Bacteria-enriched inoculant enhances banana development and influences nutrition

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Bacteria-enriched inoculant enhances banana development and influences nutrition.

Abstract — Introduction. The beneficial effects of bacteria in the soil are known, but those of commercial bacterial products, to enhance plant growth, remain unsolved. Therefore, the effect of RET-FLO PX357, a commercial mixture of microorganisms in a liquid suspension, was studied and quantified on banana crop. **Materials and methods**. A concentrated culture of this product was tested for 2 years in two commercial banana plantations in Tenerife (Canary Islands, Spain). Three treatments were used: a control, Fenamiphos nematicide (15 $L \cdot ha^{-1}$), and RET-FLO PX357 (20 $L \cdot ha^{-1}$). Parasitic root nematode number, pseudostem circumference, time to flowering, and bunch hand and finger number were determined; root, leaf and soil contents were analysed. **Results and discussion**. RET-FLO PX357 decreased *Helicotylenchus* nematode number and, in some cases, increased phosphorous, calcium and iron levels in roots and leaves; it brought about the reduction of available soil sodium concentration and electrical conductance levels; it improved pseudostem circumference and number of fingers per hand, while advancing flowering time. **Conclusion**. RET-FLO PX357 allows ecological and economical improvements, important to take into account when deciding to carry out a sustainable agriculture. (© Elsevier, Paris.)

Canary Islands / *Musa* / plant nutrition / nitrogen fixing bacteria / nematodes

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Amélioration du développement des bananiers et de leur nutrition par application d'une suspension bactérienne.

Résumé — Introduction. Les effets bénéfiques des bactéries du sol sont connues, mais ceux de préparations bactériennes commerciales, sur la croissance des plants, ne le sont pas encore. L'effet du RET-FLO PX357, un mélange du commerce constitué de microorganismes en suspension dans un liquide, a donc été étudié et quantifié en bananeraies. **Matériel et méthodes**. L'application d'une culture concentrée de ce produit a été testée, sur 2 ans, dans deux bananeraies de Tenerife (îles Canaries). Trois traitements ont été appliqués : un témoin, une application avec du Fénamiphos (nématicide) à 15 $L \cdot ha^{-1}$, et du RET-FLO PX357 à 20 $L \cdot ha^{-1}$. Le nombre de nématodes parasites des racines, ainsi que la circonférence du pseudotronc, le temps de floraison et les nombres de mains et de doigts des régimes ont été déterminés ; la composition des racines, des feuilles et du sol a été analysée. **Résultats et discussion**. Le RET-FLO PX357 a provoqué la diminution du nombre de nématodes du genre *Helicotylenchus* et, dans certains cas, l'augmentation de la teneur des racines et des feuilles en phosphore, calcium ou en fer ; il a provoqué la réduction de la teneur du sodium disponible dans le sol et la diminution de la conductance électrique ; il a amélioré la circonférence du pseudostem et le nombre de doigts par main, tout en diminuant le temps de floraison. **Conclusion**. Le produit testé présente des avantages écologiques et économiques qui devront être pris en compte lors de la mise en place une agriculture durable. (© Elsevier, Paris.)

Canaries (îles) / *Musa* / nutrition des plantes / bactérie fixatrice d'azote / nématode

1. introduction

Conventional agricultural systems rely on the use of chemical fertilizers and pesticides, which frequently leads to over-exploitation of cultivated soils, resulting in mineralization, organic matter decrease and loss of fertility. These practices negatively affect soil microorganisms, which play important roles in the soil nutrient balance. Since organic material is normally not added to such soils, there is a reduction of soil energy and a decrease of bacterial population [1].

Rhizobacteria (root colonizing bacteria) from products enriched with bacteria can have beneficial effects on the host-plant. Beneficial bacteria are called 'plant growth promoting bacteria' (PGPR) [2], because they induce plant development after inoculation in the plant growth media. Two benefits of PGPR on crops are growth promotion and biological control [3–5]. Schippers [6], Weller [7], and Stirling [8] have published interesting reviews on these subjects.

Nematodes are common organisms in soils and they constantly compete with other living organisms which limit their population. Some microflora and microfauna species are antagonistic to or are active regulators of nematodes. These organisms are principally bacteria, procaryotes, fungi, predator nematodes and many species of protozoa, microarthropods and arachnids of the soil. Research on rhizobacteria as biological agents for the control of phytopathogenic nematodes has been initiated and some success has already been reported [9].

The beneficial effects of bacteria in the soil are known, but the success of commercial bacterial products to enhance plant growth when added to the soils remains unsolved.

The objective of this study was to determine the effect of the biological product RET-FLO PX357 on banana crop, and to quantify the beneficial effects on the plant. This product is a concentrated culture of microorganisms in a liquid suspension that contains an association of different species of microorganisms, mainly nitrogen-fixing bacteria, humus-producing bacteria, moulds that produce antibiotics, algae, and actinomycetes.

2. materials and methods

The study was carried out in two commercial banana (cv. Dwarf Cavendish) plantations located in south-west (farm A) and north-east (farm B) Tenerife (Canary Islands, Spain). Both soils were anthropogenized soils, with a loamy texture (43.05% sand, 31.35% silt, 25.60% clay) in farm A, and with a clay texture in farm B (22.09% sand, 28.53% silt, and 49.38% clay).

Four plots, with 250 banana plants each (1300 m^2) in each farm, were subjected to the following treatments: a) control; b) Fenamiphos nematicide (15 $L{\cdot}ha^{-1}$); and c) 20 $L{\cdot}ha^{-1}$ RET-FLO PX357. Groups of plants in each plot were used as replicates. These treatments were applied by drip irrigation. All the plots received the same fertilization. The trials lasted 2 years and the treatments were applied in November of the first and second year.

2.1. parasitic root nematode population

Banana roots samples were taken in each plot several times during the banana culture. Root nematode number was counted following the maceration-filtration and direct counting technique [9].

2.2. pseudostem circumference measurement

The circumference of the banana pseudostem was measured at a height of 1 m from the soil, on 20 plants from each treatment in both farms, which had the same phenological characteristics at the beginning of the study. These measurements were taken three times along the second year of treatment in both farms.

2.3. time to flowering

Data on time to flowering were collected at the time of first appearance of flowering, from the same plants used to measure the pseudostem circumference.

2.4. root sampling for nutrient analysis

Three samples from each treatment were taken at the time of maximum flowering of the second year of treatment in each farm. Each sample consisted of five fully developed roots from four plants.

2.5. leaf sampling

At the same time as the root sampling, three leaf samples were taken from each treatment at time of flowering. The third youngest leaf, counting from the most recently emerged leaf with a minimum lenght of 1 m, was chosen from four plants per sample. At a point half-way along the leaf, a 10-cm wide strip was cut from the leaf lamina on both sides of the main vein. The inner half of the leaf strip was used for analysis.

2.6. root and leaf analysis

The samples were washed in distilled water, and dried in an oven at 80 °C, after which they were ground to a powder. 0.2 g of this powder was used to determine nitrogen (N) by the Kjeldahl [10] method. One g of the powder was ashed in an oven at 480 °C and then solubilized by dry ashing with 6 mol hydrochloric acid. Potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn) and copper (Cu) were determined by atomic absorption spectrophotometry, and phosphorus (P) by the vanadate-molybdate method [11].

2.7. soil analysis

Soil samples were taken at the same time of root and leaf samples, and they were taken from the same sites of the plants used to obtain root and leaf samples. Available K, Ca, Na, and Mg were extracted with a solution of 1 N ammonium acetate at pH 7, with a ratio of soil to extractant of 1:5, shaken for 30 min, and determined by atomic absorption spectrophotometry. The pH was measured in water in a ratio of 2:5, shaken and allowed to settle for 10 min. Electrical conductivity was measured in the saturated water extract. Total calcium carbonate was obtained by the Bernard procedure, described by Drouineau and Gouny [12]. Organic matter was determined by the Walkley and Black method as modified by the Comisión de métodos analíticos del instituto de Edafología y agrobiologia "José M. Albareda" [13].

2.8. hand number per bunch and finger number per hand

Hand number per bunch was counted in the same 20 plants from each treatment used to measure pseudostem circumference. Finger number per hand was counted from the second hand of the bunches, as recommended by Deullin and Monnet [14] and Fernandez et al. [15].

2.9. statistical analysis

Data were subjected to analysis of variance with Statgraphics statistical programme.

3. results and discussion

Only the results of the second year of experiments are discussed, because enriched-bacteria commercial products need some time to stabilize their action; moreover, on the basis of general observations about bacterial effects in soils, we may assume that, after 2 years, the results should be more consistent.

3.1. root nematode populations

Figure 1 shows the mean annual population of total root nematode *Helicotylenchus*. In both farms, roots from the RET-FLO PX357 20 $L \cdot ha^{-1}$ treatment had fewer nematodes than the control, and performed better than the nematicide treatment. No difference was detected in *Pratylenchus* or in *Meloidogyne* nematode populations. The lower number of nematodes observed in farm A is due to better nematode control management in years previous to our experiment.

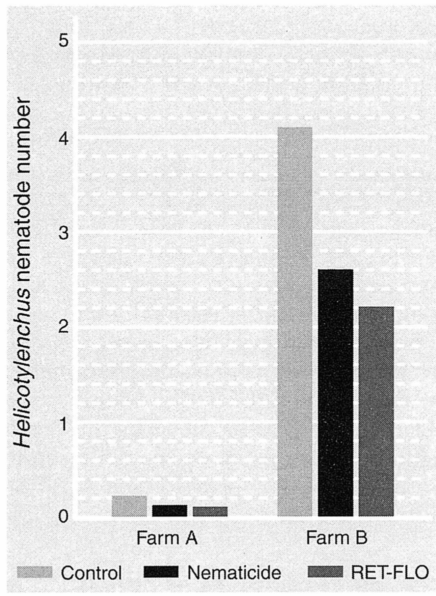

Figure 1.
Mean accumulated annual population of total root nematode *Helicotylenchus*, in farms A and B, according to control or treatments with nematicide or RET-FLO PX357 (biological product).

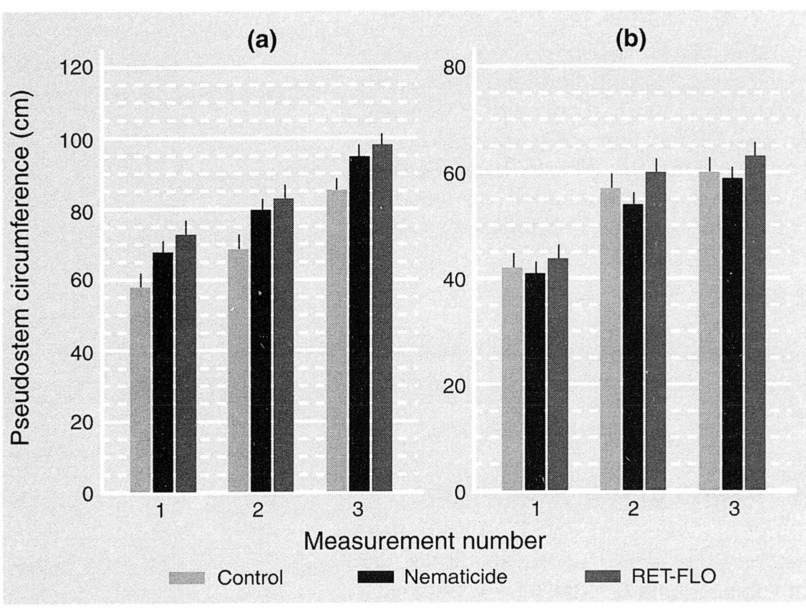

Figure 2.
Banana mean pseudostem circumference, according to control or treatments with nematicide or RET-FLO PX357 (biological product), in farm A (see a) and farm B (see b).

3.2. pseudostem circumference

The results are shown in *figure 2a* (farm A) and *figure 2b* (farm B).

In the first measurement, RET-FLO PX357 produces significantly larger mean pseudostem circumference (72.87 cm) than the other treatments in farm A, meanwhile, in farm B, the same trend is observed, but without statistical significance. In the second measurement, RET-FLO PX357 generates greater mean pseudostem circumference (83.47 cm) than the control in farm A, but it is not significantly different from that of the nematicide treatment. In farm B, the control and the nematicide treatments have significantly lower pseudostem circumferences than RET-FLO PX357 ($p = 0.002$). In the third measurement, pseudostem circumference means behave in a similar way than in the first measurement in farm A. RET-FLO PX357 treatment performed significantly better ($p = 0.005$) than nematicide and control treatments in farm B, where no difference between mean pseudostem circumference from nematicide and control treatments was observed.

These results show that the vigour of RET-FLO PX357 treated plants is greater than those which received nematicide or the control, because, in many regions and in most of the banana varieties, pseudostem circumference is highly correlated with the development of the plant and fruit weight [16–18].

3.3. time to flowering

Time to flowering is one of the factors that farmers consider to be most significant, because it is closely related to the time of cropping, and hastening this process will result in shorter time to harvest. The time to flowering was recorded in each of the marked plants during the second year of this trial (*figures 3a, 3b*).

RET-FLO PX357 application advanced flowering time in both farms. In farm A (*figure 3a*) on June 20, 13% of the plants of the RET-FLO PX357 treatment had flowered, whereas no plants had flowered in the other treatments. At the end of June, RET-FLO PX 357 showed the greatest difference (46% of flowered plants), compared to nematicide treatment (6.7% of flowered plants) and control (no flowering at all). These differences decreased with time but the plants treated with RET-FLO PX357 attained 100% flowering 10 to 20 d before the others.

In farm B (*figure 3b*), 60% of plants from RET-FLO PX357 treatment had flowered on October 20, in contrast with the 35% of plants from nematicide treatment and 40% from the control. On November 10, plants treated with RET-FLO PX357 reached 95% of flowering, whereas plants from the control and nematicide treatments only showed 55% and 65% flowering, respectively. From November 10 onwards, it was not possible to go on counting the flowered plants because Panama disease began to kill the plants that remained to flower in the control and nematicide treatments. In any case, these data confirm the advance in time to flowering caused by RET-FLO PX357 application observed in farm A.

3.4. plant nutrition

As a general pattern, nitrogen, phosphorus, calcium and manganese levels were higher in leaves than in roots. The contrary was observed for potassium and iron. Banana plants from farm A treated with RET-FLO PX357 showed higher root P level ($p = 0.006$), meanwhile leaf and root Ca content of the control was significantly lower in farm A (*tables I, II*). In both farms, the mean root K level was greater in the RET-FLO PX357 treatments, though differences were not significant. The same trend showed the Fe levels of leaves and roots from both farms. On the other hand, RET-FLO PX357 treatment reduced root Na concentrations in both farms, but without statistical significance, while this tendency appeared in leaves only in farm A. Reduction of Na uptake is positive for bananas, since it may produce harmful effects [19]. No clear behavior of N, Mn, Cu, and Zn was detected.

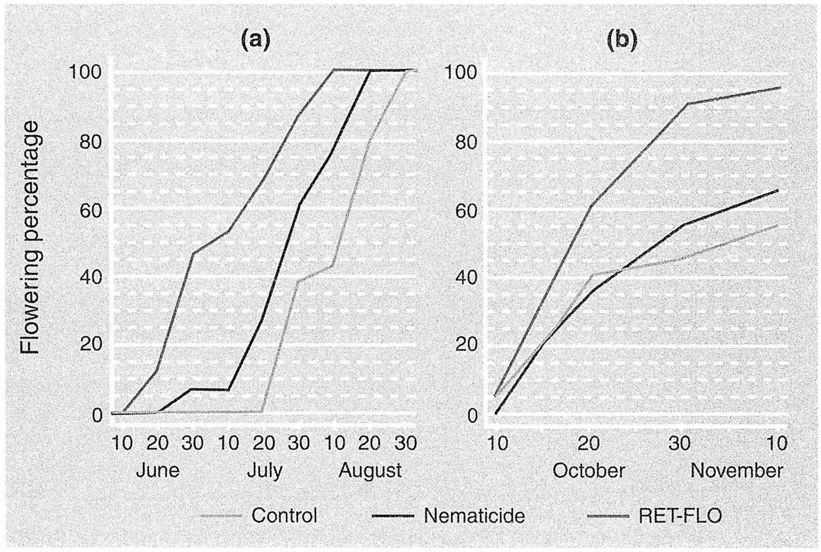

Figure 3.
The effect of the treatments with nematicide or RET-FLO PX357 (biological product), on banana flowering distribution (%) along the season, a) in farm A, b) in farm B.

3.5. soil analysis

RET-FLO PX357 treatments showed the lowest soil pH values (farm A only) (*table III*). $CaCO_3$ absence in the soils of farm A receiving RET-FLO PX357 may indicate a solubilization capacity of the microorganisms from this product. In farm B, there was no $CaCO_3$ at all due to better soil conditions. In both farms, RET-FLO PX357 reduced available Ca,

Table I.
Mean macro-nutrient content (%) in roots and leaves of bananas, according to the treatment (control, nematicide or RET-FLO 20 L·ha^{-1}) applicated to the plants.

(a) *Roots*

Treatment	N		P		K		Ca		Mg	
	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B
Control	1.40	1.73	0.16 a	0.13	6.71	4.90	0.56 a	0.90	0.54	0.63
Nematicide	1.33	1.62	0.15 a	0.12	5.51	5.39	0.75 b	0.96	0.64	0.68
RET-FLO PX357	1.63	1.66	0.25 b	0.14	7.26	5.69	0.72 b	0.92	0.62	0.71

(b) *Leaves*

Treatment	N		P		K		Ca		Mg	
	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B
Control	2.96	3.11	0.20	0.21	3.51	3.61	1.77 a	1.13	0.63	0.53
Nematicide	3.22	3.00	0.21	0.27	3.23	3.59	2.21 b	1.00	0.63	0.52
RET-FLO PX357	3.20	3.08	0.21	0.25	3.44	3.88	2.24 b	1.11	0.59	0.53

Values within the same column and plant part followed by different letters are significantly different at $p = 0.05$ level.

Table II.
Mean micro-nutrient content (ppm) in roots and leaves of bananas, according to the treatment (control, nematicide or RET-FLO 20 L·ha^{-1}) applicated to the plants.

(a) *Roots*

Treatment	Fe		Mn		Zn		Cu		Na	
	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B
Control	1 306	604	38	28	18	16	11	11	8 400	400
Nematicide	3 530	866	85	30	20	15	15	13	8 233	480
RET-FLO PX357	4 153	932	81	31	19	15	17	12	5 717	396

(b) *Leaves*

Treatment	Fe		Mn		Zn		Cu		Na	
	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B
Control	149 ab	184	173	86	20	20	10	12	810 ab	457
Nematicide	120 a	193	170	89	18	20	10	10	1 074 b	493
RET-FLO PX357	168 b	220	170	82	21	19	13	12	242 a	495

Values within the same column and plant part followed by different letters are significantly different at $p = 0.05$ level.

Table III.
Means of soil analysis in the trials located in farm A and farm B, according to the treatment (control, nematicide or RET-FLO) applicated to the plants.

Treatment	Location	pH	Organic matter (%)	P_2O_5 (ppm)	$CaCO_3$ (%)	Available cations (meq·100 g^{-1})				$dS.m^{-1}$
						Ca	Mg	Na	K	EC[1]
Control	Farm A	8.1	2.9	317	0.16	17.0	8.2	3.5	4.4	3.5
	Farm B	7.9	3.7	290	0.00	25.9	16.7	2.9	2.7	2.2
Nematicide	Farm A	7.5	3.2	274	0.10	15.6	8.3	2.7	4.5	2.9
	Farm B	7.6	5.6	295	0.00	25.4	13.1	2.5	3.7	1.9
RET-FLO PX357	Farm A	7.2	2.6	282	0.00	15.7	7.3	2.9	3.5	2.4
	Farm B	7.7	5.5	293	0.00	19.8	14.5	2.4	4.3	1.5

[1] EC = electrical conductivity.

Mg and Na content of the soils, and it also decreased the electrical conductivity (EC) levels by amounts varying from 0.7 to 1.1 $dS{\cdot}m^{-1}$ when compared to the control. The reduction of the available Na and EC values could be beneficial to banana plants which are susceptible to high salt contents in the soil. The other soil parameters did not show a clear behavior (*table III*).

3.6. hand number per bunch and finger number per hand

There was no difference among treatments in the number of hands per bunch in both farms, but RET-FLO PX357 produced higher mean finger number per hand compared to the the nematicide treatment in farm A (*table IV*). In farm B, though ANOVA analysis did not show differences among treatments, two-sample analysis revealed higher number of fingers per hand in plants treated with RET-FLO PX357 than those from the control ($t = 1.684$; $p = 0.102$).

As far as economical implications are concerned, it is more interesting to display data as percentage differences, because they better show the possible increase in benefits. In that respect, treatment with RET-FLO PX357 increased the number of fingers per hand by 6.6% (farm A) and by 8.1% (farm B) when compared with the control. The differences with the nematicide treatment are 7.7% and 6.1% in farms A and B, respectively.

4. conclusions

RET-FLO PX357 control of *Helicotylenchus* population means that it may exert the function of a nematicide without its drawbacks. Simultaneously it advances flowering time and augments the number of fingers per hand of the bunch. These are ecological and econo-

Table IV.
Average hand number per bunch and finger number per hand from the different treatments in farms A and B.

Treatment	Hand number per bunch		Finger number per hand	
	Farm A	Farm B	Farm A	Farm B
Control	12.3	11.4	26.7 ab	22.7
Nematicide	12.7	11.3	26.4 b	23.2
RET-FLO PX357	12.7	11.7	28.6 a	24.7

Values within the same column followed by different letters are significantly different at p = 0.07 level.

mical improvements which are important to take into account when deciding to carry out a sustainable agriculture.

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references

[1] Sikora R.A., Interrelationship between plant health promoting rhizobacteria, plant parasitic nematodes and soil microorganisms, Med. Fac. Landbouww. Rijksuniv. Gent. 53 (1988) 867–878.

[2] Kloepper J.W., Schroth M.N., Plant growth promoting rhizobacteria on radishes, in: Inra Angers (Ed.), Proceedings of the 4th International Conference on Plant Pathogenic Bacteria, Gibert-Clarey, Tours, France, vol. 2, 1978, pp. 879–882.

[3] Kloepper J.W., Zablotowicz R.M., Tipping E.M., Lifshitz R., Plant growth promotion mediated bacterial rhizosphere colonizers, in: D.L. Keister, P.B. Cregan (Eds.), Proceedings of the Beltsville Symposia in Agricultural Research, No 14, United States, 1991, pp. 315–326.

[4] Kloepper J.W., Plant growth-promoting rhizobacteria as biological control agents of soilborne diseases, in: The Biological Control of Plant Diseases, FFTC Book Series, No 42, United States, 1991, pp. 142–152.

[5] Lifshitz R., Simonson C., Scher F.M., Kloepper J.W., Rodrick-Semple C., Zaleska I., Effect of rhizobacteria on the severity of *Phytophthora* root-rot of soybean, Can. J. Plant Pathol. 8 (1986) 102–106.

[6] Schippers B., Biological control of pathogens with rhizobacteria, Phil. Trans. R. Soc. Long. B. 318 (1988) 283–292.

[7] Weller D.M., Biological control of soilborne plant pathogens in the rhizosphere with bacteria, Annu. Rev. Phytopathol. 26 (1988) 379–407.

[8] Stirling G.R., Biological control of plant parasitic nematodes, CAB International, Wallinford, United Kingdom,1991.

[9] Rodriguez R., Los nematodos de la platanera en Canarias (1963-1984), Xoba, Monografía 4, Caja Insular de Ahorros de Canarias, Las Palmas, Spain, 1990.

[10] Cottenie A., Soil and plant testing as a basis of fertilizer recommendation, FAO Soils Bulletin 38/2, Rome, Italy, 1980.

[11] Chapman H.D., Pratt P.F., Methods of analysis for soils, plants and waters, University of California, Division of Agricultural Sciences, United States,1961.

[12] Drouineau D., Gouny P., La mesure du pouvoir chlorosant des sols calcaires, in: Extraits du Congrès international des engrais chimiques, octobre 1951, Rome, Italie, 1951.

[13] Comisión de métodos analíticos del instituto de edafología y agrobiologia “José M. Albareda”, Determinaciones analíticas en suelos. Normalización de métodos, pH, M.O. y nitrógeno, Anal Edaf. Agrob. 32 (1973) 1153–1172.

[14] Deullin R., Monnet J., Mesure de la plénitude de la banane, Fruits 15 (1960) 205–221.

[15] Fernandez M., Robles J., Alvarez C.E., Diaz A., Relación entre la composición mineral y el desarrollo de los frutos del plátano, Anal. Edaf. Agrob. 39 (1980) 2199–2213.

[16] Lahav E., Effect of different amounts of potassium on growth of the banana, Trop. Agric. 49 (1972) 321–335.

[17] Fernandez E., García V., Étude sur la nutrition du bananier aux îles Canaries. I. Effets de la nutrition azotée sur la circonférence du pseudo-tronc, Fruits 27 (1972) 509–512.

[18] Diaz A., Estudio de la relación suelo-planta en la fertilidad y nutrición potásica del plátano, Tesina, University of La Laguna, Spain, 1973.

[19] Lahav E., Fertilising for high yield, Banana, International Potash Institute, Bulletin 7, Switzerland, 1983.

Mejora del desarrollo y nutrición del banano mediante la aplicación de una suspensión bacteriana.

Resumen — Introducción. Se conocen los efectos beneficiosos que tienen las bacterias en el suelo, pero los de preparaciones bacterianas comerciales en el crecimiento de las plantas no se conocen aún. Se procedió pues, al estudio y la cuantificación del efecto del RET-FLO PX357, una mezcla comercial constituida de microorganismos en suspensión en un líquido, en los bananales. **Material y métodos**. La aplicación de un cultivo concentrado de este producto ha sido probada, durante dos años, en Tenerife (Islas Canarias). Se efectuaron tres tratamientos: uno testigo, una aplicación con Fenamiphos (nematicida) de 15 $L \cdot ha^{-1}$ y una con RET-FLO PX357 de $20 \cdot L\ ha^{-1}$. Se determinó el número de nematodos parásitos del sistema radical así como el diámetro del seudotallo, el tiempo de floración y el número de manos y dedos por racimo. Se analizó la composición de las raíces, de las hojas y del suelo. **Resultados y discusión**. El RET-FLO PX357 provocó la disminución de los nematodos del género *Helicotylenchus* y, en algunos casos, el aumento del contenido de P, Ca o Fe en las raíces y hojas; provocó la reducción de contenido de Na disponible en el suelo y la disminución de la conductancia eléctrica; incrementó el diámetro del seudotallo y el número de dedos por mano a la vez que reducía el tiempo de floración. **Conclusión**. El producto probado presenta una serie de ventajas ecológicas y económicas con las que habrá que contar para la puesta en marcha de una agricultura sostenible. (© Elsevier, Paris.)

Canarias / *Musa* / nutrición de las plantas / bacteria fijadora del nitrógeno / nematodos