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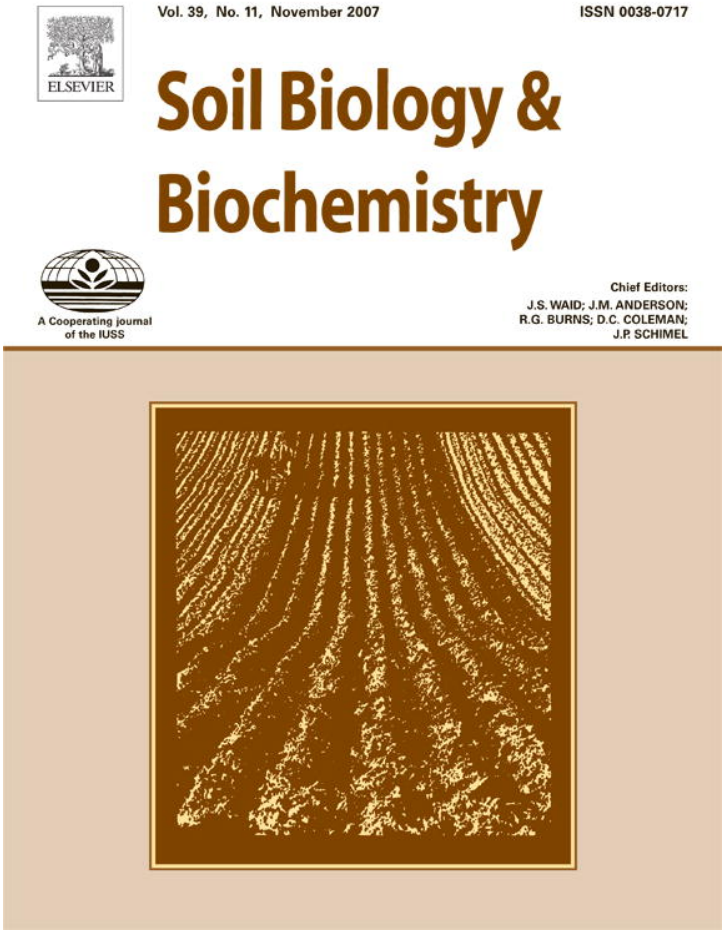


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# Variation of *Pythium*-induced cocoyam root rot severity in response to soil type

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**Abstract**

In Cameroon, andosols are suspected to be suppressive to cocoyam (*Xanthosoma sagittifolium*) root rot disease (CRRD) caused by the Oomycete pathogen *Pythium myriotylum*. To determine factors involved in disease suppressiveness, andosols were studied in comparison to ferralsols known to be disease-conducive. Soil samples were collected from six sites of which three were in andosols around Mount Cameroon (Boteva, Njonji, and Ekona) and the three others in ferralsols (Bakoa, Lapkwang, and Nko'o canane). Greenhouse plant experiments were used to assess soil suppressiveness. Soils were artificially infested with two levels of *P. myriotylum* inoculum (100 and 300 mycelia strands $g^{-1}$ soil) prior to planting cocoyam. Disease severity was significantly higher in ferralsols than in andosols. Andosols partly lost their suppressiveness as a result of autoclaving and could recover suppressiveness following recolonisation by their original microflora. Soil microbial groups implicated in the disease suppression were investigated by assessing the effect of fungicide, bactericide, and pasteurisation on andosol suppressiveness. Andosols suppressiveness was significantly reduced following pasteurisation and treatment with fungicide and bactericide. The possible influence of microbial biomass on andosol suppressiveness was investigated by comparing microbial populations of suppressive andosols to those in andosols that had lost suppressiveness. A comparative analysis of suppressive and conducive soil properties was performed to identify soil variables, which may contribute to soil suppressiveness. Soil chemical analysis results showed that organic matter content was higher in andosols than in ferralsols. In addition, the content of mineral nutrients such as Ca, K, Mg and N, was higher in andosols than in ferralsols. These soil variables negatively correlated with disease severity. By contrast, sand and clay, which were higher in ferralsols than in andosols, were positively related to disease severity. This study has confirmed the suppressive nature of andosols from Mount Cameroon to CRRD. The results suggest that high organic matter content is likely mediating *P. myriotylum* suppression in andosols by improving soil structure, increasing soil nutrient content and microbial biomass, and sustaining microbial activity.
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*Keywords:* Andosols; Ferralsols; *Pythium myriotylum*; Root rot disease; Suppressive soils; *Xanthosoma sagittifolium*

## 1. Introduction

Cocoyam (*Xanthosoma sagittifolium* (L) Schott) is an herbaceous monocotyledonous plant belonging to the Araceae family (Coursey, 1968; Purseglove, 1972). It is typically a tropical rainforest plant requiring warm weather (20 to 35 °C), high rainfall (1400–2000 mm) and adequate soil moisture for optimal growth and tuber yield. Light texture and well-drained soils are best for cocoyam growth but this crop can tolerate heavy texture soils with pH values ranging from 5.5 to 6.5 (Onwueme, 1978). The importance of cocoyam mostly relies on its edible tubers and leaves, which are staple food for more than 400 millions people in the tropics and subtropics (Onokpise et al., 1999; Reyes Castro et al., 2005). In the African continent, cocoyam is widely cultivated in West and Central Africa with Cameroon, Ghana, Nigeria, Gabon, and Equatorial Guinea as the major producing countries (Knipscheer and Wilson, 1981).

Unfortunately, this valuable crop is highly susceptible to the Oomycete pathogen *Pythium myriotylum* (Nzietchueng,

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1983; Pacumbaba et al., 1992; Tambong et al., 1999; Perneel et al., 2006). *P. myriotylum* can attack cocoyam at various growth stages. Early infection of cocoyam (at the emerging root stage) by *P. myriotylum* induces stunting, while late infection (at 5 to 6 months after planting) reduces the number of feeder roots, causing chlorosis and poor yields. In Cameroon, yield losses in some plantations due to this soil-borne disease were estimated as high as 90% (Nzietchueng, 1983).

*P. myriotylum* is difficult to control in cocoyam using classical control methods such as chemicals (Nzietchueng, 1983). Additionally, no resistant cocoyam variety has been developed yet. A reliable short-term disease control strategy that can reduce *Pythium*-induced cocoyam yield losses at an economically acceptable level and provide sustainable production of this vital crop is of urgent need.

Field observations seem to indicate that the two main soil types ferralsols and andosols (FAO soil classification system) used for cocoyam production in Cameroon interact differently with the cocoyam pathogen. Ferralsols (also referred to as ferallitic soils) are the predominant soil type. They are weathered soils composed mainly of kaolinite clay, sesquioxides and gibbsite. Ferralsols have a low nutrient content, and are characterized by Al and Mn toxicity, high P-fixation, and low water retention. In contrast, andosols are relatively fertile soils developed from volcanic ash. The clay fraction consists of allophane. They have a high nutrient content, Mn toxicity, high P-fixation, medium water and nutrient retention (Müller-Sämann and Kotschi, 1994). In Cameroon, cocoyam can continuously be grown on the andosols for about five years without noticeable disease outbreaks if the plot is from virgin land, whereas in all other surrounding ferralsols, replanting of cocoyam results very often in high disease severity. Andosols from Mount Cameroon area become progressively conducive as from about five years of continued cropping of cocoyam because of land use intensity.

This research was undertaken based on the hypothesis that andosol suppressiveness is due to identifiable soil variables. Knowledge of such variables may be helpful for developing a sustainable management of andosol suppressiveness, and to generate a satisfactory level of CRRD suppression in ferralsols.

The objectives of this study were to confirm suppressiveness of andosols from Mount Cameroon to CRRD and to identify essential factors that induce soil suppressiveness.

## 2. Materials and methods

### 2.1. Soil types and soil sampling

Soil samples were collected from six sites: three in andosols around Mount Cameroon (Boteva, Njonji, and Ekona) and three in ferralsols (Bakoa, Lapkwang, and Nko'o canane) (Table 1). Soil cores (20 cm depth) were taken randomly at diverse points of virgin plots at each site and were composited. A subsample of 500 g, made from composite soil sample at each site, was separately transferred into clean plastic bags and taken to the laboratory for physical and chemical analysis. Similarly, soil cores (15 cm depth) were taken at diverse points of virgin plots in Boteva and Njonji and within *Pythium*-infected cocoyam plot in Ekona, and separately composited. Then a subsample of 500 g of each composite soil sample was separately stored in sterile plastic bags and refrigerated for further microbial analysis.

### 2.2. Plant material

The white cocoyam type, which is highly susceptible to *P. myriotylum* attack, (Tambong et al., 1999) was micropropagated to produce enough cocoyam plantlets (Zok et al., 1997). These cocoyam plantlets were then acclimatised at 25–26 °C in the greenhouse for eight weeks before use in the various plant experiments.

### 2.3. Culture of the pathogen, preparation of inoculum and inoculation procedure

*P. myriotylum* was cultured and inoculated on cocoyam as described by Tambong et al. (1999). Briefly, *P. myriotylum*

Table 1
Origin of soils used in this study

| Soil type | Agro-ecological zone[a] | Location | Altitude (m) | Geographical coordinates | |
|---|---|---|---|---|---|
| | | | | N | E |
| Andosols | IV | Boteva | 808 | 4°13′351″ | 9°17′047″ |
| | IV | Njonji | 50 | 4°10′506″ | 9°00′117″ |
| | IV | Ekona | 400 | 4°15′225″ | 9°20′100″ |
| Ferralsols | V | Bakoa | 458 | 4°33′775″ | 11°10′453″ |
| | V | Lapkwang | 752 | 4°46′445″ | 11°08′884″ |
| | V | Nko'o canane | 671 | 3°45′864″ | 12°13′351″ |

[a]Zone IV = evergreen humid forest, monomodal rainfall pattern, 3000 mm average annual rainfall; Zone V = semi-deciduous forest and transitional savannah, bimodal rainfall pattern and an annual rainfall of 1600 mm.

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isolates CMR5, which was isolated from an infested cocoyam field in Cameroon and tested for aggressiveness to cocoyam (Perneel et al., 2006), was grown on potato dextrose agar (PDA) at 28 °C for 5 days. *Pythium* cultures (mycelium and agar) were blended in sterile distilled water (a 90 mm plate 200 $ml^{-1}$) for 3 min to produce a stock suspension of mycelia strands. Required concentrations (in mycelia strands $ml^{-1}$) in the various experiments were microscopically determined using a haemocytometer. Inoculation consisted in manually mixing a given volume of mycelia suspension with soil prior to planting cocoyam.

### 2.4. Greenhouse disease suppression assays

Virgin soils from Boteva and Njonji (andosols), and from Bakoa, Lapkwang, and Nko'o canane (ferralsols), were artificially infested with two levels of *Pythium* inoculum (100 and 300 mycelia strands $g^{-1}$ soil). Infested soils were dispensed into 1000 ml plastic pots. Each treatment combination (soil type × inoculum level) was applied to 5 plants (1 plant per pot). Pots were placed on the top of a greenhouse bench in a completely randomised design arrangement at 26–28 °C. Pots were watered when necessary to provide sufficient soil moisture. Disease severity was determined the tenth day after inoculation based on a 0–4 rating scale defined as follows: 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of foliage with yellowing. From these data a disease index was calculated as follows:

$$\text{Disease index} = (1 \times n1 + 2 \times n2 + 3 \times n3 + 4 \times n4)/(4 \times N) \times 100,$$

where $N$: total number of plants; $n1$: number of plants with scale 1; $n2$: number of plants with scale 2; $n3$: number of plants with scale 3; $n4$: number of plants with scale 4. The experiment was conducted three times and for each repetition soil samples were re-collected at the various sites.

### 2.5. Determination of the nature of CRRD suppression

To test whether suppressiveness was microbial in nature, a greenhouse experiment involving autoclaved and non-autoclaved soils from Boteva and Njonji, and autoclaved soils treated with soil extract from their corresponding non-autoclaved soils were artificially infested with a suspension of mycelia strands (300 propagules $g^{-1}$ soil). Treatment with soil extract was prepared as follows: 50 g of soil was suspended in 250 ml sterile water, stirred for 2 min, filtered using sterile cheesecloth, and then thoroughly mixed with 500 ml of sterile soil. The mixture was used to fill a single pot and incubated for 24 h before use. Inoculation of soils with *P. myriotylum* and evaluation of disease were conducted as previously described with 5 plants per treatment. The experiment was carried out three times.

### 2.6. Bioassays for identification of microbial groups responsible for soil suppressiveness

To identify groups of micro-organisms which might be responsible for disease suppression, a greenhouse plant experiment was carried out with the following treatments: (i) Boteva and Njonji soil untreated (controls); (ii) soils treated with benlate (fungicide; active ingredient is benomyl) at 2.5 mg $g^{-1}$ soil and incubated for 24 h; (iii) soils treated with streptomycin sulphate + penicillin G at 2.5 mg $g^{-1}$ soil and incubated for 24 h; and (iv) soils pasteurised at 60 °C for 5 days. Both fungicide (benlate) and bactericide (streptomycin sulphate and penicillin G) were first suspended in sterile distilled water and applied as a drench to the soil. Then 1000 ml-pots were filled with the various treated soils. Inoculation of soils with *P. myriotylum* and evaluation of disease were conducted as previously described with five plants per treatment. The experiment was carried out four times.

### 2.7. Determination of physical and chemical soil properties

A 500 g composite air-dried soil sample from each location was analysed for its physical and chemical properties. Soil texture was determined by the pipette method and according to the International Society of Soil Science (ISSP) system. Soil pH was measured in a soil-water suspension (1:2.5, weight/volume). Organic carbon was determined after sulfo-chromic acid Kjeldahl digestion and spectrometric analysis (Heanes, 1984). Briefly, 100–500 mg of various finely ground soil samples were transferred into digest tubes. Thereafter, corresponding volume of standard solution of sucrose and some drops of potassium dichromate solution and concentrated sulphuric acid were added to the digest tubes, and mixed properly. The digest tubes were heated for 30 min, allowed to cool, and then the content was diluted with distilled water. Soil samples were transferred into centrifuge tubes for centrifugation. Finally, the concentration of C could be determined from a standard curve using a spectrophotometer at 600 nm.

Total nitrogen was determined from a wet sulphuric acid digest (Buondonno et al., 1995). Briefly, 200–500 mg of each finely ground soil sample was introduced into a digest block together with a mixture of sulphuric acid and selenium dioxide. Thereafter, hydrogen peroxide was added and the mixture was heated to 300–320 °C until the digest was colourless. Digested material was allowed to cool, diluted first with deionised water and then successively with two reagents composed of sodium salicylate, sodium citrate, sodium tartrate and sodium nitroprusside (reagent 1), and sodium hydroxide and sodium hypochlorite (reagent 2). After one hour, the nitrogen concentration was determined from a standard curve using a colorimetric spectrophotometer at 655 nm.

Exchangeable bases (Ca, Mg and K) and P were extracted from soils using Mehlich-3 procedure (Mehlich, 1984). A Mehlich-3 extract solution, a P stock solution

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(1000 ppm) and a stock solution containing Ca, Mg, K and Na (1000 ppm each) were prepared. For extraction, 3 ml of each soil sample was introduced into a 50-ml centrifuge tube, including 5 soil check samples for quality control. Then 30 ml of Mehlich-3 extractant solution was added to each soil sample, capped securely, and shaken for 5 min. Thereafter samples were allowed to stand for 10 min, centrifuged for 5 min at 3000 rpm before transferring into tubes for analysis. For analysis purposes, working standards were made from stock solutions previously prepared using Mehlich extractant solution and diluted strontium chloride as diluent. Soil samples were diluted with strontium chloride in a matrix to match standards for Ca, Mg, K, and Na. All soil samples were read on an atomic absorption spectrophotometer and the concentrations of various elements were determined from a standard curve.

Available P was extracted and analysed using the molybdate blue procedure based on the method of Murphy and Riley (1962). This procedure involves reduction of the phospho-molybdate complex by ascorbic acid in a reaction catalysed by antimony. To analyse Mehlich soil extracts, Murphy and Riley stock solution, working solution and standards for P were prepared according to Murphy and Riley (1962). One ml of various Mehlich-3 soil extracts and the standard were introduced together with Murphy and Riley working solution into test tubes. Various mixtures were allowed to stand for 30 min before reading absorbance at 360 nm.

### 2.8. Evaluation of cocoyam field soils for suppressiveness to CRRD

To identify changes in soil properties, which may contribute to loss of suppressiveness in andosols, CRRD severity, physical and chemical properties, and microbial populations in cocoyam field soils from Boteva and Njonji (disease-suppressive soils) were compared to those in Ekona cocoyam field soil (disease-conducive soil). Two cocoyam fields were established on virgin andosols in Boteva, and in Njonji. Another cocoyam field was established in Ekona. All the three fields had the same size (20 m × 6 m). White cocoyam was planted in the same manner in all fields. Clean cocoyam suckers were introduced into holes (40 cm × 40 cm × 20 cm) at 1 m apart and in a completely randomised design. Expected number of cocoyam plants per field was 120. At 4 months after planting, CRRD was scored on 80 plants excluding plants from border rows. Then the percentage of yellow leaves was taken as the disease severity value of an individual cocoyam plant. Average field disease severity was determined based on disease severity values from 80 plants.

### 2.9. Determination of microbial populations

Microbial populations were determined using dilution plating. Ten grams of each composite soil sample was suspended in 90 ml sterile solution of NaCl ($8.5\,g\,l^{-1}$). From this suspension, a 10-fold serial dilution was prepared. A 100 µl of appropriate dilutions were plated in triplicate on different media. The four target microbial groups and their corresponding culture media were as follows: (1) total aerobic heterotrophic bacteria ($10^{-1}$th-strength tryptic soy agar (TSA); 4 g of TSA, 13.5 g of agar, and 100 mg of cycloheximide per liter of deionised water; (2) actinomycetes (actinomycete isolation agar; 2 g of sodium caseinate, 0.1 g of asparagine, 4 g of sodium propionate, 0.5 g of dipotassium phosphate, 0.1 g of magnesium sulphate, 1 mg of ferrous sulphate, 5 g glycerol, and 15 g of agar per liter of deionised water); (3) fluorescent pseudomonads (King's B medium; 20 g of Proteose Peptone No. 3, 1.5 g of $K_2HPO_4$, 1.5 g of $MgSO_4$ $7H_2O$, 15 ml of glycerol, 13.5 g of agar, and 100 mg of cycloheximide per litre of deionised water); (4) *Trichoderma* spp. in *Trichoderma* semi-selective medium (Chung and Hoitink, 1990). Dilution plating was carried out three times using different soil samples that were collected at three different times. Plates were incubated at room temperature (about 23 °C) and inspected for microbial growth after 48–72 h (bacteria) or 10 days (fungi).

### 2.10. Statistical analysis

Data were subjected to statistical analysis using SPSS software version 12.0. Analysis of variance was performed where applicable and differences between treatments were determined using Duncan's multiple-range test. A nonparametric procedure was used for data that could not satisfy the assumptions required for analysis of variance, and significant differences between treatment means were determined using Kruskall–Wallis and Mann–Whitney tests. Pearson's correlation coefficients were used to characterize the relationship between disease index and soil variables, and the relationship among soil variables themselves.

## 3. Results

### 3.1. Soil response to Pythium infection

Cocoyam planted in uninfested soils remained healthy until the end of the experiment. Infestation of soils with *Pythium* induced cocoyam root rot at both levels of inoculum, irrespective of soil types. The disease index was significantly ($P = 0.05$) lower in cocoyam planted in virgin andosols compared to those planted in ferralsols at lower inoculum level ($100\,propagules\,g^{-1}$ soil) (Fig. 1a). Andosol suppressiveness persisted even at triple inoculum dose (Fig. 1b), whereas in ferralsols plants were heavily affected with increased inoculum level.

### 3.2. Effects of autoclaving and microbial recolonisation of autoclaved andosols on disease suppressiveness

The disease index significantly increased in cocoyam planted in Boteva and Njonji virgin soils following

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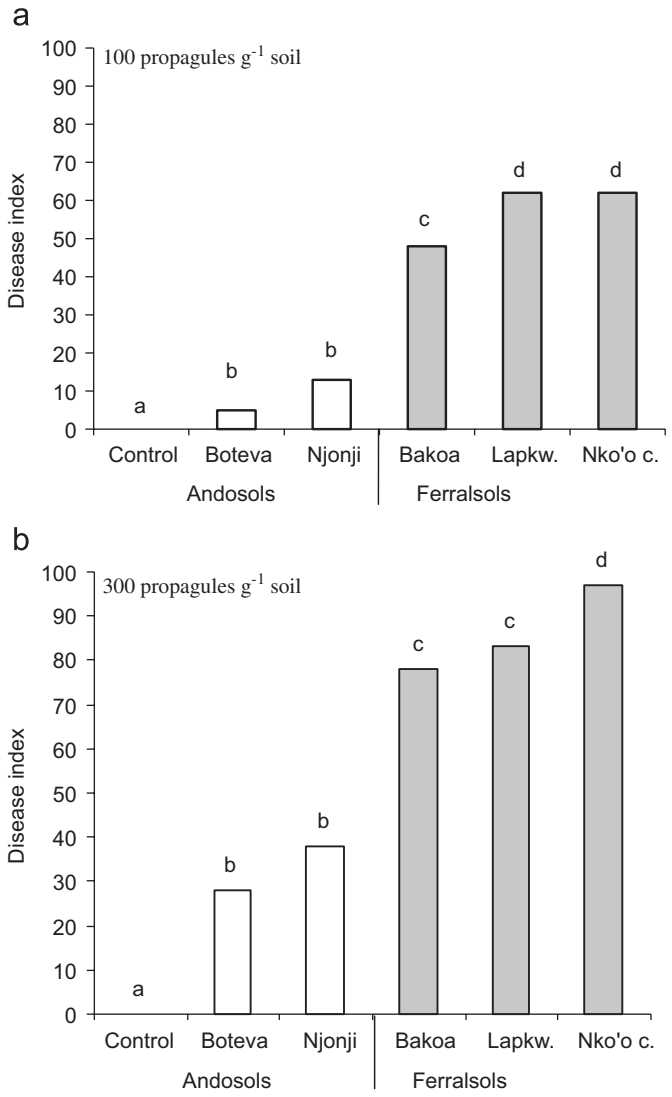


Fig. 1. Effect of *Pythium myriotylum* on cocoyam planted in virgin andosols and ferralsols. Two independent experiments (a) and (b) were conducted with two levels of inoculum, 100 and 300 propagules $g^{-1}$ soil, respectively. Potted soils were infested with *P. myriotylum* inoculum before planting cocoyam plantlets. The experimental unit consisted of five plants. Pooled data of three replications (5 plants × 3) are shown. Disease was evaluated 10 days following inoculation. Bars with the same letter are not significantly different at $P = 0.05$ according to Mann–Whitney test.

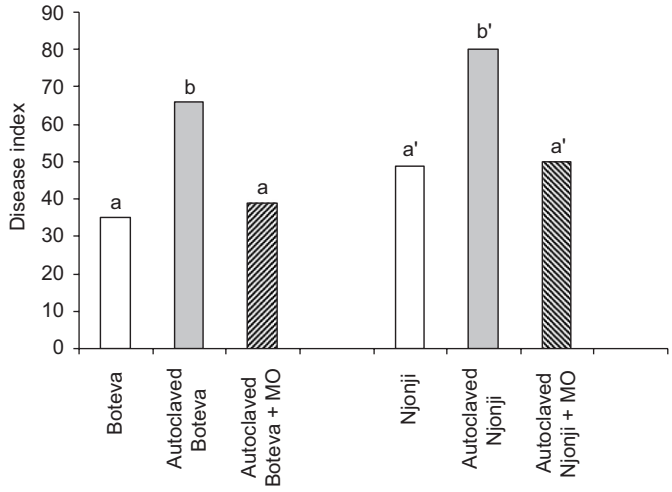


Fig. 2. Effect of autoclaving and microbial recolonisation on the disease index of cocoyam root rot disease in suppressive andosols. Potted soils were infested with *Pythium myriotylum* inoculum (300 propagules $g^{-1}$ soil) before planting cocoyam plantlets. The experimental unit consisted of five plants. Pooled data of three replications (5 plants × 3) are shown. Disease was evaluated 10 days following inoculation. Bars with the same letter are not significantly different at $P = 0.05$ according to Mann–Whitney test.

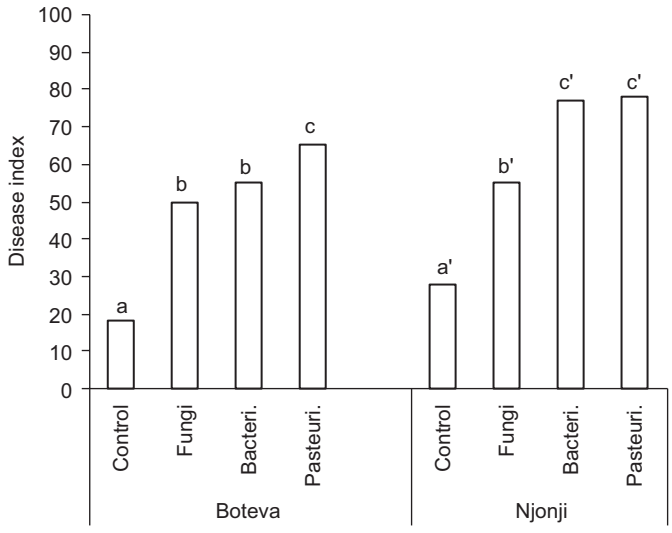


Fig. 3. Effect of pasteurisation, fungicide or bactericide treatment on the disease index of cocoyam root rot disease in suppressive andosols. *Pythium myriotylum* inoculum was applied at the dose of 300 propagules $g^{-1}$ soil. The experimental unit consisted of five plants. Disease was evaluated 10 days following inoculation. Pooled data from 4 replications (5 plants × 4) are shown. Bars with the same letter are not significantly different at $P = 0.05$ according to Mann–Whitney test.

autoclaving (Fig. 2). However, the presence of healthy cocoyam plants in andosols following autoclaving suggests that andosol suppressiveness was not totally abolished. The disease indices in autoclaved Boteva and Njonji andosols decreased significantly following colonisation by their original microflora (Fig. 2), indicating a partial recovery of andosol suppressiveness.

### 3.3. Microbial groups associated with soil suppressiveness

Disease indices increased significantly in pasteurised, fungicide or bactericide treated Boteva and Njonji andosols, indicating a partial loss in suppressiveness. This decrease in andosol suppressiveness was significant ($P = 0.05$) for all treatments (Fig. 3), suggesting that andosol suppressiveness is likely due to activities of the various targeted microbial groups.

### 3.4. Physical and chemical properties of suppressive and conducive soils to CRRD

Results of soils analyses are shown in Table 2. All soils were acidic with pH values ranging from 4.7 to 5.2.

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Table 2
Physical and chemical properties of andosols and ferralsols used in this study

| Soil variable | Andosols | | Ferralsols | | |
|---|---|---|---|---|---|
| | Boteva | Njonji | Lapkwang | Bakoa | Nko'o canane |
| Clay (%) | 2.35 | 14.40 | 28.00 | 12.70 | 39.00 |
| Silt (%) | 80.65 | 62.70 | 23.00 | 15.77 | 16.80 |
| Sand (%) | 17.00 | 22.90 | 49.00 | 71.50 | 44.30 |
| Textural class | Si[a] | Si[a] | SCL[a] | SL[a] | SC[a] |
| pH ($H_2O$) | 4.95 | 4.70 | 5.10 | 5.20 | 4.90 |
| Ca (cmol $kg^{-1}$) | 8.90 | 11.50 | 12.00 | 3.50 | 1.10 |
| Mg (cmol $kg^{-1}$) | 2.90 | 2.60 | 3.70 | 1.00 | 0.50 |
| K (cmol $kg^{-1}$) | 0.20 | 0.50 | 0.65 | 0.10 | 0.11 |
| P (g $kg^{-1}$) | 4.10 | 7.20 | 17.00 | 4.00 | 7.10 |
| Organic C (%) | 8.10 | 8.75 | 3.10 | 0.90 | 0.85 |
| Total N (%) | 0.80 | 0.90 | 0.40 | 0.13 | 0.14 |
| ECEC (cmol $kg^{-1}$)[b] | 10.80 | 15.60 | 17.20 | 5.39 | 2.80 |

[a]Si = silt; SCL = sandy clay loam; SL = sandy loam; SC = sandy clay.
[b]Effective cation exchange capacity.

Table 3
Correlations between physical or chemical characteristics of soil and disease severity

| Soil variables | Pearson correlation coefficient ($r$) | |
|---|---|---|
| | Disease severity at 100 propagules $g^{-1}$ soil | Disease severity at 300 propagules $g^{-1}$ soil |
| pH ($H_2O$) | 0.01 | −0.10 |
| Clay (%) | 0.82 | 0.84 |
| Silt (%) | −0.95** | −0.97** |
| Sand (%) | 0.77 | 0.78 |
| Organic C (%) | −0.91** | −0.94** |
| Total N (%) | −0.91** | −0.95** |
| P (mg $kg^{-1}$) | 0.50 | 0.35 |
| Ca (cmol $kg^{-1}$) | −0.45 | −0.61 |
| K (cmol $kg^{-1}$) | −0.08 | −0.53 |
| Mg (cmol $kg^{-1}$) | −0.35 | −0.53 |
| ECEC (cmol $kg^{-1}$) | −0.33 | −0.50 |

Disease severity was measured 10 days following inoculation based on the rating scale from 0 to 4: 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of foliage with yellowing. Pearson correlation was determined based on data of five soil samples from the two soil types and the disease index calculated from the disease severity scores.
**Significant correlation at $P = 0.05$ and 0.01.

The two disease suppressive andosols were light texture soils (silt and silt loam), whereas all conducive ferralsols were from heavier textural classes including sandy clay loam, sandy loam, and sandy clay. Organic carbon content in suppressive soils (8.10–8.75%) was higher as compared to that in conducive soils (0.85–3.10%). Total nitrogen was also higher in disease suppressive soils (0.80–0.90%) than in conducive soils (0.13–0.40%). Mineral nutrient content including Ca, K, and Mg was higher in suppressive soils than in conducive soils from Bakoa and Nko'o canane.

### 3.5. Relationship between soil variables and CRRD severity

To determine the extent to which soil factors were related to disease suppression, analysis of correlations between soil variables and disease severity was conducted. At both levels of inoculum (100 and 300 propagules $g^{-1}$ soil), the following soil variables correlated negatively with the disease severity (Table 3): percentage of silt ($r = -0.95$** and $-0.97$**, respectively); organic carbon ($r = -0.91$** and $-0.94$**, respectively); total nitrogen ($r = -0.91$** and $-0.95$**, respectively); and to lesser extent Ca ($r = -0.45$ and $-0.61$, respectively) or ECEC ($r = -0.33$ and $-0.5$, respectively). Soil variables that positively correlated with disease severity were percentage of sand ($r = 0.77$ and 0.78, respectively) and percentage of clay ($r = 0.82$ and 0.84, respectively).

### 3.6. Relationship among soil variables

All soil variables that negatively correlated to CRRD severity such as Ca, N, silt, and ECEC were positively associated with soil organic carbon. By contrast, variables that positively correlated to CRRD severity, such as percentage of sand or clay, were negatively associated to soil organic content (Table 4).

### 3.7. Soil properties and CRRD severity in suppressive and conducive andosols

As expected, CRRD was more severe in the degraded andosol at Ekona than in virgin andosols in Boteva and Njonji. Diseased andosol (Ekona andosol) had a higher proportion of clay compared to both disease-suppressive soils (Table 5). Organic matter content (as expressed by the percentage of organic carbon) and total nitrogen were lower in the disease conducive andosol. Concentration of cations such as Ca and Mg were higher in suppressive than in conducive andosols.

All targeted microbial groups were found in the two groups of soils. Three microbial groups including total heterotrophic bacteria, fluorescent pseudomonads and *Trichoderma* spp. were significantly higher in one or both suppressive soils than in the disease-conducive andosol from Ekona. But their respective populations size were still high in disease-conducive andosol. This suggests that loss in suppressiveness in Ekona andosol may be due to low microbial activity instead of reduced microbial biomass (Table 6).

## 4. Discussion

In Cameroon, field observations have revealed that cocoyam can continuously be grown on andosols from volcanic origin for about five years without noticeable disease outbreaks, whereas in the surrounding ferralsols, replanting of cocoyam rapidly results in high disease severity. The objectives of this study were to confirm that andosols from Mount Cameroon are suppressive to

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Table 4
Correlation of soil properties

| Factors correlated | pH | Ca (cmol kg$^{-1}$) | Mg (cmol kg$^{-1}$) | K (cmol kg$^{-1}$) | P (kg mg$^{-1}$) | Org. C (%) | N (%) | ECEC (cmol kg$^{-1}$) | Clay (%) | Silt (%) | Sand (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH ($H_2O$) | 1.00 | −0.26 | 0.08 | 0.20 | 0.14 | −0.66 | −0.67 | −0.23 | −0.01 | 0.57 | 0.78 |
| Ca | | 1.00 | 0.95* | 0.87 | 0.51 | 0.71 | 0.76 | 0.99** | −0.37 | 0.54 | −0.50 |
| Mg | | | 1.00 | 0.82 | 0.58 | 0.61 | 0.65 | 0.94* | −0.34 | 0.50 | −0.46 |
| K | | | | 1.00 | 0.82 | 0.37 | 0.44 | 0.94* | 0.10 | 0.13 | −0.24 |
| P | | | | | 1.00 | −0.15 | −0.10 | 0.61 | 0.51 | −0.33 | 0.11 |
| Org. C | | | | | | 1.00 | 0.99** | 0.62 | −0.65 | 0.95* | −0.88* |
| N | | | | | | | 1.00 | 0.67 | −0.60 | 0.93* | −0.87 |
| ECEC | | | | | | | | 1.00 | −0.25 | 0.42 | −0.41 |
| Clay (%) | | | | | | | | | 1.00 | −0.72 | 0.34 |
| Silt (%) | | | | | | | | | | 1.00 | −0.89* |
| Sand (%) | | | | | | | | | | | 1.00 |

*Correlation coefficients differ significantly at $P = 0.05$.
**Correlation coefficients differ significantly at $P = 0.01$.

Table 5
Variation in andosol properties and their influence on disease suppression

| Variables | Suppressive field soils | | Conducive field soil |
|---|---|---|---|
| | Boteva | Njonji | Ekona |
| Diseased plants (%) | 5.60 | 7.30 | 41.00 |
| Silt (%) | 76.60 | 50.30 | 40.41 |
| Sand (%) | 20.41 | 37.10 | 6.15 |
| Clay (%) | 2.99 | 12.60 | 53.44 |
| pH ($H_2O$) | 5.15 | 5.12 | 4.72 |
| Ca (cmol kg$^{-1}$) | 16.47 | 14.03 | 4.16 |
| Mg (cmol kg$^{-1}$) | 3.14 | 3.56 | 1.72 |
| K (cmol kg$^{-1}$) | 0.22 | 0.25 | 0.38 |
| Na (cmol kg$^{-1}$) | 0.00 | 0.00 | 0.01 |
| P (mg kg$^{-1}$) | 6.57 | 4.39 | 6.37 |
| Organic C (%) | 6.04 | 5.26 | 1.93 |
| Total N (%) | 0.73 | 0.63 | 0.22 |
| C:N ratio | 8.25 | 8.38 | 8.72 |
| ECEC (mg kg$^{-1}$) | 19.86 | 17.87 | 6.59 |

Two cocoyam fields were established on virgin andosol plots at Boteva, and at Njonji (disease-suppressive soils). Another cocoyam field was established on a degraded andosol at Ekona (disease-conducive soils). The number of yellow leaves and total number of leaves per plant were determined at 4 months after planting. The percentage of yellow leaves was taken as the disease-severity value of an individual cocoyam plant. Average field disease severity was determined based on disease-severity values from 80 plants.

Table 6
Populations of four target microbial groups in suppressive and conducive andosols from Mount Cameroon

| Soils | Microbial population[1] ($\log_{10}$ cfu g$^{-1}$ soil) | | | |
|---|---|---|---|---|
| | Total bacteria | Actinomycetes | Fluo. pseudomonads | *Trichoderma* spp. |
| Ekona | 5.63 b | 5.83 a | 4.10 b | 2.10 b |
| Boteva | 6.13 a | 5.86 a | 4.30 a,b | 2.63 a |
| Njonji | 6.15 a | 5.93 a | 4.43 a | 2.46 a |

Microbial populations were determined using dilution plating. A 100 µl of each appropriate dilution was plated in triplicate on Petri dishes containing various semi-selective media. The counting experiment was conducted three times. Counts were log-transformed before analysis of variance. Population means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

CRRD and to identify essential factors that induce soil suppressiveness.

In greenhouse trials with *Pythium*-infested virgin soils, cocoyams planted in andosols were significantly less affected by CRRD compared to plants grown in ferralsols, showing that both soil types interact differently with the cocoyam pathogen. No single diseased plant was found on uninfested soils, indicating that the virgin soils used in the experiment may not contain *P. myriotylum*. Bouhot and Joannes (1979) already established that the saprophytic growth of *Pythium* in soil before infection, rather than the initial amount of *Pythium* inoculum, determines the ultimate extent of crop damage. Soils with properties that are inhibitory to the saprophytic growth of *Pythium* spp. are prone to suppress the diseases they cause in plants. Under conditions in which the experiment was conducted (refer to methodology) other factors than soil properties known to influence the pathogenicity of *Pythium* spp. such as moisture, temperature, and light (Hendrix and Campbell, 1973; Schmidt et al., 2004) could not account much for the observed variation of disease severity between the two soil types. Increasing inoculum level reduced significantly andosols suppressiveness, but plants on ferralsols were more strongly affected. Indeed, suppression of saprophytic activities does not influence much the disease severity in conditions of sudden increase of inoculum. It is likely that *Pythium* spp. would be progressively suppressed in time because of lack of saprophytic activity. These results indicate that virgin andosols from Mount Cameroon area are effectively suppressive to *P. myriotylum*.

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A number of soils naturally suppressive to *Pythium* spp. have been reported worldwide including soils from France (Bouhot and Joannes, 1979), San Joaquin valley of California (Martin and Hancock, 1981; Martin and Hancock, 1986), the tropical grassland in South Kahala region on the island of Hawaii (Kao and Ko, 1986), and the traditional Chinampa agricultural system in the Valley of Mexico (Lumsden et al., 1987). In most of the above-cited cases, soil suppressiveness was linked to the soil microbial populations, fertility level, organic matter content, and soil nature (texture). This is the first work showing that andosols of Mount Cameroon area are naturally suppressive to *P. myriotylum* on cocoyam. It should be noted that soil suppressiveness is lost if cocoyam is cultivated continuously for a longer period (more than 5 years). It is not known yet if other *Pythium* spp. are suppressed in these soils.

Virgin andosols became conducive following autoclaving, possibly because of alteration of their properties that could suppress saprophytic activity of *P. myriotylum*. Recovery of suppressiveness following recolonisation by andosols resident microflora indicates that soil properties that might have suppressed the saprophytic growth of *P. myriotylum* were biological. However, the disease suppression was not totally biological in nature since soil suppressiveness was just partly removed following autoclaving. Thus physical and chemical properties may be important factors for andosols suppressiveness.

The comparative analysis of soil properties between virgin andosols and ferralsols shows that on one hand, silt, organic carbon, total nitrogen, and to a lesser extent Ca and Mg were higher in suppressive soils than in conducive soils. These variables correlated negatively with the disease severity. On the other hand, sand and clay were relatively higher in ferralsols and were positively correlated to the disease severity. From literature, high silt content favours plant diseases caused by *Pythium* spp. Sugarcane root rot caused by *Pythium arrehenomanes* was severe in silt loam soil (Dissanayake et al., 1998). Johnson et al. (1978) reported abundant *Pythium ultimum* in cotton seedlings growing in silt loam soils. In this study, the high soil silt content in the CRRD suppressive andosols appears to contradict results obtained in previous work. However, in andosols (volcanic soils) silt is associated to allophane (a typical clay of volcanic soils) and both soil components do not dissociate in water solution during physical analysis. Therefore, in this study this soil variable may be misleading. It may not be considered as a good indicator of soil suppressiveness since its content in andosols was probably overestimated. But it is possible that the complex "pseudosilt" which makes soil lighter, suppresses the *P. myriotylum* growth. To our knowledge the direct effect of this complex on *Pythium* spp. has never been reported. The positive association of clay with CRRD should not be surprising since soils with high clay content are prone to long periods of water saturation, and Pythium root rot is prevalent in wet soils (Cook et al., 1980; Martin and Loper, 1999). Knudsen et al. (2002) reported a similar effect of soil clay content on Pythium damping-off in sugar beet. In contrast to clay, sand improves soil drainage and soil with high sand content suppressed Pythium damping-off in sugarcane (Knudsen et al., 2002). The positive correlation of sand content to disease severity in this study is probably because sandy soils are poor in organic matter.

High soil organic carbon content is an indicator of soil microbial activity (Hu et al., 1997). Furthermore, activities of the disease-suppressive soil micro-organisms are organic matter dependant (Hoitink and Boehm, 1999). Biological control of *Pythium*-induced plant diseases in both container media and the field appears to be mediated by organic matter (Boehm et al., 1997; Chen et al., 1988). Soil organic matter content influences soil functions such as moisture retention, infiltration, and nutrient retention and release, which may affect plant development. Thus, increased organic matter content may be partly responsible for soil suppressiveness.

High nitrogen supply generally tends to increase the disease incidence. For plant diseases caused by *Pythium* spp., Huber and Watson (1974) showed that nitrogen could variably influence the disease severity depending on the form in which it was available in soil. They demonstrated that a high supply of $NO_3$-N increases severity of diseases caused by *Pythium* spp., whereas $NH_4$-N reduces it. In the natural environment, nitrogen supply depends qualitatively and quantitatively on soil organic matter. The negative association of soil nitrogen content with the disease severity suggests that the quality of organic matter in andosols also accounts for its suppressiveness. Higher Ca content in suppressive soil retains much attention considering its involvement in the natural suppression of *Pythium splendens* in pasture soils in South Kohala in Hawaii (Kao and Ko, 1986). Nyochembeng et al. (2002) showed that calcium affects sporangia of *P. myriotylum* by enhancing zoospore production. Based on these findings we may speculate that *Pythium* active propagules may not persist in soil with high calcium content since structures of propagation such as sporangia are rapidly replaced by short living propagules (zoospores). However, further studies are still needed to elucidate the possible role of calcium on cocoyam disease suppression. Effective cation exchange capacity (ECEC) and particularly nutrients, such as K and Mg, were negatively correlated to the disease severity. This indicates that suppressive soils were more fertile than conducive soils. Plants grow much faster in fertile soils and this may enable them to escape some soil-borne diseases (Agrios, 1997).

Correlating soil variables among themselves (Table 4), it appears that all soil variables which are negatively associated with CRRD severity such as percentage of Ca, Mg, N, ECEC are positively associated with organic carbon, whereas soil variables that promote high CRRD severity such as percentage sand and clay correlate negatively to soil organic carbon content. These results indicate that high soil organic matter content is the key

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factor determining andosol suppressiveness. At this step of the study it appeared important to determine factors that were associated with suppressiveness loss in andosols from Mount Cameroon. Andosol from CRRD-free fields (suppressive) and andosol from infected cocoyam field (conducive) did no longer belong to the same textural class. Suppressive andosol belonged to a lighter textural class (silty) while disease-conducive andosols were sandy clay, indicating changes in soil physical properties. Organic carbon and mineral nutrient content were lower in the conducive andosol than in suppressive andosols. This indicates that changes in physical properties from lighter to heavier textural classes, and in chemical properties such as reductions of organic matter and soil nutrient content are likely responsible, at least partly, for loss in suppressiveness.

Identification of microbial groups, which contribute to soil suppressiveness is a step forward in understanding soil mechanisms of CRRD suppression. Andosol exposure to either benlate (fungicide), streptomycin and penicillin G (bactericides), or to pasteurisation increased the disease severity, indicating that soil suppressiveness was reduced following these different treatments. But suppressiveness was not totally removed since compared to the control, a significant number of healthy cocoyam plants were still present in treated soils. The results obtained suggest that CRRD suppression in andosol could not be attributed to a specific group of micro-organisms (specific suppression). CRRD suppression probably resulted from overall soil micro-organism activities (general suppression). Similar results were reported by Postma et al. (2005) with *Pythium aphanidermatum* in cucumber. Rockwool suppressiveness to *Pythium aphanidermatum* was not removed after treating rockwool with antibiotics (vacomycin and cefotaxine), fungicide (benlate) and pasteurisation. In the present study total removal of soil suppressiveness could not be expected as suppressiveness was shown to be due partly to soil abiotic factors. Hoitink and Boehm (1999) reported that the general suppression phenomenon was responsible for suppression of Pythium damping-off in peat and compost-based soilless container mixes. In the tested andosols, factors such as high organic matter content and fertility level suggest that *P. myriotylum* might be reduced through "general suppression" (Weller et al., 2002).

Population densities of some groups of micro-organisms (heterotrophic bacteria, fluorescent Pseudomonads, *Trichoderma* spp.) were significantly higher in suppressive andosols than in conducive andosol. In many instances, high populations of fluorescent pseudomonads in soil correlated with soil suppressiveness (Chapon et al., 2002; Raaijmakers and Weller, 1998; Weller et al., 2002). Recently, phenazine-producing *Pseudomonas aeruginosa* PNA1 was shown to be antagonistic to *P. myriotylum* in cocoyam (Tambong and Höfte, 2001). Perneel et al. (2007) have isolated fluorescent pseudomonas strains antagonistic to *P. myriotylum* from healthy cocoyam rhizosphere in Cameroon. *Trichoderma* spp., one of the more numerous microbial groups in suppressive andosols, are common inhabitants in natural ecosystems. Their ability to control plant-pathogenic Oomycetes has been widely reported (Howell, 2002; Harman et al., 2004). To our knowledge, control of *P. myriotylum* in cocoyam by *Trichoderma* spp. has not yet been reported. Further studies are needed to elucidate their possible contribution to actual andosol suppressiveness. However, andosol suppressiveness loss might not be due to decreased microbial biomass since targeted micro-organism populations were still high in disease-conducive andosols at Ekona. Possibly, soil micro-organisms were less active in the conducive andosol as a result of unfavourable changes in abiotic soil properties. The on-going assessment of andosol microbial diversity and quantitative evaluation of microbial activities both in suppressive and conducive andosols might elucidate much better the role of soil micro-organisms in andosol suppressiveness.

To conclude, this study has confirmed suppressiveness of andosols from Mount Cameroon to CRRD. Disease suppression was shown to be due to abiotic and biotic soil properties. Light texture of andosols contributes to CRRD suppression, whereas its degradation under cultivation is likely to induce conduciveness to CRRD. The high mineral content of andosols may also contribute to suppressiveness through enhanced plant growth. High microbial populations in suppressive soils are associated to suppression of the cocoyam pathogen, and thereby contribute to soil suppressiveness. Organic matter, because of its influence on soil structure, density of soil microbial populations, and soil nutrient availability, appears as the key factor inducing suppressiveness in andosols from Mount Cameroon. Based on this knowledge, research has been initiated to evaluate the use of various composts, made from locally available agricultural waste, for their potential to control CRRD in ferralsols and conducive andosols.

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