one damaged bud prevents the formation of the inflorescence. Important reductions are then recorded in the quantity of pods and, consequently, in seed production. Likewise, Ananthakrishnan and Sivaramakrishnan (2017) report this insect as an important pest in such legume in the Western Ghats, known as the Sahyadry Mountains, in India.

Regarding the losses due to the bacterial affectation by the species *P. carotovorum* subsp. odoriferum, associated to the pentatomid *L. viridis*, they were estimated from 1 175 total sampled pods. From them, 301 were infected by the bacterium. Thus, in one hectare with 500 trees, 59 000 pods and 767 000 seeds could be affected, which would represent a deficit of 51 kg, and a decline in seed production of 26 %, percentage that is in the range of 17 – 49, obtained by Delgado *et al.* (1989). These authors evaluated the affectations caused by the bacterium on the seeds of different *L. leucocephala* varieties, in which cultivar Peru was included.

The damage caused by the bacterium, due to a large extent to its penetration through the lesions made by the pentatomid, and to its possible transmission by it, is sufficiently significant to consider the behavior of this insect as pest of interest for the crop. This is directly related to the importance of insects as transmitters of bacterial diseases, because it is reported that 15 of the more than 200 diseases disseminated by insects and arachnids in 2015, had bacterial origin (Ritacco, 2015).

Conclusions

Associated to the reproductive organs of *L. leucocephala*, the micromoth *I. lassula*, due to its direct affectation on the inflorescences, and the

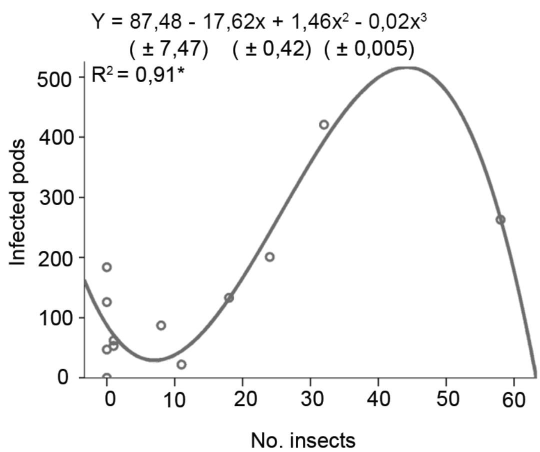

Figure 3. Relation between the number of *L. viridis* and pods infected by *P. carotovorum* subsp. odoriferum..

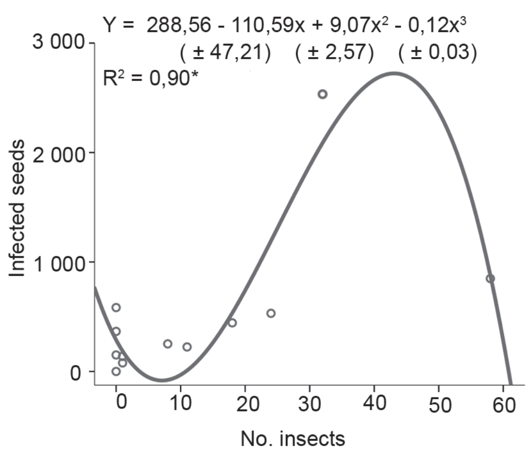

Figure 4. Relation between the number of *L. viridis* and seeds infected by *P. carotovorum* subsp. odoriferum.

pentatomid *L. viridis* in the pods, were detected with potentiality to become pests. The latter, indirectly, also affects seed production, behaving as a transmitter of the bacterium *P. carotovorum* subsp. odoriferum; in addition to the losses it can cause, according to the estimation that was made.

This suggests the need to continue conducting new studies that allow to obtain more information about the biological cycle of the researched insects and their synchrony with the reproductive phenophase of the forage plant, in order to design efficiently an agroecological strategy that is effective for their management, and thus avoid yield losses.

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Authors' contribution

- Osmel Alonso-Amaro. Generated the idea of the research, searched for bibliographic information, executed the experiments with the corresponding measurements, participated in the identification of the studied organisms, wrote the manuscript, and made its revision during the edition process until its publication.
- Rayner Núñez-Águila. Carried out the identification of the micromoth, in order to corroborate the determination of the species, and contributed criteria about its behavior for the results and discussion.
- Vicente Horacio Grillo-Ravelo. Made the identification of the pentatomid, in order to corroborate the determination of the species, and contributed criteria about its behavior for the results and discussion.
- Juan Carlos Lezcano-Fleires. Contributed in the search for bibliographic information, and in making measurements, participated in the identification of the bacterium, and collaborated with the revision of the manuscript during the edition process until its publication.
- Moraima Suris-Campos. Contributed to concrete the idea of the research, revised the experimental methodology for the execution of this study, and collaborated with the revision of the manuscript during the edition process until its publication.

Conflict of interests

The authors declare that there are no conflicts of interests among them.

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