# Estimation of the population density of arbuscular mycorrhizal fungi in soils used for intensive banana cultivation in Martinique

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## Estimation of the population density of arbuscular mycorrhizal fungi in soils used for intensive banana cultivation in Martinique.

**Abstract — Introduction**. In Martinique, banana is grown mostly in intensive cropping systems. This study was undertaken to determine the mycorrhizal status of soils used for intensive banana cultivation and to examine the relationship of arbuscular mycorrhizal (AM) fungi population densities to the edapho-climatic characterisitics of the fields sampled. **Materials and methods**. Banana fields were sampled in ten locations represented by one of five different soil types. In each location, a composite soil sample was constituted and analysed to determine texture, pH, percentage of organic matter, cationic exchange capacity (CEC) and N and P content. The altitude and rainfall of each location were also recorded. The population density of AM fungi in each soil sampled was estimated by the most probable number (MPN) method. The relationship of population density to soil characteristics was assessed using Pearson correlation analysis. **Results**. AM fungi were present in all the soils studied. Mean propagule estimates per 100 g dry soil varied between 1.5 and 102. With the exception of the CEC, no correlations were observed between the chemical properties of the soils studied, rainfall and the AM fungi population density. **Discussion**. Our results indicate that all the soils studied contain AM fungi. The populations size are, however, low in comparison to the values generally reported in cultivated soils. **Conclusion**. A thorough research should be conducted to elucidate the relationships existing between the edapho-climatic characteristics of the fields sampled and the AM fungi population densities recorded. Field studies based on a multivariate analysis could be a possible way for identifying the factors which account for the observed variations in AM fungal propagules density. (© Elsevier, Paris)

**Martinique / *Musa acuminata* / arbuscular mycorrhizae / population density / soil testing**

## Évaluation de la densité de population des champignons mycorhiziens à arbuscules (chMA) dans des sols utilisés pour la culture intensive de bananiers en Martinique.

**Résumé — Introduction**. En Martinique, le bananier est principalement exploité en système de production intensif. Cette étude a été entreprise pour déterminer le statut mycorhizien des sols exploités par de tels systèmes de culture du bananier et pour examiner les relations existant entre les populations de chMA et les caractéristiques édapho-climatiques des plantations prospectées. **Matériel et méthodes**. Des bananeraies ont été échantillonnées en dix endroits représentatifs de cinq types de sol différents. À chaque site, un échantillon de sol composite a été constitué et analysé pour déterminer sa texture, son pH, le taux de matière organique, la capacité d'échange cationique (CEC) et les teneurs en azote et phosphore. L'altitude et les précipitations de chacun de ces sites ont également été relevées. La densité de population des chMA de chacun des sols échantillonnés a été évaluée par la méthode du nombre le plus probable. Le rapport entre la densité de population et les caractéristiques des sols a été évalué à partir d'une analyse des corrélations de Pearson. **Résultats**. Des chMA ont été trouvés dans tous les sols étudiés. Le nombre moyen de propagules par 100 g de sol sec varie de 1,5 à 102. À l'exception de la CEC, aucune corrélation n'est apparue entre les caractéristiques chimiques des sols étudiés, les précipitations et la densité des chMA. **Discussion**. Tous les sols étudiés au cours de ces travaux contiennent donc des chMA. Leurs populations sont cependant peu élevées par rapport aux valeurs généralement obtenues en sols cultivés. **Conclusion**. Une étude approfondie devrait être menée afin d'expliquer les rapports existant entre les caractéristiques édapho-climatiques des parcelles échantillonnées et les densités de populations des chMA mesurées. Des études in situ basées sur des analyses multivariables pourraient permettre d'identifier les facteurs impliqués dans les variations de densité des propagules des chMA. (© Elsevier, Paris)

**Martinique / *Musa acuminata* / mycorhize arbusculaire / densité de population / analyse du sol**

## 1. introduction

Arbuscular mycorrhizal (AM) fungi are root symbiotic micro-organisms that colonize nearly all plant taxa and soils. Their well known benefits on plant mineral nutrition and growth has led to attempts to introduce or manage them for increased crop production [1]. In the field, the AM fungi populations may contribute to nutrient acquisition and growth with consequent increase in yield [1]. Mc Gonigle [2] evaluated 78 field trials and observed that inoculation with AM fungi resulted in an average yield increase of 37%. Ross and Harper [3] obtained important yield increase of soybean inoculated with AM fungi in sterilised soils. Saif and Khan [4] observed also an important growth increase in barley plantlets pre-inoculated with an AM fungi and transplanted in the field in comparison with non-inoculated plants. They attributed this result both to the pre-inoculation of plantlets and to the low indigenous AM fungal population (< 1 spore·g$^{-1}$ of soil). In case of low native AM fungal populations, inoculation of selected species or enhancement of the natural population, by adequate soil management, may thus be considered. However, as mass-production of AM fungi inoculum for large-scale use under field conditions remains difficult, management of the indigenous population appears an appropriate option at this moment [1].

Banana plants form associations with AM fungi [5]. Results to date demonstrated both their presence in intensive and extensive cropping systems [6, 7] and their beneficial effects on plant growth and mineral nutrition [8–10] as well as protection against root pathogens [11]. Notwithstanding these benefits, little information is available about the mycorrhizal status of soils used for intensive banana cultivation. Decades of intensive cropping through repeated applications of fertilisers and pesticides may have reduce the mycorrhizal population of these soils. Therefore, disturbance resulting from management practices and environmental stresses may have altered the viability and diversity of the microbial communities in the soil [12]. Consequently, assessing the potential benefits of the existing population of AM fungi on banana cultivation system first requires a determination of the AM fungi population size of the soils concerned.

This study was designed to determine the mycorrhizal population density of various soils used for intensive banana cultivation in Martinique and to examine the relationship of population density values with location characteristics, including rainfall, soil texture, pH and soil nutrient status.

## 2. materials and methods

### 2.1. study locations and soils

The study was conducted in Martinique, an island that is part of the volcanic Caribbean arc situated to the north of the Venezuelan tip of South America. Banana fields were sampled from a north to south transect on the Atlantic side of the island to encompass either rainfall extremes and a variety of soil types (*table I*). Ten fields were selected on one of five different soil types (*table I*), all of which being cultivated for banana during the last decade. The following soil types were considered: Udivitrand, Hapludand, Humitropept, Kandiudult and Ustert [13] which correspond respectively to weakly developed soil derived from ash and pumice, andosol derived from ash and pumice, andic brown soil, ferrisol, and vertisol (*sol peu évolué dérivé de cendres et ponces, andosols dérivés de cendres et ponces, sol brun andique, ferrisol* and *vertisol*) identified in the regional soil classification proposed by Colmet-Daage and Lagache [14]. Rainfall data were taken from the weather stations closest to each sampling site and were approximate means of values recorded in the last 10 years (*table I*). Soils were collected in January 1993. In each banana field, a composite soil sample was constituted as follows: at random points within the field, approximates of 2 kg soil containing fibrous roots were collected in the root zone of flowering banana plants to a depth of 25 cm. The soil samples were then thoroughly mixed and subsequently air-dried and passed through a 2 mm sieve.

**Table I.**
Soil chemical analysis and average rainfall of the study fields.

| Field location | Soil type | Altitude (m) | Rainfall ($mm \cdot year^{-1}$) | Soil texture | Organic matter (%) | Nitrogen ($\mu g \cdot g^{-1}$) | Phosphorus ($\mu g \cdot g^{-1}$) | pH ($H_2O$) | Cationic exchange capacity ($Cmol \cdot kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Macouba | Udivitrand | < 120 | 2 170 | sandy | 2.33 | 2 380 | 280 | 5.4 | 15.6 |
| Gradisse | Udivitrand | < 120 | 2 440 | sandy | 2.85 | 2 730 | 170 | 5.2 | 15.3 |
| Chalvet | Udivitrand | < 120 | 2 260 | sandy | 1.29 | 1 260 | 140 | 5.5 | 9.0 |
| Longchamp | Hapludand | > 300 | 4 590 | sandy | 6.10 | 6 060 | 220 | 5.3 | 25.2 |
| Balisier | Hapludand | > 300 | 3 600 | sandy | 2.89 | 2 810 | 300 | 4.7 | 12.6 |
| Bellevue | Humitropept | 170 – 200 | 2 700 | sandy loamy | 1.39 | 1 570 | 30 | 5.1 | 19.8 |
| Bellevue | Humitropept | 170 – 200 | 2 700 | sandy loamy | 2.66 | 3 050 | 10 | 5.1 | 20.3 |
| St-Michel | Humitropept | 170 – 200 | 2 700 | sandy loamy | 2.91 | 3 340 | 30 | 4.5 | 25.8 |
| Grand Fond | Kandiudult | < 100 | 1 850 | clay | 1.15 | 1 240 | 40 | 4.2 | 11.6 |
| Simon | Ustert | < 100 | 1 480 | clay | 2.82 | 2 910 | 10 | 6.1 | 37.6 |

The parent material of the soil types are respectively recent volcanic ash and pumice for the Udivitrand and Hapludant, andesitic ash and tuff for the Humitropept and volcanic alluvial deposit for the Kandiudult and Ustert. Annual rainfall values were approximate means of values recorded in the last 10 years.

A portion of this composite sample was used to determine textural and chemical properties (*table I*). The soil chemical properties were determined according to the Afnor [15] standard methods.

## 2.2. assessment of mycorrhizal population density

The population density of AM fungi in each soil was estimated by the most probable number (MPN) method [16]. This method, also known as the "dilution method", was developed for estimating the population size of micro-organisms on the basis of the highest dilution at which growth could be observed. Adapted to AM fungi, this method involves the determination of the presence or absence of the fungi in the root system of a susceptible plantlet grown on successive dilutions of the soil to be tested. A 2-fold dilution series was made of each soil by mixing the original soil with a sterilised (Gamma Ray, 10 kGy) sample of the same soil. Twelve successive dilutions were prepared with three replicates for each dilution. Plastic multipots (Somapo-Sopirec, Diemeringen, France) were filled with 40 g of each soil dilution, layered between 10 g of an autoclaved (121 °C for two separate 1 h periods) calcined clay, Oil Dri US-special Typ/IIIR (Oil Dri Company, Chicago) [17]. Each multipot contained the dilution series for one soil. Control pots, i.e., containing only sterilised soil (Gamma Ray, 10 kGy) were also included in the experiment to check that the soil sterilisation procedure worked.

One seed of leek (*Allium porrum* L., var. Bleu de Solaise) was germinated in the upper layer of the Oil Dri of each pot. The pots were then placed in a growth chamber (24/18 °C day/night; 16 h day; 112 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity; 80% relative humidity). Plants were watered every three days with deionized water. No nutrient solution was added during the experiment. After 6 weeks, the entire root system from each plant was collected and stained with trypan blue [18]. Each root system was mounted on microscope slides and scored for the presence or absence of AM fungi colonization. A single entry point was sufficient to consider colonization as being present.

## 2.3. statistical analysis

The numbers of infective AM fungi propagules, in the 10 fields sampled, for the

2-fold dilution series with three replicates per dilution were calculated using table VIII2 of Fischer and Yates [19] and were statistically analysed by calculating the 95% confidence limits using Cochran's table 1 [20]. In addition, analysis of variance (Anova) was achieved to compare the MPN values among the five soil types. Correlations between MPN values, rainfall and soil chemical properties were determined by Pearson's correlation coefficients. All the statistical data analysis were performed with the SAS V6.12 system.

## 3. results

The AM fungal population density recorded in the Ustert was significantly higher in comparison to the propagule number scored in the Udivitrand, Hapludand and Humitropept ($p < 0.05$) (*table II*). No significant differences were observed between the three latter soil types (*table II*), although, within the Udivitrand and Hapludand, AM fungal population density were found to be significantly ($p < 0.05$) lower in Gradisse and in Balisier respectively in comparison with the other locations associated with these two soil types (*table II*). The Kandiudult had intermediate propagule density as compared to the Udivitrand, Hapludand and Humitropept, and the Ustert respectively (*table II*). No root colonization was further observed in the leek plants grown in the control pots demonstrating that the gamma irradiation disinfection process was efficient.

The selected locations refer to distinct soil and climate conditions. Rainfall was highest in the Udivitrand, Hapludand and Humitropept with values exceeding 2 000 mm·year$^{-1}$ at low altitude (< 120 m) and exceeding 3 600 mm·year$^{-1}$ above 30 m (*table I*). In the two other soils (Ustert and Kandiudult), rainfall values approximated 1 480 and 1 850 mm·year$^{-1}$ (*table I*) respectively. No significant correlation was observed between mean annual rainfall and AM fungal population density ($r = -0.51$; $p < 0.05$). A significant positive correlation ($r = 0.66$; $P < 0.05$) was observed between the AM fungal population density and the cationic exchange capacity (CEC), whereas no significant relationship was stated with the other soil characteristics, although AM fungi population density was the highest in the soil having the lowest Olsen P content.

**Table II.**
Mean propagule population density in field sites cropped to banana.

| Field location | Soil type | Propagules·100 g$^{-1}$ | Confidence limits (95%) |
|---|---|---|---|
| Macouba | Udivitrand[a] | 12.6[cde] | 5.6–28.1 |
| Gradisse | Udivitrand[a] | 1.5[ab] | 0.7–3.3 |
| Chalvet | Udivitrand[a] | 15.9[cde] | 7.1–35.5 |
| Longchamp | Hapludand[a] | 15.9[cde] | 7.1–35.5 |
| Balisier | Hapludand[a] | 2.1[ab] | 0.9–4.7 |
| Bellevue | Humitropept[a] | 6.3[abcd] | 2.8–14.0 |
| Bellevue | Humitropept[a] | 7.9[bcd] | 3.5–17.6 |
| St Michel | Humitropept[a] | 4.9[abc] | 2.2–10.9 |
| Grand Fond | Kandiudult[ab] | 31.8[def] | 14.3–70.9 |
| Simon | Ustert[b] | 102.0[fg] | 45.7–227.4 |

The 95% confidence limits were calculated according to Cochran's Table 1 [20].
Values of mean propagules per 100 g soil followed by the same letter are not significantly different ($p < 0.05$).
Soil types were compared using variance analysis (ANOVA). The values within this column followed by the same letter are not significantly different ($p < 0.05$).

## 4. discussion

Our results indicate that, although being intensively cultivated in the last decade, all the soils studied contain AM fungi. However, as measured by the leek bioassay, the AM fungal population size varied among the selected soils. The AM fungal population densities appeared lower on the recent sandy soils derived from volcanic ash and pumice (Udivitrand and Hapludand) and on the soils derived from andesitic ash and tuffs (Humitropept) in comparison to the population densities recorded in the clayey Kandiudult and Ustert. We assume that the significant differences observed between this latter and the Udivitrand, Hapludand and Humitropept soils could be related to soil textural properties as already demonstrated by Cortes [21] and Sieverding [22]. These authors observed that heavy tropical clayey soils could be higher in spore density than sandy soils. Further experiments with an increased number of banana fields sampled on clayey and sandy soils should, however, be conducted to ascertain this observation.

AM fungal population density was only significantly related to the CEC and not to any of the other chemical characteristics considered in the study. However, the lack of significant correlations between these elements and the AM fungal population density may partly have been due to the restricted number of fields sampled. For the same reason, the relationship observed between the CEC and the AM fungal population density may have been an artifact. The relationships between AM fungi population density and chemical properties of the soils may, furthermore, be extremely variable as discussed by Abbott and Robson [23]. High AM fungi population densities have been observed over a wide range of soil pH [24] and soil phosphate level [25]. Many complex interactions between mineral elements may also occur in soil, making it not surprising that few marked correlations between soil chemical properties and AM fungi population density were observed in our experiment.

It appears that the AM fungi population densities observed in our study are low in comparison with the values generally reported in uncultivated and cultivated soils, both in tropical and temperate ecosystems. Fischer et al [26] recorded values up to 63 propagules per 100 g soil in tropical pasture. Powell [27] scored values between 600 and 1 900 propagules under temperate pasture soils and observed in the same soils but eroded and without vegetation, average AM fungi population densities varying from 20 to 100 propagules per 100 g soil. In cultivated soils, population densities are frequently observed to be lower in comparison with uncultivated soils with the exception of bare soils or soils inhospitable to plant growth [1]. Values observed in our experiment were close to those reported by Gianinazzi et al. [24] in cultivated soils from Burgundy but lower in comparison to agricultural sandy and loamy soils from India [28] and to silt-loamy soils cultivated for Fescue in western Kentucky [29]. Results obtained on soils under citrus in Dominica, Grenada and St Lucia, three neighbour islands of Martinique, showed also a well established mycorrhiza colonization in citrus roots [30] but no propagule densities were recorded in these soils making it difficult to determine some similarities with the values recorded in our experiment.

## 5. conclusion

The results reported here outline, for the first time, the population density of AM fungi in different soils cropped for intensive banana. The MPN technique appears a valuable tool for assessing mycorrhizal populations. However, even though the 2-fold dilution series appear appropriate in regard to the generally low AM propagule density recorded in the banana fields, uncertainty remains concerning the effects of the edaphoclimatic conditions on population density. The lack of correlations may be partly due to the restricted number of fields sampled. A thorough and systematic survey involving a large number of banana fields would probably strengthen the results obtained in this study.

In Martinique, agricultural practices include high fertilisation, chemical control of plant pathogens and weed control by

means of herbicides. These treatments are known to influence both AM fungi population density and diversity and the extent to which the fungi colonise plant roots [1]. Field studies based on a multivariate analysis could represent another possible way of identifying biotic and abiotic factors as well as agricultural practices, which account for the observed variations in AM fungal propagules density. In addition to a multivariate analysis approach, mycorrhizal populations should be assessed in other ways, such as spore density and root colonization. As stated by An et al. [29] wet sieving of spores from field soils and MPN bioassay of propagules may produce different and useful information.

The combination of these different approaches may give a broad overview of the situations existing in soils used for intensive banana cultivation in Martinique and could be a prerequisite to elucidate the complicated soil – plant – AM fungi – agricultural practices interactions encountered in these situations.

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## Evaluación de la densidad de poblaciones de hongos micorrizas arbusculares (hMA) en suelos utilizados para el cultivo intensivo de plátanos en Martinica.

**Resumen — Introducción**. En Martinica, el plátano se explota sobre todo en sistema de producción intensivo. Se emprendió este estudio para determinar el estatuto micorriziano de los suelos explotados por semejantes sistemas de cultivo del plátano y para examinar las relaciones que existen entre las poblaciones de hMA y las características edafo climáticas de las plantaciones prospectadas. **Material y métodos**. Se muestrearon platanales en diez lugares representativos de cinco tipos de suelo diferentes. En cada sitio, una muestra de suelo compuesto fue constituida y analizada para determinar su textura, su pH, la tasa de materia orgánica, la capacidad de intercambio catiónica (CEC) y los contenidos de nitrógeno y fósforo. También se registraron la altitud y las precipitaciones de cada uno de estos sitios. Se evaluó la densidad de población de hMA de cada uno de los suelos muestreados mediante el método del número más probable. La relación entre la densidad de población y las características de los suelos fue evaluada a partir de un análisis de las correlaciones de Pearson. **Resultados**. Se encontraron hMA en todos los suelos estudiados. El número promedio de propagulos por 100 g de suelo seco varió de 1,5 a 102. Con excepción de la CEC, ninguna correlación apareció entre las características químicas de los suelos estudiados, las precipitaciones y la densidad de hMA. **Discusión**. Por lo tanto, los suelos contienen hMA. Sus poblaciones son no obstante bajas respecto a los valores generalmente logrados en suelos cultivados. **Conclusión**. Se debería de llevar a cabo un estudio indagado para explicar las relaciones que existen entre las características edafo climáticas de las parcelas muestreadas y las densidades de poblaciones de hMA medidas. Estudios in situ basados en análisis multivariables podrían permitir identificar los factores implicados en las variaciones de densidad de los propagulos del hongo. (© Elsevier, Paris)

**Martinica / *Musa acuminata* /micorrizas arbusculares / densidao de la población / análisis del suelo**