

## Grain production and incidence of galling nematodes in common bean

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### Abstract

The interaction environmental genotype in common bean crops (*Phaseolus vulgaris* L.) and their reaction to *Meloidogyne* spp., are aspects insufficiently addressed despite the influence they have on the productive results of this crop. The productive potential of five common bean crops was determined, in agroecosystems in Pinar del Río, Cuba, and the incidence of a population of *Meloidogyne* spp. in the vegetative phase of the crop. To this end, field experiments were established in 'San Juan and Martínez' and 'Sandino', on Ferralitic yellowish and Fluvisol soils, respectively. Commercial crops (treatments) were used: 'BAT 304' (commercial production witness), 'CUL 156', 'Buenaventura', 'Delicias 364' and 'Chevere'. A test was also developed, under semi-controlled conditions with pots of 1.5 kg and substrate based on ferralitic yellowish + peat soil (70% + 30%), with and without inoculation of *Meloidogyne* spp. (1.5 J<sub>2</sub>-eggs g<sup>-1</sup> substrate) in the crops referred. The differences between crops and their interaction with agroecosystems showed a marked influence on grain production, although the best results were obtained with the crops 'CUL 156' and 'Buenaventura', because they exceeded by 25% the agricultural yield of the commercial production witness, in the agroecosystem of 'San Juan and Martínez' and 'Sandino', respectively. It was confirmed that the cultivars evaluated are hosts of *Meloidogyne* spp., although 'Buenaventura' expressed lower infection index for the level of inoculum used, while the inverse relationship between weight and the number of galls and ootheca in the radical system of plants, suggested a harmful effect of these phytonematodes on the vegetative phase of the crop.

**Keywords:** *Meloidogyne* spp., *Phaseolus vulgaris* L., agroecosystems, cultivars.

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## Introduction

Grain production is a global and national priority for its importance in human and animal feed. In this plays an important role the crop of the common bean (*Phaseolus vulgaris* L.) due to its nutritional value, wide distribution and consumption (Calero *et al.*, 2018; Aguilar *et al.*, 2019; Martínez *et al.*, 2019). 67% of the world's crop production, estimated at 30.4 million tons, concentrated in eight countries: India, Myanmar, Brazil, the United States of America, China, Tanzania, Mexico and Uganda, all with production above one million tons (FAO, 2018).

In Cuba, more than 147 thousand hectares were harvested in 2018, with a total production of 161.5 thousand tons and average agricultural yield of 1.09 t ha<sup>-1</sup> (ONEI, 2019), although they do not yet meet the needs of the population (Martínez *et al.*, 2017; Hernández-Ochandía *et al.*, 2018). If it is intended to improve the productive results in this legume, it is important that producers expand their germplasm with crops recommended due their higher agricultural yield.

The regionalization studies are necessary due the genotype-environment interaction that occurs in that crop (Martínez *et al.*, 2019). However, in Cuban agriculture there are more than 30 commercial crops in the production of common bean (MINAG, 2018), although they have been little studied under local agroclimatic conditions of Pinar del Río.

Another factor influencing the cultivation of common bean in Cuba is the involvement by pest organisms, particularly plant parasitic nematodes (Hernández-Ochandía *et al.*, 2016), recognizing *Meloidogyne* spp. (galling nematodes) among them., they are causing considerable damage on the development of plants (Hernández-Ochandía *et al.*, 2018a) although it is often neglected, that is, these organisms are underestimated by technicians and producers in this crop.

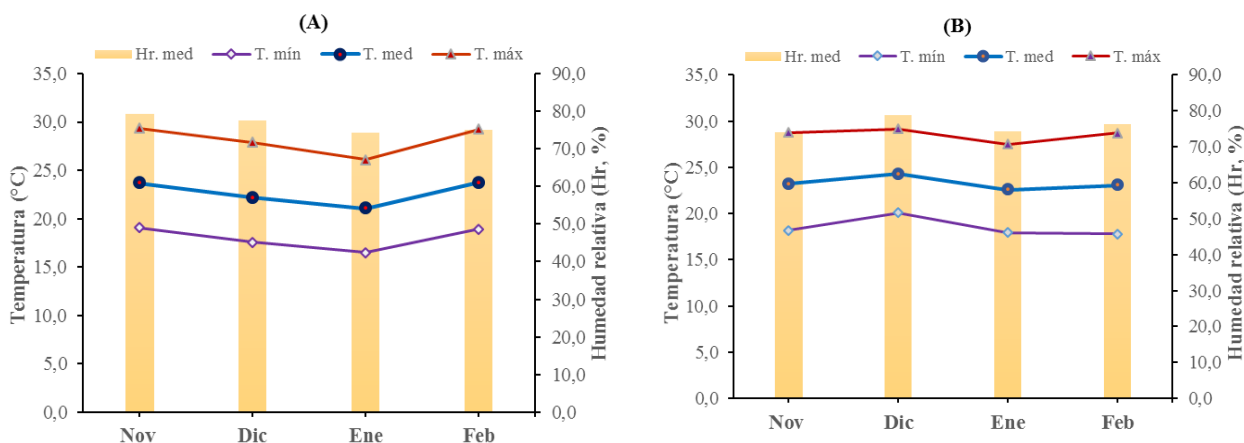
Considering the above, the objective was to determine the productive potential of common bean crops, in agroecosystems in Pinar del Río, Cuba and the incidence of a population of *Meloidogyne* spp., in the vegetative phase of the crop.

## Materials and methods

### Description of scenarios for field experiments

Field test were conducted in the period November 2017 to February 2018, on the popular councils 'Rio Seco' (22° 18' 13" north latitude and 83° 47' 39" longitude west) of 'San Juan and Martínez' and 'Sandino' (SJM) and 'Manuel Lazo' (22° 02' 95" latitude north and 84° 21' 22" longitude west) of 'Sandino' (SDN), in Pinar del Río, Cuba. The soil of the localities was classified as Ferralitic Yellowish (pH<sub>(KCl)</sub>= 5.1 and MO= 1.84%) and Fluvisol (pH<sub>(KCl)</sub>= 4.71 and MO=1.51%), respectively, according to Cuba's soil classification key (Hernández *et al.*, 2015).

Weather conditions during tests in San Juan and Martínez (Figure 1A) and Sandino (Figure 1B), were characterized by average temperatures of 22.9 °C and 23.3 °C, relative humidity of 77.9% and 75.9% and accumulated precipitation of 150.5 mm and 142.5 mm, according to data obtained at the Meteorological Stations number 314 (San Juan and Martínez) and number 313 (Sandino) of the Provincial Meteorological Center.



**Figure 1.** Average temperature values (minimum, mean, maximum) and relative humidity in ‘San Juan and Martínez’ (A); and ‘Sandino’ (B).

### Crops used and field experiments

Five crops (treatments) of common bean (Table 1) were used, distributed in a random block design with four replicates. The cultivated area, in each locality, was 560 m<sup>2</sup> with experimental units of 28 m<sup>2</sup>. Uniformity in crop management was ensured in the two locations. The planting was carried out manually, at 0.6 m between grooves and 0.07 between plants.

**Table 1.** Description of the crops used in the experiments.

Crops	Color	Type of growth	Potential agricultural yield (t ha <sup>-1</sup> )
‘BAT 304’ *	Black	Indeterminate prostrate	2.1
‘CUL 156’	Black	Indeterminate shrubby	3.17
‘Buenaventura’	Red	Indeterminate shrubby	2.93
‘Delicias 364’	Red	Indeterminate shrubby	2.8
‘Chévere’	White	Indeterminate prostrate	3.1

\* = witness to commercial production of common bean in studio locations (Faure *et al.*, 2013).

All cultural attentions were made as established in the technical guide for the cultivation of beans in Cuba (Faure *et al.*, 2013). Fertilization was used with formula 12-6-16-3 (N-P-K-Mg) at planting time, dose 0.4 t ha<sup>-1</sup>, and a foliar application of fitomas E (1.5 L ha<sup>-1</sup>) prior to flowering. The surface method by groove was used for irrigation. Celest Top FS 31.2 pesticides (thiamethoxam 26.2 + difenoconazole 2.5 + fludioxonil 2.5), calcium hydroxide (CaOH), mixture duple E 3.125 (acephate3 + cypermethrin 0.125) and Kospi SC 13 (Imidacloprid 10.0 + bifenthrin 3) were used to protect the crop.

## Variables evaluated in field experiments

Yield assessments and their components were carried out at harvest time. 10 randomly representative plants were selected per replicate in each crop. The variables analyzed were: number of legumes/plant (u), full legume index (%) -valued as a proportion of legumes with more than 50% seed curd-, number of seeds/legume (u), legume mass (g), seed/legume mass (g), mass of 100 seeds (g) and agricultural yield ( $t\ ha^{-1}$ ).

In the calculation of mass-related variables, an OHUS Adventurer<sup>®</sup> Pro digital technical balance was used precision 0.01 g. To determine agricultural yield, 6 m<sup>2</sup> of the center was harvested in each replica, the plants were threshed and the grains dried up to 14% moisture.

## Experiment for interaction crops-*Meloidogyne* spp.

The experiment was conducted between February and march 2018, under semi-controlled conditions of the University of Pinar del Río, Cuba, with location at 22° 24' 48" north latitude and 83° 41' 16" west longitude. Environmental conditions were characterized by temperatures between 26 and 36. 2 °C, with an average of 30.2 °C and relative humidity from 54 to 82%. A Weather Pocket Station brand LM-8000 DC9V was used for variable monitoring.

A completely random design was used with factorial arrangement. Two factors were considered: crops ('BAT 304', 'CUL 156', 'Buenaventura', 'Delicias 364' and 'Chévere') and nematodes (with and without inoculation of *Meloidogyne* spp.) and five replicates per treatment. The planting was carried out in pots of 1.5 kg of substrate, which was made from yellowish ferralitic soil (Hernández *et al.*, 2015) + peat (70% + 30%), previously disinfested with 4% formaldehyde, with  $pH_{(KCl)}=5.84$  and  $MO=6.67\%$ . The *Meloidogyne* spp. inoculum, from a population associated with the cultivation of common beans in the soil used, was obtained according to the Hussey and Barker methodology (1973).

The same it was applied five days after planting at the rate of 1.5 J<sub>2</sub>-eggs g<sup>-1</sup> of substrate. 35 days after germination, morphological variables in bean silvers of the cultivars evaluated were analyzed: stem length (cm), stem diameter (mm), radical system length (cm), number of secondary roots and total fresh mass (g). Also, it is quantified, in the radical system of plants, the infestation by *Meloidogyne* spp., expressed in number of oothecas per gram of root, and the number of nodules by native strains of *Rhizobium* spp. Observation and direct counting were performed with the help of the Novel<sup>®</sup> stereoscope.

## Statistical analysis of the results

With the data obtained in the experiments, the assumptions of normality and uniformity of variance were checked using the Kolmogorov-Smirnov and Levene tests, respectively. Variance analysis was applied, in correspondence with experimental designs, and Tukey's test for mean comparison, at a confidence level of 95% ( $p \leq 0.05$ ). Principal component analysis was also performed for the crops-*Meloidogyne* spp. interaction experiment. Minitab 17 statistical software was used for Windows.

## Results and discussion

### Effect of interaction crops agroecosystems on common bean yield

The results showed highly significant differences for all variables analyzed in the two factors included, except for agricultural yield in agroecosystem (Table 2); however, only significant interaction was found for the number of legumes/plant (L/P), legume mass (ML) and agricultural yield (RA). This demonstrates the importance of environmental genotype interaction studies in the selection of crops for local agroclimatic conditions and constitutes the first report on the subject for these localities in Pinar del Río, Cuba.

**Table 2. Bifactorial variance analysis (F-value) for yield components.**

Factors	L/P	ILL	S/L	MI	MS/L	M100S	RA
Crops	38.34**	24.36**	44.56**	18.01**	35.42**	39.24**	20.5**
Agroecosystems	17.73**	60.1**	11.59**	9.58**	26.53**	6.53**	2.04 <sup>ns</sup>
Crops*agroecosystems	6.19**	1.93 <sup>ns</sup>	0.54 <sup>ns</sup>	3.28*	2.35 <sup>ns</sup>	1.79 <sup>ns</sup>	9.89**
EEM	0.22	1.38	0.09	0.02	0.02	0.32	0.04

L/P= legumes/plant; ILL= full legume index; S/L= seeds/legume; ML= legume mass; MS/L= seed/legume mass; M100S= mass of one hundred seeds; RA= agricultural yield; \*\*, \* = significant differences for  $p \leq 0.01$  and  $p \leq 0.05$ ; <sup>ns</sup>= non-significant; EEM= standard error of the mean.

Some authors have found highly significant differences in comparing yield and its components in the different common bean crops (Izquierdo *et al.*, 2018; Romero *et al.*, 2019), hence the importance of assessing the potential they express in the agroclimatic conditions evaluated. With regard to the components of agricultural yield, it can be seen that the differences between cultivars (Table 3) indicate better results with 'CUL 156' for the number of legumes/plant, seeds/legume, legume mass and seed/legume mass.

**Table 3. Average values of yield components by crop and agroecosystem.**

Factors	L/P (u)	ILL (%)	S/L (u)	ML (g)	MS/L (g)	M100S (g)
Crops						
'BAT 304'	8.96 c	88.72 a	5.06 b	1.07 c	0.8 c	16.13 c
'CUL 156'	13.86 a	65.13 c	7.56 a	1.52 a	1.35 a	17.79 bc
'Buenaventura'	11.23 b	74.32 b	4.39 c	1.34 b	1.02 b	23.19 a
'Delicias 364'	10.27 b	76.04 b	4.86 b	1.08 c	0.85 c	17.54 bc
'Chévere'	8.91 c	79.21 b	4.76 bc	1.29 b	0.91 c	19.09 b
Agroecosystems						
San Juan and Martínez	12.6	61.6	5.68	1.42	1.16	20.8
Sandino	9.28	85.99	4.69	1.15	0.84	18.18

Different letters in the same column indicate significant differences ( $p \leq 0.05$ ). L/P= legumes/plant; ILL= full legume index; S/L= seeds/legume; ML= legume mass; MS/L= seed/legume mass; M100S= mass of one hundred seeds

It should be noted that a better result in the number of legumes/plant is key to the production of the crop due to its recognized contribution to agricultural yield (De la Fé *et al.*, 2016); Izquierdo *et al.*, 2018). A study of 15 common bean crops in eastern Cuba indicated values among 13.9 and 23.6 legumes/plant (Estrada *et al.*, 2016), result that corroborates the variability of this component between crops and agroecosystems.

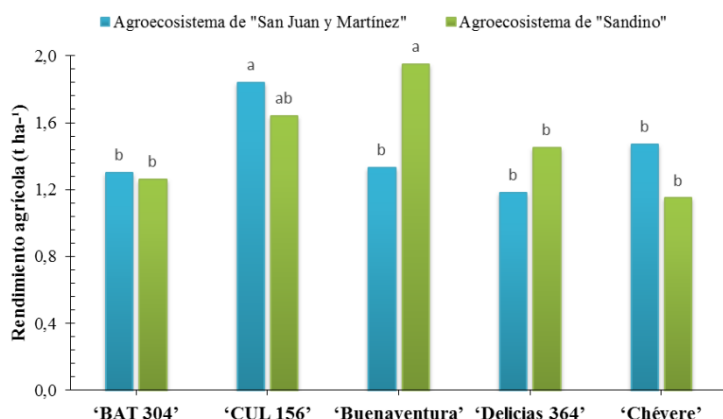
The number of seeds/legume contrasted with other research developed in Cuba, as Maqueira *et al.* (2017) reached values below 4 seeds/legume in 'CUL 156' for the same planting date, and Calero *et al.* (2018) obtained between 3 and 4 seeds/legume with 'Buenaventura'. The full legume index yield presented significantly higher values in the commercial production witness 'BAT 304', although all exceeded 60%.

Higher values in this index suggest a higher proportion of photosynthates towards seed production (Flores *et al.*, 2018) and at the discretion of the authors, presents a direct relationship with the harvest index in the crop; however, grain production does not always achieve higher results with more efficient crops, because the agricultural yield potential of each one influences, although it is indispensable for the production of seeds in this crop. The dry mass of 100 seeds exceeded what was established for this component in the crops 'Buenaventura' and 'Chévere', the other cultivars expressed more than 75% of their average mass (Faure *et al.*, 2013).

The best results among agroecosystems for yield components, with the exception of the full legume index, were obtained in 'San Juan and Martínez', with values that exceed by 14% and 38% those obtained in 'Sandino'. However, in this town, the full legumes index exceeded by 28.4% the value achieved in 'San Juan and Martínez', where greater vegetative development was achieved and as a result, a lower proportion of photosynthates intended for seeds in the reproductive phase of cultivation.

In agricultural yield (Figure 2) it could be found that the commercial production witness 'BAT 304' was significantly surpassed by the cultivars 'CUL 156' and 'Buenaventura' in the agroecosystems of 'San Juan y Martínez' and 'Sandino', respectively, with increases of more than 25% in grain production. It is noted that only the crop 'Buenaventura' obtained significant differences between agroecosystems, although, in general, the black crops ('BAT 304' and 'CUL 156') and white ('Chévere') achieved higher average agricultural yields in 'San Juan and Martínez' and the red ('Buenaventura' and 'Delicias 364') in 'Sandino', regardless of statistical differences.

It was appreciated that the cultivars 'BAT304' (in both agroecosystems) and 'Buenaventura' (in 'Sandino'), exceeded 60% their potential agricultural yield (Table 1). However, the average agricultural yield, in all crops, exceeded that achieved by the crop of common bean in Cuba ( $1.09 \text{ t ha}^{-1}$ ), according to data from the National Bureau of Statistics and Information (ONEI, 2019), a result of great importance not only in grain production, but for the selection of scenarios for the seed production of these crops, since it is known that, in most common bean-producing areas, potential yields are never achieved (Domínguez *et al.*, 2016).

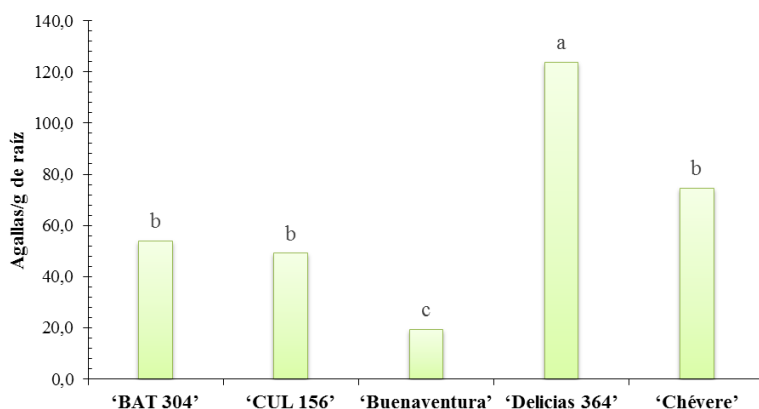


**Figure 2. Effect of interaction crops agroecosystems on the agricultural yield of the crop.** Different letters on the bars indicate significant differences for  $p \leq 0.05$ .

Studies developed in Cuban agroecosystems, which included crops ‘CUL 156’, ‘Buenaventura’ and ‘Delicias 364’, report better productive results in the latter, with agricultural yield values above  $1.5 \text{ t ha}^{-1}$  (Izquierdo *et al.*, 2018); however, De la Fé *et al.* (2016) obtained between 1 and  $3.2 \text{ t ha}^{-1}$  in 14 crops recently introduced in production, while Martínez *et al.* (2015) exceeded  $2 \text{ t ha}^{-1}$  with the crop ‘Chévere’.

### Effect of the population of *Meloidogyne* spp. on common bean crops

It is noted that the five crops used are hosts of *Meloidogyne* spp. (Figure 3) and allow their reproduction, because the external oothecas was observed in the root system, confirming the criterion of several authors who consider these phytonematodes as pest organisms on common bean (Hernández-Ochandía *et al.*, 2016; Al-Hazmi *et al.*, 2017).



**Figure 3. Galling capacity of *Meloidogyne* spp., in common bean crops.** Different letters on the bars indicate significant differences for  $p \leq 0.05$ .

The best result was obtained in the crop ‘Buenaventura’, with damage less than 50% of what was expressed by other crops evaluated, while the best host attitude was appreciated in the crop ‘Delicias 364’, surpassed twice the infestation of ‘BAT 304’. The above should be deepened in



resistance/susceptibility studies to define reference crops in common bean-nematodes (*Meloidogyne* spp.) interaction studies. However, the above is a scientific reference for this crop in the conditions of Pinar del Río, Cuba.

Multivalent analysis for interaction crops *Meloidogyns* spp. in vegetative development he showed two new main components (CP) extracted that explained, as a whole, 86.9% of the variance of the results, with extraction coefficients >0.65 (Table 4).

**Table 4. Correlation matrix between the main extracted components and the original variables for the crops-*Meloidogyne* spp. interaction.**

Original variables	Main components		Extraction
	CP1	CP2	
Stem diameter	0.188	0.902	0.914
Total fresh mass	0.905	0.309	0.849
Total dry mass	0.965	-0.089	0.938
Galls/g root	-0.693	0.387	0.951
Oothecas/g root	-0.917	0.234	0.897
Rhizobium nodules/g root	0.426	0.623	0.665
Variance explained	52.8	34.12	86.92

Extraction method= principal component analysis.

The first principal component (CP1) was determined by total fresh and dry mass and *Meloidogyne* spp. infestation (galls and oothecas per gram of root), showing that the phytomass of bean plants increased with the reduction of the number of oothecas and galls, suggesting a harmful effect of phytonematodes on common bean crops, which is accentuated by the complete reproduction of the female, as a high correlation was obtained (>0.9 in module) negative between the number of oothecas per gram of root and the fresh and dry fitomasas, result that corroborates what has been expressed by several authors on the parasitic action of *Meloidogyne* spp. in common bean (Al-Hazmi *et al.*, 2017; Hernández-Ochandía *et al.*, 2018a).

This result suggests deepening the tolerance of cultivars against different levels of *Meloidogyne* spp. inoculums, both vegetatively and reproductively, to define their impact on growth and yield. The second principal component (CP2) was related to the original stem diameter variables and number of native rhizobia nodules, which expressed a positive correlation, derived from the effect of biological nitrogen fixation resulting from common bean-Rhizobium symbiosis.

## Conclusions

The differences between crops and their interaction with agroecosystems showed a marked influence on grain production, although the best results were obtained with the cultivars ‘CUL 156’ and ‘Buenaventura’, as they exceeded by 25% the agricultural yield of the commercial production witness, in the agroecosystem of ‘San Juan and Martínez’ and ‘Sandino’, respectively.



All the cultivars evaluated were hosts of *Meloidogyne* spp., although a lower galling index was reached in 'Buenaventura' and it was found that the increase in the number of galls and oothecae, in the root system of plants, reduced phytomass in the vegetative phase of the crop.

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