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*Full Length Research Paper*

# Antagonist potential of *Trichoderma* indigenous isolates for biological control of *Phytophthora palmivora* the causative agent of black pod disease on cocoa (*Theobroma cacao* L.) in Côte d'Ivoire

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**The biodiversity of *Trichoderma* isolates from cocoa rhizosphere in cocoa production areas of Côte d'Ivoire, and their antagonist potential with *Phytophthora palmivora* using *in vitro* assays and bioassays, were investigated and screened for field trials. A total of 135 isolates were analysed at the species level by using sequence analysis of ITS1 and 2 of the rRNA region and a fragment of translation elongation factor 1a (*tef*1) gene. Sixty-four isolates were identified as *T. virens*, 60 as *T. harzianum,* 7 as *T. spirale*, two as *T. asperellum* and two unidentified. Forty-three *Trichoderma* isolates were *in vitro* confronted with *P. palmivora* on agar plates, and their antagonist activity was further evaluated by the damages on leaf discs and detached pods. Twenty-five isolates reduced the mycelial growth of *P. Palmivora* more than 50%. The isolate T17 assigned to *T .virens* was the best to reduce mycelium growth upto 97.9%. All of *Trichoderma* isolates with the exception of isolate T39 reduced foliar sensitivity to *P. palmivora*. Tweenty-six *Trichoderma* isolates reduced the pod sensitivity to *P. palmivora* more than 50%. Based on the combined analysis, *T. virens* T7, *T. harzianum* T40, *T. asperellum* T54 and *T. spirale* T4 isolates were selected for field trials.**

**Key words:** Biodiversity, antagonist, *in vitro* assays and bioassays, *Phytophthora Palmivora*, *Trichoderma*, cocoa rhizosphere, Côte d'Ivoire.

## INTRODUCTION

*Theobroma cacao* L., chocolate tree, is a forest species of the Malvaceae (Whithlock et al., 2001), native of tropical rainforest America (Wood and Lass, 1985). With a world production of about 43% (ICCO, 2000), Côte d'Ivoire is the number one cocoa producing country. The average yields of dry bean in farms, which range from 250 to 450 Kg/ha, are relatively low (Braudeau, 1969; Mossu, 1990; Keli et al., 2005). However, in research station, they reach 2.5 t/ha (Clement et al., 1996). One of the major constraints responsible for these low yields is the severity of insects and pathogens attacks especially mirids and fungal pathogens such as *Phytophthora palmivora* (Braudeau, 1969; Mossu, 1990). *P. palmivora* causes black pod, main disease of Ivorian cocoa field that cause yield losses by 15-20% (Kebe, 1994).

From the 1990s, appearance of *Phytophthora megakarya* at the Eastern border between Côte d'Ivoire and Ghana increased these losses from 30 to 45% (Kebe, 1999). The control of the black pod disease became a

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national priority. Initially, chemical fungicides were applied to control the disease. These are very costly and represent a health risk for the farmers. Likewise, they were not very well accepted by consumers (Anonyme, 2006). Additionally, difficulties of their application as well as both the incidence of their residue on cocoa quality and negative impact on the environment do not encourage their recurrent use (Pereira, 1985). As complement to these ones, the genetic control allowed the selection of resistant varieties to black pod (Clement et al., 1993). However, in field conditions, such resistance was proved to be partial rather than total (Cilas et al., 1996).

Thus, due to insufficiencies of two above-mentioned methods, a strategy of integrated control was proposed in Côte d'Ivoire to overcome this disease. This is less costly, less constraining and respectful of the environment (Hebbar and Lumsden, 1999). This strategy includes the cultural practices, plant breeding and use of native antagonists to *Phytophthora* sp. Usually, fungal species of genus *Hypocrea/Trichoderma* were described as potential antagonists of plant pathogens (Viterbo et al., 2002; Benitez et al., 2004; Harman et al., 2004).

However, their efficiency is closely linked with local conditions. Thus, in Latin America, preliminary works reported on the antagonist potential of some species of *Trichoderma* against *Phytophthora* sp. (Hebbar et al., 1999; Krauss and Soberanis, 2001; Krauss et al., 2003). Likewise, in Cameroon, *T. asperellum* is an antagonist of *P. megakarya* causing black pod disease (Tondjé et al., 2007). In Côte d'Ivoire, there could exist among the *Trichoderma* species living in cocoa rhizosphere a natural antagonist to *P. palmivora*. The search for, isolation, evaluation and identification of this antagonist would allow the reducing of the inoculum pressure, so that of level of yield losses caused by *P. palmivora*. Knowledge of this antagonist will be an additional element for the strategy of integrated control.

In the present study, we concentrated on the isolation, identification of *Trichoderma* isolates existing in Ivorian cocoa rhizosphere and evaluated their antagonist potential against *P. palmivora* by *in vitro* and *in vivo* tests on leaves and on pods. We also proposed the best *Trichoderma* isolates which antagonist potential will be tested in cocao field trials.

## MATERIALS AND METHODS

### Plant materials, fungus materials and culture medium of *Phytophthora palmivora*

Plant materials consisted in six clones belonging to Forastero genetic group. It consist of NA32, IMC67, T85/799, SCA 6, P7 Upper Amazon clones and only one Lower Amazon clone, IFC5. Their susceptibility to *P. palmivora* has been previously shown (Kebe et al., 1996; Tahi et al., 2000). Thus, NA32 and IFC5 were identified as susceptible, while IMC67 and T85 / 799 were found to be fairly resistant. SCA6 and P7 expressed resistance to black pod. All these clones were planted in Plots B10 and C2 / 2 on station of Bingerville.

Fungal materials constituted of isolate of *P. palmivora* named BL7 / 11-2 and these of *Trichoderma* isolates. *P. palmivora* strain BL7/11-2 was isolated from a naturally infected cocoa pod in a plot BL7 planted in 1986 at the Bingerville station in Côte d'Ivoire. Water agar and pea agar as culture media were used for isolation of *P. palmivora*. About 1 cm side cubic plug of rotten pod was taken and placed onto 1.5% (w/v) water agar plate, and incubated at 26°C for 4 days. A 6 mm diameter agar plug was transferred on pea agar plate once and was for local using (Huguenin and Boccas, 1971). On this pea agar medium, three rounds of subculturing were carried out. Before the use, pathogenicity of isolate was re-established by regular inoculation in laboratory of green cocoa pods.

Isolates of *Trichoderma* spp. used in this study were collected from a soil in a main cocoa production area in Côte d'Ivoire. Soil samples were implemented in Bingerville (5.21 N, 3.54 W) and Divo (5.50 N, 5.22 W) stations from 2003-2004. These two stations belong to Eastern and Central areas of the country. In the east, including station of Abengourou (7.15 N, 3 W), South-Western and Central areas, soil samples were collected from 2006 - 2007 in farmer's field. These *Trichoderma* spp. were isolated on a *Trichoderma* selective medium E (TME) (Papavizas and Lumsden, 1982) and then stored in Ependorf tubes containing glycerol at 50% on small pieces of TME agar at –20°C. A total of thirty sites were visited.

### Collecting sites of samples of *Trichoderma* spp.

Soil samples were collected in experimental and farmer's fields in three main cocoa production areas in Côte d'Ivoire (Figure 1). Eastern area is the border of Ghana. Its ferralitic soil comes from schist. The total annual rainfall is lower than 1300 mm. In the south-west area, samples were taken from Soubré, Meagui and San Pedro. Its ferralitic soil is supported by gneiss. Its rainfall is higher than 1400 mm. In the centre, soil samples were performed in Divo, Yamoussoukro, Bouafflé and Daloa. Cocoa plantations are located there on a ferralitic soil stemmed from granite. Rainfall of this area stretches out from 1200 to 1500 mm. Furthermore, it is worth of knowing that prospected areas have a humid equatorial climate. Their annual mean temperature arises to 26.1°C.

### Collection and isolation techniques of samples

In each location, soil samples were collected in three cocoa fields. But, in areas where black pod disease pressure was high, numbers of visited cocoa field reach eight, in the hope of meeting more antagonists at *Phytophthora* sp., In one cocoa field, the soil samples were taken at the tree foots bearing some healthy pods, within a radius from 60-80 cm around trunk in parts of field homogeneous shade. Soil samples were taken in 10-15 cm depth in rhizosphere along the diagonal and four taken from 100-200 g of soil were implemented, after the removal of litter. Soils sampled in different cocoa field of a location were bulked for constituting a single sample. This one was subdivided into two parts. For one of them, the artificially infected cocoa pod plugs by *P. palmivora* as bait to *Trichoderma* were buried there in sterile polyethylene bags for 30-day period. Other part did not contain some infected pod plugs by *P. palmivora*. Sole soil samples containing the baits were used for isolating *Trichoderma*.

After 30-days of incubation at 26°C, the isolation of *Trichoderma* was performed from pod plugs previously buried in sterile polyethylene bags. Rotten cocoa pod plugs as bait (10 g) were ground in sterile mortar, mixed in 100 ml sterile distilled water and serially diluted ($10^{-1}$ to $10^{-7}$). For each dilution, aliquot (100 µl) was pipetted, deposited in the centre of the Petri plates and spread onto the surface of each of 4 plates containing *Trichoderma* selective medium E (TME) agar. After inoculation, the Petri plates were incubated at 26°C for 7days.

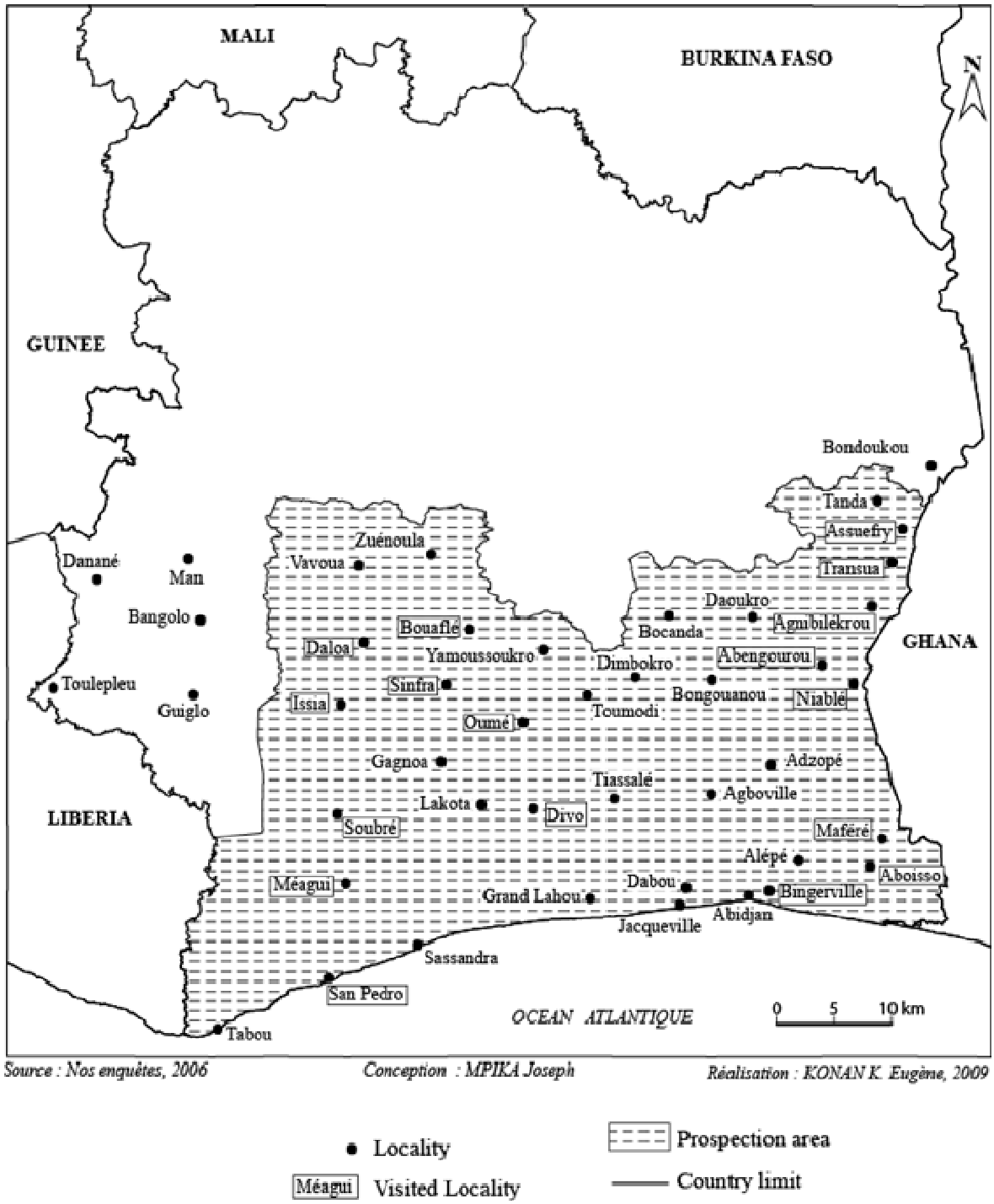


**Figure 1.** Prospected areas for the collection of soil samples in Côte d'Ivoire (hatched).

**Identification of *Trichoderma* isolates**

Isolation of genomic DNA, and amplification of gene fragments comprising the internal transcribed spacer regions 1 and 2 (ITS1 and 2) of the nuclear rRNA and the 5.8S rRNA gene, as well as the fourth large intron of the gene encoding translation elongation factor 1-alpha (*tef1*), and amplicon sequencing was performed as described by Druzhinina et al. (2005). For species identification, ITS1 and 2 sequences were subjected to analysis by oligonucleotide DNA BarCode program *TrichO*KEY (http://www.isth.info/tools/molkey/index.php/) (Druzhinina et al., 2005). In cases of ITS alleles shared between 2 or more taxa, the result was re-checked by analysis of the large intron of *tef1* using sequence similarity search against a database of type sequences implemented in *Tricho* BLAST (www.isth.info/tools/blast). For analysis of unusual ITS1 and 2 or *tef1* alleles, sequences were automatically aligned with

ClustalX and visually edited in GeneDoc 2.6. Potentially unique alleles were then compared to NCBI Gen Bank, and a database of fungal strains of Vienna University of Technology which currently contains more than 3100 *Hypocrea/ Trichoderma* strains with more than 4100 sequences.

### Inoculum preparation of *P. palmivora* and *Trichoderma*

Zoospore suspensions of *P. palmivora* as inocula were used in bioassay, that is, leaf disc and detached pod. It was obtained on a 16-day-old colony culture of *P. palmivora* after an incubation period of 6-days in darkness, followed by an alternation of 12 h / 12 h darkness and fluorescent light for 10 days (Tahi et al., 2000). In order to obtain zoospore release, cultures of *P. palmivora* were flooded with sterile distilled water at 4°C and incubated with incandescent light for at least 40 min. The zoospore suspension was then adjusted to 3 x $10^5$ zoospores $ml^{-1}$.

Conidial suspension of *Trichoderma* was obtained by gently scraping with a spatula onto one 10-day-old pea extract agar culture and put into a test tube containing 10 ml of sterile distilled water. The tube was agited on a vortex mixer for 30 s. Aqueous suspension of conidia was filtered through three layers of sterile cheesecloth to remove mycelium and the agar fragments from. Regarding counting, it was performed using a MALASSEZ hemacytometer (SOVIREL, France), and spore suspension was adjusted to $10^8$ spores $ml^{-1}$ as recommended by Dubos (1986).

### Direct confrontation of *Trichoderma* and *P. palmivora* in plates

Each *Trichoderma* isolate and *P. palmivora* were separately inoculated onto pea extract agar at 26 ± 2°C for 4 days. After the incubation period, mycelial discs of *Trichoderma* (6 mm in diameter) was placed on one edge of the agar plate and mycelial disc (6 mm in diameter) of *P. Palmivora*, obtained from actively growing colonies was placed on the opposite side of the plate. Petri dishes were incubated at 26 ± 2°C for 7 days. Growth of *P. palmivora* was recorded by measuring the diameter of the colonies each day. Percentage inhibition (I) of colony growth of *P. palmivora* was calculated as described by Whipps (1997):

$$I(\%) = (1 - Cn/Co) \times 100$$

Where I(%) represent the inhibition average percentage, Cn is the average radial growth of *P. palmivora* in the presence of *Trichoderma* and Co is the average radial growth of *P. Palmivora* without *Trichoderma* (control). I is the mean value of replicates per isolate.

### Evaluation of phytopathogenicity ability of *P. palmivora* after *in vitro* confrontation with *Trichoderma*

The pea extract agar plates were inoculated with a 6 mm diameter disc of *P. palmivora* and a mycelial disc (6 mm in diameter) of each *Trichoderma* isolates. The plates were incubated at 26 ± 2°C for 7 weeks. After the meeting of mycelia of *Trichoderma* and *P. palmivora*, four mycelia discs (6 mm in diameter) were removed and introduced in opening the same diameter carried out on a detached pod and represented one replicate. In total, 4 replicates were performed twice. The inoculated pods were placed in humidified plastic bags (80%) and incubated at 26 ± 2°C for 6 days.

After the incubation period, the presence or absence of symptoms of black pods on inoculated pods was observed. When the symptom is observed, it reveals survival of *P. palmivora* after the confrontation expressing his phytopathogenicity. If at least one pod is infected by the black pod disease, taking of mycelial discs were pursued seven weeks after the meeting of *P. palmivora* with that of *Trichoderma* isolates. The surviving (%) was evaluated according to time by the detached pod number presenting symptoms on the whole of inoculated pods.

### *In vivo Trichoderma* antagonist effect at *P. Palmivora*

The *in vivo* antagonist effect of *Trichoderma* was evaluated on leaf discs and detached pods on cocoa tree respectively according to Nyassé's et al. (1995) and Iwaro's et al. (1997), Iwaro's et al. (2000) methods. Nyassé's et al. (1995) method is used for early screening of cocoa varieties for resistance to *Phytophthora* sp. This method was adapted to evaluate effect of *Trichoderma* on *P. palmivora*. The leaves of six clones were used for leaf assay. For this purpose, the leaves of about 2 months were collected from each of six clones. These leaves were cleaned with sanitary towels and eight leaf discs were obtained from each leaf by using a cork borer of 15 mm diameter. Eight leaf discs of each clone and of each tested isolate were placed upside down on wetted plastic foam in each of four trays of 70 x 60 x 10 cm. 48 leaf discs of the 6 clones per trays were used per isolate of *Trichoderma*. An equivalent number of control leaf discs were only treated with *P. palmivora*. Thus, 43 isolates of *Trichoderma* were tested.

Leaf discs were simultaneously inoculated by depositing one drop of 10 µl of the suspension of $10^8$ spores $ml^{-1}$ of *Trichoderma* and the suspension of 3 x $10^5$ zoospores $ml^{-1}$ of *P. palmivora* in the centre of each leaf disc. The trays covered with plastic sheet were incubated in the dark at 26 ± 2°C for 7days. After incubation, Scoring on the following scale ((Nyassé et al., 1995) : 0 = no symptoms and 5 = true patch (necrosis). For antagonistic activity of *Trichoderma*, average foliar sensitivity scores of treated discs were evaluated compared with these of untreated discs with *Trichoderma* that constituted the controls.

On pods, only NA32 susceptible clone at *P. palmivora* was used. These pods came from manual pollination with pollen from susceptible clone IFC5, in order to make sure of homogeneity of plant material and age of used pods. About 4-month-old pods were harvested, rinsed in two changes of sterile distilled water, placed in trays lined on wetted plastic foam in a completely randomised design and conserved for 24 h. The next day, each pod was inoculated by spraying about 1 ml of a suspension of $10^8$ spores/ml of *Trichoderma* by means of a chromist atomiser (cat. No 51901 spray unit, Gelman Sciences, Ann Arbor, Michigan). After, each pod was sprayed again with suspension of 3 x $10^5$ zoospores $ml^{-1}$ of *P. palmivora* at 4 h interval, in the same conditions. In a tray, 43 treated and 3 untreated pods were placed on 7 lines. Each pod was treated by one sole *Trichoderma* isolate. This was quadruplicated. Each tray, three control pods were only sprayed with *P. palmivora*.

The trays were incubated at 28 ± 2°C for 6 days, after the attack severity of the inoculated pods was assessed by means of Iwaro's scoring scale varying from 1 (no symptom) to 8 (lesion fusion). Regarding antagonist effect of *Trichoderma* isolates, average scores of sensitivity at *P. palmivora* of treated pods were evaluated, compared with these of untreated control. These pod sensitivity scores led to the calculation of the percentage inhibition according to followed formula (Whipps, 1997):

$$I(\%) = (1 - Nn/No) \times 100.$$

Where Nn is the average score of pod sensitivity at *P. palmivora* in the presence of antagonist and No, the average score of pod sensitivity at *P. palmivora* in absence of *Trichoderma* isolate.

### Statistical analyses

Evaluation of the antagonist effect of *Trichoderma* isolates on radial growth of *P. palmivora* was carried out by average comparison of

**Table 1.** Colonies for unit and number of isolates as a function of *Trichoderma* species, location and area of sample.

| Area | Location | *Trichoderma* species* | Colony forming unit/g | Number of isolates |
|---|---|---|---|---|
| **East** | **Abengourou** | *T. virens* | $2 \times 10^7$ | 8 |
| | | *T. harzianum* | $10^7$ | 7 |
| | **Alepé** | *T. harzianum* | $2 \times 10^2$ | 7 |
| | | *T. virens* | $10^6$ | 1 |
| | | *T. spirale* | $10^5$ | 1 |
| | **Assueffry-Ngam** | *T. virens* | $10^6$ | 2 |
| | | *T. harzianum* | $3 \times 10^6$ | 7 |
| | **Bingerville** | *T. virens* | $10^5$ | 19 |
| | | *T. harzianum* | $3 \times 10^6$ | 4 |
| | | *T. asperellum* | $10^6$ | 2 |
| | | *T. spirale* | $2 \times 10^7$ | 2 |
| | **Niablé** | *T. harzianum* | $3 \times 10^7$ | 10 |
| | | *T. virens* | $10^7$ | 3 |
| | **Noë –Maféré-Aboisso** | *T. harzianum* | $2 \times 10^7$ | 9 |
| | | *T. virens* | $2 \times 10^7$ | 3 |
| | **Transua** | *T. harzianum* | $10^6$ | 4 |
| | | *T. virens* | $10^6$ | 4 |
| | | *T. spirale* | $10^5$ | 1 |
| **Centre-West** | **Soubré-Méagui** | *T. harzianum* | $4 \times 10^7$ | 11 |
| | | *T. virens* | $10^7$ | 4 |
| | **San –Pédro Gagnoa** | *T. harzianum* | $2 \times 10^6$ | 6 |
| | | *T. virens* | $10^5$ | 1 |
| **Centre** | **Divo** | *T. virens* | $3 \times 10^7$ | 12 |
| | | *T. harzianum* | $4 \times 10^5$ | 1 |
| | | *T. spirale* | $2 \times 10^6$ | 4 |

the percentage inhibition of *P. palmivora* growth. On leaf discs from six clones, the antagonist action of *Trichoderma* isolates at *P. palmivora* was evaluated also by average separation of average foliar sensitivity scores. Thus, every group of identified clones was used for comparing the average foliar sensitivity scores induced by *P. palmivora* in the presence of *Trichoderma* isolates. On pods, these scores allowed to calculating the inhibition percentage for which the averages of *Trichoderma* isolates were compared. All averages were separated according to Duncan's test at 5% level. For the selection of *Trichoderma* isolates, the threshold of reliability coefficient was established at 30% (Issali et al., 2008 a). In order to normalize the distributions and equalize the variances, the percentages of inhibition underwent the arcsin√ transformation, whereas square root transformation was applied at the leaf sensitivity scores. All of the data were analysed by SPSS version 10.1.3 and Xlstat version 7.5.3 softwares.

## RESULTS

### Isolation and molecular identification of *Trichoderma* isolates from cocoa plantations

In a total of 90 soil samples collected in three main cocoa production areas, 135 isolates of *Trichoderma* (*Hypocrea*) were obtained (Table 1). Thus, in Eastern area, 94 isolates were obtained, against 22 in centre west. In centre, 17 isolates were counted. Regarding characterization, the oligonucleotide BarCode program *TrichO*Key identified 64 isolates as *H. virens/T. virens*, 60 as *T. harzianum,* 7 as *T. spirale*, and 2 as *T. asperellum*. Out of 135 isolates, two were unclearly identified as species. With the exception of *T. asperellum*, which was only encountered in Bingerville, *T. harzianum* and *T. virens* were found in all visited locations. However, *T. spirale* was present in Alepé, Bingerville, Transua and Divo.

In research station, there was a predominance of *T. virens*, whereas in farmer's plots *T. harzianum* was important. The Colony forming unit per gramme of soil relatively varied as a function of *Trichoderma* species and of locations.

### Effect of *Trichoderma* Isolates on mycelial growth of *P. Palmivora*

Due to the numerous collected isolates, 44 coming from Bingerville and Divo stations were used. The results showed that the mycelial growth of *P. palmivora* was inhibited by all of the *Trichoderma* isolates. Reduction of

mycelial growth was correlated with *Trichoderma* isolate which significantly exhibited an antagonism effect against the pathogen. On the whole, at four days of confrontation, *T. virens* T17 reduced mycelial growth of *P. palmivora* more than 90%, while that of *T. virens* T45 was the lowest. Twenty five isolates (58.13%) provided inhibitory rate ranged from 52.26 to 97.86%, while in eighteen isolates, these ones were lower than 50% (Table 2). More specifically, two groups of isolates were identified as a function of their percentage inhibition. The first includes *T. virens* T17 (The second is represented by 42 other isolates). The dispersion of observations around average stretched out from 1.69 to 4.81%. After four days, all of *Trichoderma* isolates invaded the colonies of *P. palmivora*.

### Phytopathogenicity ability of *P. palmivora* after its *in vitro* confrontation with *Trichoderma* on green mature pods

Inoculated pods with mycelial discs of *P. palmivora* coming from direct confrontation with *Trichoderma* isolates produced some percentages of rotten pods relatively different from one species to another (Table 3). Thus, for *P. palmivora* confronted with *T. virens*, three groups were identified, based on to the survival (%). The First was composed of *T. virens* T2, *T. virens* T6, *T. virens* T7, *T. virens* T9, *T. virens* T10, *T. virens* T15, *T. virens* T17 and *T. virens* T20 isolates, characterized by a destruction of *P. palmivora*, from the first week. Consequently, they induced no symptom of black pod. The second consisted of *T. virens* T12, *T. virens* T19, *T. virens* T24, *T. virens* T16, *T. virens* T18 and *T. virens* T8 isolates marked by a mycelium elimination of *P. palmivora* from the second to the sixth week. The third represented by the rest of *T. virens* isolates that did not produced an effect on pathogenecity of *P. palmivora*. However, *P. palmivora* in the presence of other isolates generated on all inoculed pods from symptom black pod disease. This corresponded to 25 to 100% survival. Isolates of other three species, with the exception of T4 as *T. spirale* and T5 as *T. asperellum* that respectively destroyed mycelium of *P. palmivora* up to 0% during the first and second week , did not produced an effect on pathogenecity of *P. Palmivora.* In the presence of these isolates, *P. palmivora* had 100% survival after eight weeks of direct confrontation on plates.

### *In vivo* antagonistic effect of *Trichoderma* isolates against *P. Palmivora*

The mean of scores and reliability coefficient for 6 clones evaluated by leaf test are presented in Table 4. The comparison of scores for sensitivity among the leaf discs of treated clones and untreated controls showed a clear reduction of the susceptibility to the pathogen. Three clones groups were identified based on to the susceptible end of the disease rating scale, with however a difference in their composition (Table 4).

These clones groups identified enabled the assessment of the effect *Trichoderma* isolates on *P. palmivora*. From the susceptible clone (IFC5) on one hand, T85/799, IMC67, NA32 and P7, and on the other hand, the foliar sensitivity score, the lowest was recorded with *T. virens* T21, while the highest was with *T. spirale* T39 of which average was inferior to that of untreated leaf discs. Globally, two groups of *Trichoderma* isolates were identified, with a difference in the order of their classification. The first consisted of all isolates, with the exception of *T. spirale* T39. (Table 5). In the presence of all isolates of *Trichoderma*, the mean of scores of sensitivity to *P. palmivora* of these four clones were inferior to those of control. In regard to resistant clone (SCA6), the weakest sensitivity score was recorded with *T.harzianum* T36, while the strongest was with *T. spirale* T39 of which average was inferior to this of control. The effect of all *Trichoderma* isolates on *P. palmivora* was not significantly different. Average of scores for foliar sensitivity to *P. palmivora* in the presence of *Trichoderma* were, in the whole, lower than control as well. The gaps between score average and individual scores varied from 9.22% to infinite.

Generally, lesions slowly developed in detached pods sprayed with *Trichoderma* isolate and inoculated with *P. palmivora*. This revealed a reduction of the susceptivity of pods to *P. palmivora* by *Trichoderma*. Again, all of the *Trichoderma* isolates prevented the occurence of lesions and reduced the size of rot pods, but individual results varied between 19.55 and 84% (Table 6). The lowest inhibition rate was obtained with *T. virens* T21 isolate, while the highest was with *T. virens* T7. This inhibitory action varies according to the species: in *T. virens*, inhibitory rates ranged from 84 to 19.55% (isolates T7 and T26, respectively). In *T. spirale* and *T. asperellum*, the extremes lay between 74.81 and 75.24% (isolates T4 and T54, respectively) to 28.85 and 27.86% (isolates T39 and T5). In *T. harzianum* they ranged from 80.55 to 30.68% (isolates T40 and T35, respectively).

However, 50% of the isolates showed biocontrol activity higher than 51% which was most pronounced (> 75%) with three isolates *T. virens* (Isolates T7, T19 and T8). On pods of NA32 susceptible clone, antagonist effect of all *Trichoderma* on *P. palmivora* isolates did not significantly vary. Dispersion of observations stretched out from 10.61 to 26.86%.

## DISCUSSION

The diversity of *Trichoderma* isolates existing in Ivorian cocoa field, their antagonist potential against *P. palmivora* by *in vitro* and *in vivo* tests on leaves and on pods were evaluated to select the best in order to test them in

**Table 2.** Classification of inhibitory effect of *Trichoderma* isolates on mycelium growth of *P. palmivora* at the fourth day of confrontation.

| Isolate | Transformed average * | RC (%)* | Untransformed average (%) |
|---|---|---|---|
| *T. virens* T17 | 1.424 a | 1.69 | 97.86 |
| *T. virens* T29 | 1.082 b | 2.22 | 77.95 |
| *T. virens* T27 | 1.024 bc | 2.34 | 72.96 |
| *T. virens* T32 | 0.990 cd | 2.42 | 69.89 |
| *T. virens* T8 | 0.958 cde | 2.51 | 66.92 |
| *T. virens* T9 | 0.940 def | 3.19 | 65.22 |
| *T. virens*T 51 | 0.938 def | 2.56 | 65.02 |
| *T.* asperellumT54 | 0.928 defg | 2.59 | 64.07 |
| *T. spirale* T4 | 0.925 defg | 2.59 | 63.78 |
| *T.asperellum* T5 | 0.922 defg | 2.60 | 63.49 |
| *T. virens* T13 | 0.918 defg | 2.61 | 63.11 |
| *T. spirale* T38 | 0.902 efgh | 2.66 | 61.55 |
| *T. spirale* T34 | 0.900 efgh | 2.67 | 61.36 |
| *T. virens* T7 | 0.886 efghi | 2.71 | 59.99 |
| *T. virens* T55 | 0.883 efghi | 2.72 | 59.70 |
| *T. virens* T15 | 0.877 fghi | 2.74 | 59.11 |
| *T. harzianum* T36 | 0.876 fghi | 2.97 | 59.01 |
| *T. virens* T19 | 0.867 fghi | 2.77 | 58.12 |
| *T. spirale* T39 | 0.867 fghi | 2.77 | 58.12 |
| *T. virens* T18 | 0.862 fghi | 2.78 | 57.63 |
| *T. virens* T56 | 0.862 f ghi | 2.78 | 57.63 |
| *T. virens* T58 | 0.849 fghi | 2.83 | 56.34 |
| *T. harzianum* T44 | 0.843 g hi | 3.08 | 55.75 |
| *T. spirale* T46 | 0.825 hij | 2.91 | 53.96 |
| *T. harzianum* T40 | 0.808 ijk | 2.97 | 52.26 |
| *T. virens* T24 | 0.766 jkl | 3.13 | 48.06 |
| *T. virens* T33 | 0.765 jkl | 3.40 | 47.96 |
| *T. virens* T30 | 0.764 jkl | 3.14 | 47.86 |
| *T. virens* T2 | 0.762 jkl | 3.41 | 47.66 |
| *T. virens* T16 | 0.761 jkl | 3.15 | 47.56 |
| *T. virens* T10 | 0.757 jkl | 3.17 | 47.16 |
| *T. virens* T3 | 0.746 klm | 3.22 | 46.06 |
| *T. virens* T31 | 0.730 klmn | 3.29 | 44.47 |
| *T. virens* T21 | 0.724 lmn | 3.31 | 43.88 |
| *T. virens* T25 | 0.722 lmn | 3.32 | 43.68 |
| *T. virens* T28 | 0.712 lmn | 4.78 | 42.69 |
| *T. virens* T26 | 0.708 lmn | 3.39 | 42.29 |
| *T. harzianum* T42 | 0.707l lmno | 4.81 | 42.19 |
| *T. virens* T20 | 0.696 lmno | 3.45 | 41.11 |
| *T. harzianum* T35 | 0.677 mno | 3.55 | 39.24 |
| *T. virens* T6 | 0.669 mno | 3.89 | 38.47 |
| *T. virens* T12 | 0.654 no | 3.98 | 37.01 |
| *T. virens* T45 | 0.615 o | 3.90 | 33.29 |

Transformed average*: Values followed by the same letter are not significantly different according to Duncan's test at 5 % threshold.
RC (%)*: Reliability coefficient in percentage.

large scale field trials. The pod fragments baits artificially infected by *P. palmivora*, buried in soil samples led to the identification of 135 *Trichoderma* isolates. Most of implemented works reported the isolation of *Trichoderma* from

**Table 3.** Further effect of *Trichoderma* on phytopathogenecity of *P. palmivora* on detached cocoa pods.

| Isolates of *Trichoderma* | Survival (%) of *P. plamivora* according to the time of confrontation (week) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ***Trichoderma virens*** | | | | | | | |
| T2 | 0 | | | | | | |
| T6 | 0 | | | | | | |
| T9 | 0 | | | | | | |
| T20 | 0 | | | | | | |
| T17 | 0 | | | | | | |
| T7 | 0 | | | | | | |
| T15 | 0 | | | | | | |
| T10 | 0 | | | | | | |
| T12 | 50 | 0 | | | | | |
| T19 | 25 | 25 | 25 | 0 | | | |
| T24 | 100 | 50 | 25 | 0 | | | |
| T16 | 25 | 25 | 25 | 25 | 0 | | |
| T18 | 25 | 25 | 25 | 25 | 0 | | |
| T8 | 50 | 50 | 50 | 50 | 50 | 0 | |
| T55 | 25 | 25 | 25 | 25 | 25 | 25 | |
| T51 | 50 | 25 | 25 | 25 | 25 | 25 | |
| T58 | 50 | 50 | 25 | 25 | 25 | 25 | 25 |
| T30 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| T25 | 100 | 50 | 50 | 50 | 50 | 25 | 25 |
| T56 | 100 | 100 | 50 | 50 | 25 | 25 | 25 |
| T32 | 100 | 50 | 50 | 50 | 25 | 25 | 25 |
| T29 | 100 | 50 | 50 | 50 | 50 | 50 | 25 |
| T33 | 100 | 50 | 50 | 50 | 50 | 50 | 50 |
| T31 | 100 | 100 | 100 | 100 | 50 | 50 | 25 |
| T27 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| T45 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| T28 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| ***Trichoderma spirale*** | | | | | | | |
| T4 | 0 | | | | | | |
| T34 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 38 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| T39 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| ***Trichoderma harzianum*** | | | | | | | |
| T42 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| T44 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| T40 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| T36 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| ***Trichoderma asperellum*** | | | | | | | |
| T5 | 25 | 0 | | | | | |
| T54 | 100 | 100 | 100 | 50 | 50 | 25 | 25 |

baited soil samples. That was the case of these of Tondjé et al. (2007) on cocoyam tubers as bait, and of Tim et al. (2003) on infected agar plugs by *P. palmivora*. However, Kubicek et al. (2002), Wuczkowski et al. (2003) and Zhang et al. (2005) isolated *Trichoderma* from soil samples unbaited. Interest of baits lies in obtaining a picture of the spectrum of mycoparasites present in the cocoa rhizosphere (Tim et al., 2003). This would be due to

**Table 4.** Classification of averages of sensitivity scores of the treated six clones and of their controls after the simultaneous inoculation of their leaf discs by both Trichoderma isolates and *P. palmivora*.

| Clone | Transformed average* | RC (%)* | Untransformed average* | Control | Transformed average* | RC (%)* | Untransformed average* |
|---|---|---|---|---|---|---|---|
| IFC5 | 0.556 a | 4.32 | 0.309 | CIFC5 | 1.624 a | 1.85 | 2.637 |
| T85/799 | 0.486 b | 4.94 | 0.236 | CNA32 | 1.616 a | 1.86 | 2.611 |
| IMC67 | 0.437 b | 5.49 | 0.191 | CT85/799 | 1.575 a | 1.90 | 2.481 |
| NA32 | 0.435 b | 5.52 | 0.189 | CIMC67 | 1.533 a | 1.96 | 2.350 |
| P7 | 0.422 b | 5.69 | 0.178 | CP7 | 1.429 b | 2.10 | 2.042 |
| SCA6 | 0.337 c | 7.12 | 0.114 | CSCA6 | 1.279 c | 2.35 | 1.636 |

Transformed average*: Values bearing the same letter in a column are not significatively different according to Duncan's test at 5% probability.
RC (%)*: Reliability coefficient in percentage.
Untransformed average*: Each value obtained squaring transformed average.

power of chemotropism that would exercise *Trichoderma* as indicated in Chet (1997). Such attraction between *Trichoderma* species and soil pathogen fungi was illustrated not only in different natural ecosystems (Papavizas, 1985; Chet 1997), but also in experimental conditions (Cortes et al., 1998; Kulling et al., 2000). Therefore, *Trichoderma* isolates which expressed an obvious attractive potential toward *P. palmivora* could have a strong antagonist effect.

From *Trichoderma* isolates, four species that are *T. virens*, *T. harzianum*, *T. spirale* and *T. asperellum* were identified. This number is comparable to the one found in Taïwan, Thaïland and Indonesia (Kubicek et al., 2002), but superior to that of Youssouf et al. (2004) who counted two species in Egypt. However, this number is inferior to the one from China, which number was 11 (Zhang et al., 2005), that of Malaysia which was 7, and that of Singapore which was 5 (Kubicek et al., 2002). In three prospected areas of which the climatic-soil conditions were different, both *T. virens* and *T. harzianum* were predominant. Some analogous results were obtained in Egypt by Youssouf et al. (2004) who identified *T. harzianum* and *T. orientalis*. There, the climatic-soil conditions do not seem influencing the geographic distribution of these two species.

Our results showed a predominance of *T. virens* in experimental plots of research stations. Up till to-day, no study reported a strong distribution of this species on a country scale. Such predominance could be caused by the use of chemical fungicides which were applied before the strain selection was carried out for which *T. virens* would be resistant. The resistance of *Trichoderma* to pesticides was demonstrated by Roberti et al. (2006) and Kredics et al. (2003). In contrast, in farmer's plots, *T. harzianum* dominated. Several works evidenced the predominance of this species (Kulling et al., 2000; Wuczkowski et al., 2003; Zhang et al., 2005). Indeed, it is ubiquitous, a decomposer, and expresses both nutrient assimilation variability and diversity of carbon source (Klein and Eveleigh, 1998).

The few *Trichoderma* spp., which we found have also been detected in tropical soils in other studies, that is, *T. spirale* (Druzhinina et al, 2005; Kubicek et al, 2002; Zhang et al, 2005), and species which are generally believed to be cosmopolitan are *T. harzianum*, *T. asperellum*, and *T. virens*. However, a closer examination of their ITS 1 and 2 sequences revealed that the isolates of *T. harzianum* exhibited an allele which is dominant in central Africa, but rare in other geographic areas. In short, the biodiversity appears low in Côte d'Ivoire, but *T. virens* and *T. harzianum* are widely distributed in all cocoa production areas. In fact, our success in isolating *Trichoderma* contrasts to the reports of Tondjé et al. (2007), who were unable to isolate *Trichoderma* directly in cooa-agroforestry system in Cameroun.

To select the 43 *Trichoderma* isolates, antagonist effects towards *P. palmivora* were *in vitro* assessed on mycelial growth and the survival after their direct confrontation. The classification of inhibitory effect for growth of *P. palmivora* among the 43 *Trichoderma* isolates tested showed a distinct reduction of colony diameter of *P. palmivora* in presence of all isolates of *Trichoderma*. Our isolates of *T. spirale* (T4, T34, T38, T39,T46), *T asperellum* (T5,T54), *T.harzianum* (T36, T40, T44) and *T. virens* (T7, T8, T9, T13, T15,T17, T18, T19, T27, T29, T32, T51, T55, T56 T58) were more efficient providing a more 50% inhibition rate for *P. palmivora* after four days of confrontation. This antagonist activity of *T. asperellum* strains rejoin the one obtained by Tondje et al. (2004) which indicated a more inhibitory action on *P. megakarya*, virulent agent causing the black pod disease of cacao.

This action of *T. asperellum* may be the direct penetration in the sporocystes, the coil around the *P. megakarya* hyphae and the formation of appressoria on the hyphae surface causing their destruction, and the substantial activity of hydrolytic enzymes such as laminarinase. The strong sensitivity of *P. palmivora* to *T. virens* observed was also demonstrated by Krauss and Soberanis (2001). Assessment of direct inhibitory effect

**Table 5.** Classification of averages of leaf sensitivity scores of IFC5, T85/799, IMC67, NA32 and P7 clones proceed from the antagonist effect of *Trichoderma* isolates against *P. palmivora*.

| **Effect of *Trichoderma* isolates on leaf disc stemming from IFC5*** | | | | **Effect of *Trichoderma* isolates on leaf disc proceeding from T85/799, IMC67, NA32 and P7*** | | | |
|---|---|---|---|---|---|---|---|
| **Isolate *** | **Transformed average *** | **RC(%)*** | **Untransformed Average*** | **Isolate** | **Transformed average *** | **RC(%)*** | **Untransformed Average*** |
| *T.virens*T21 | 0.165 a | 76.36 | 0.027 | *T.virens*T21 | 0.105 a | 55.24 | 0.011 |
| *T.virens*T41 | 0.200 a | 63.00 | 0.040 | *T virens*T33 | 0.108 a | 53.70 | 0.012 |
| *T.virens*T9 | 0.208 abc | 60.58 | 0.043 | *T.virens*T41 | 0.113 a | 51.33 | 0.013 |
| *T.virens*T29 | 0.218 abcd | 57.80 | 0.048 | *T.virens*T55 | 0.126 a | 46.03 | 0.016 |
| *T.virens*T17 | 0.227 abcd | 55.51 | 0.052 | *T.virens*T17 | 0.139 a | 41.73 | 0.019 |
| *T.virens*T25 | 0.237 abcde | 53.16 | 0.056 | *T.virens*T58 | 0.201 ab | 40.80 | 0.040 |
| *T.virens*T31 | 0.237 abcde | 53.16 | 0.056 | *T.virens*T19 | 0.202 ab | 28.71 | 0.041 |
| *T.virens*T33 | 0.241 abcde | 52.28 | 0.058 | *T.virens*T24 | 0.204 ab | 28.43 | 0.042 |
| *T.virens*T58 | 0.260 abcdef | 68.46 | 0.068 | *T.virens*T7 | 0.208 ab | 27.88 | 0.043 |
| *T.virens*T15 | 0.276 abcdef | 45.65 | 0.076 | *T.virens*T31 | 0.218 ab | 26.61 | 0.048 |
| *T.virens*T8 | 0.277 abcdef | 45.49 | 0.077 | *T.virens*T10 | 0.230 ab | 25.22 | 0.053 |
| *T.virens*T28 | 0.293 abcdef | 43.00 | 0.086 | *T.virens*T29 | 0.231 ab | 25.11 | 0.053 |
| *T.virens*T7 | 0.297 abcdef | 42.42 | 0.088 | *T.virens*T56 | 0.231 a b | 25.11 | 0.053 |
| *T.virens*T55 | 0.316 abcdef | 39.87 | 0.100 | *T.virens*T28 | 0.243 a b | 23.87 | 0.059 |
| *T.spirale*T34 | 0.334 abcdef | 37.72 | 0.112 | *T.virens*T9 | 0.244 ab | 23.77 | 0.060 |
| *T.spirale*T4 | 0.334 abcdef | 37.72 | 0.112 | *T.virens*T4 | 0.255 ab | 22.75 | 0.065 |
| *T.virens*T19 | 0.347 abcdef | 36.31 | 0.120 | *T.virens*T25 | 0.256 ab | 22.66 | 0.066 |
| *T.virens*T10 | 0.351 abcdef | 35.90 | 0.123 | *T.virens*T8 | 0.262 abc | 22.14 | 0.069 |
| *T.virens*T56 | 0.377 abcdef | 33.42 | 0.142 | *T.virens*T2 | 0.268 abc | 21.64 | 0.072 |
| *T.virens*T2 | 0.440 abcdefg | 28.64 | 0.194 | *T.virens*T15 | 0.274 abc | 21.17 | 0.075 |
| *T.harzianum*T36 | 0.446 abcdefg | 28.25 | 0.199 | *T.harzianum*T36 | 0.281 abcd | 20.64 | 0.079 |
| *T.virens*T24 | 0.452 abcdefg | 27.88 | 0.204 | *T.spirale*T34 | 0.362 bcde | 16.02 | 0.131 |
| *T.harzianum*T44 | 0.500 abcdefgh | 25.20 | 0.250 | *T.virens*T27 | 0.373 bcdef | 15.82 | 0.139 |
| *T.virens*T20 | 0.527 abcdefgh | 23.91 | 0.278 | *T.virens*T45 | 0.417 bcdefg | 13.91 | 0.174 |
| *T.harzianum*T35 | 0.561 abcdefghi | 22.46 | 0.315 | *T.virens*T20 | 0.426 bcdefg | 13.62 | 0.181 |
| *T.virens*T18 | 0.568 abcdefghi | 22.18 | 0.323 | *T.harzianum*T35 | 0.431 bcdefg | 13.46 | 0.186 |
| *T.virens*T27 | 0.569 abcdefghi | 22.14 | 0.324 | *T.harzianum*T42 | 0.451 cdefgh | 12.86 | 0.203 |
| *T.virens*T32 | 0.582 abcdefghi | 21.65 | 0.339 | *T.virens*T18 | 0.463 defgh | 12.53 | 0.214 |
| *T.virens*T12 | 0.613 bcdefghij | 20.55 | 0.376 | *T.asperellum*T5 | 0.475 efghi | 12.21 | 0.226 |
| *T.asperellum*T5 | 0.647 cdefghijk | 19.47 | 0.419 | *T.virens*T16 | 0.488 efghi | 11.89 | 0.238 |
| *T.virens*T16 | 0.660 defghijk | 19.09 | 0.436 | *T.harzianum*T44 | 0.500 efghi | 11.60 | 0.250 |
| *T.virens*T45 | 0.672 efghijk | 18.75 | 0.452 | *T.harzianum*T40 | 0.529 efgghi | 10.96 | 0.280 |
| *T.virens*T30 | 0.684 fghijk | 18.42 | 0.468 | *T.virens*T32 | 0.564 fghi | 10.28 | 0.318 |
| *T.harzianum*T42 | 0.703 fghijk | 17.92 | 0.494 | *T.virens*T12 | 0.587 ghi | 9.88 | 0.345 |
| *T.harzianum*T40 | 0.826 ghijkl | 15.25 | 0.682 | *T.virens*T13 | 0.632 hij | 9.18 | 0.399 |
| *T.virens*T13 | 0.855 ghijkl | 14.74 | 0.731 | *T.virens*T30 | 0.656 ijk | 8.84 | 0.430 |
| *T.spirale*T46 | 0.862 ghijkl | 14.62 | 0.743 | *T.virens*T3 | 0.789 jkl | 7.35 | 0.623 |
| *T.virens*T3 | 0.910 hijkl | 13.85 | 0.828 | *T.spirale*T46 | 0.799 jkl | 7.26 | 0.638 |
| *T.virens*T6 | 0.959 ijkl | 13.14 | 0.920 | *T.spirale*T38 | 0.825 kl | 7.03 | 0.681 |
| *T.spirale*T38 | 1.023 jkl | 12.32 | 1.047 | *T.virens*T6 | 0.849 lm | 6.83 | 0.721 |
| *T.asperellum*T54 | 1.061 kl | 11.88 | 1.126 | *T.asperellum*T54 | 0.886 lmn | 6.55 | 0.785 |
| *T.virens*T51 | 1.189 l | 10.60 | 1.414 | *T.virens*T51 | 1.016 mn | 5.71 | 1.032 |
| *T.virens*T26 | 1.215 l | 10.37 | 1.476 | *T.virens*T26 | 1.052 n | 5.51 | 1.107 |
| *T.spirale*T39 | 1.582 m | 7.96 | 2.503 | *T.spirale*T39 | 1.496 o | 3.88 | 2.238 |
| Control | 1.624 m | 4.50 | 2.637 | Control | 1.538 o | 2.15 | 2.365 |

Transformed average*: Values bearing the same letter in a column are not significatively different according to Duncan's test at 5 % probability.
RC (%)*: Reliability coefficient in percentage.
Untransformed average*: Each value obtained squaring transformed average.
Effect of *Trichoderma* isolates on leaf disc coming from IFC5 on the one hand, T85/799, IMC67, NA32 and P7 on the other hand*: By reason of the existence of distinct sensitivity of tested clones, *Trichoderma* isolates effect was separately compared for every group identified.

of *T. spirale* on mycelial growth of *P. palmivora* was not reported previously. This study also shows an interest for isolates of *T. spirale* in the selection of antagonist candidate for the biocontrol of *Phytophthora* sp.

Nevertheless, the isolates of *T. harzianum* T35, T42 and T42 revealed a lesser inhibitory activity on the mycelial growth of *P. Palmivora*. This activity contrast with the potent agents for the biocontrol of plant pathogens known as filamentous fungus *T. harzianum* (Harman et al, 2004). But, *T. harzianum* grew considerably faster on pea media agar, invaded and sporulated the same on the *P. palmivora* colonies showing an important advantage in the competition for space and nutrients with *P. palmivora*.

The macroscopic observations on the inoculated pods with mycelial discs of *P. palmivora* come from direct confrontation with *Trichoderma* isolates showed an important damage of mycelium of *P. palmivora* illustrated by the no symptom of the black pod disease. But, the mycoparasitic capacity by *Trichoderma* spp. is strain dependent. Results indicated that *T. harzianum* has the potential to provide the survival rate of *P. palmivora* of 100% though their strains invaded the *P. palmivora* colony in plates. This fongistatic activity of *T. harzianum* contrast with the necrotrophic mycoparasitic activity recognized towards of other phytopathogenic fungi (Harman and Kubicek, 1998; Howell, 2003). This fongistatic activity observed should be due to their ability to colonize and the competition for nutrients on culture media. Likewise, the secretion of lytic enzymes, antibiotic and proteins by *T. harzianum* on culture media inhibited mycelial growth but did not destroy the cell wall of *P. Palmivora*. The cellulose, essential component of the cell wall from Oomycetes, including *P. Palmivora*, would constitute an obstacle to the utilization of intracellular contents of *P. palmivora* by strains of *T. harzianum*.

However, one must take into consideration that *T. harzianum* has been described as a complex of several cryptic species (Chaverri et al., 2003), and it is possible that the ability of biocontrol is only a property of some of them. In support of this hypothesis, it has been found that > 90% of the published biocontrol strains of *T. harzianum* belong to a single ITS1 and 2 allele (C.P. Kubicek and I.S. Druzhinina, manuscripy in preparation), which is different from the one shown by the isolates obtained in the present study. Thirteen strains of *T. virens*, one isolate of *T. asperellum* T5 and *T. spirale* T4 for their part presented a fungistic activity than a more necrotrophic mycoparasitic capacity against *P. Palmivora* after their direct confrontation. This was confirmed by no observation of black pod on the inoculated cocoa pods with mycelial discs taken in the direction of *P. Palmivora*. Apart from these ones, others isolates showed a biotrophic mycoparasitism as also illustrated by Whipps (2001) and Chet (1997). Like for mycelial growth and survival of *P. palmivora*, all the strains of *Trichoderma* revealed antagonism but not allowing selection of the limited number as candidates for evaluation of efficiency in field trials.

Bioassays performed by artificial inoculation of leaf discs with *P. palmivora* and *Trichoderma* induced only a low attack; indeed the foliar sensitivity scores, for these six tested clones, were inferior to 1.7. However for leaf discs inoculated by *P. palmivora* only, this value is higher. These low foliar sensitivity scores to *P. palmivora* in relation to control explained the inhibitory action of *Trichoderma* isolates. These isolates significantly reduced the extent and frequency of necrosis caused by *P. palmivora*. Previous studies have shown that the leaf discs treated with *Trichoderma* enabled the reduction of the foliar sensitivity to *Phytophthora megakarya* (Tondje et al., 2005). The reductory action could result from germination of *Trichoderma* spores on foliar area which probably inhibit or trouble the germination of zoospores of *P. Palmivora*. This beneficial ability by colonization with our *Trichoderma* isolates stimulate or enhance the defense system consequently reinforcing the intrinsic resistance level of all tested plant material to the penetration and propagation of *P. Palmivora*.

Some analogous results were obtained on cocoa leaf discs by Tondje et al. (2005). Such reinforcement was evidenced by Bigirimana et al. (1997), Howell et al. (2000), Sid Ahmed et al., 2000 and Harman et al. (2004) on bean, cotton pepper and maize, respectively, treated with *T. virens* and *T. harzianum* against attacks of *Rhizoctonia* sp, *Collectrotrichum* sp and *Phytophthora* sp.

The inhibitory activity of the *Trichoderma* isolates were observed on pods. The cocoa pods treated with *Trichoderma* isolates and inoculated with *P. palmivora* showed varing levels of susceptibility to *P. palmivora*. Four days after inoculation, pods treated with *T. virens* significantly inhibited *P. palmivora* but an heterogeneity of the inhibittory activity of the *T. virens* population was observed. This heterogeneity indicated by Howell et al. (2000) is caused by the capacity difference of *T. virens* isolates to produce biologically active compounds and to be mycoparasitic. *T. virens* T20, *T. virens* T8, *T. virens* T19 and *T. virens* T7 showed strong ability to reduce the pod susceptivity to *P. Palmivora*. Thus, the pods treated with *T. virens* reinforced the resistance to the penetration and post-penetration to *P. palmivora*. This beneficial effect was expressed by reduction of the frequency and spread of the established lesions to *P. palmivora* on cocoa pods. Tondje et al. (2007) revealed the reduction of necrosis caused by *P. megakarya* on fragment pods treated with *T. asperellum* onto the Petri dishes. However, the pods treated with *T. harzianum* T40, *T. asperellum* T54 and *T. spirale* T4 significantly reduced the extent of necrosis caused by *P. Palmivora*. According to the induction of plants defense responses mediated by the antagonistic *Trichoderma* well documented (Yedidia et al., 1999, Sid Ahmed et al, 2000, Hanson and Howell, 2004), our isolates are inductor of the intrinsic resistance of cocoa pods and leaf discs to *P. Palmivora*. The mycoparasitic activity of *Trichoderma* towards to *P. Palmivora* on the pods in

**Table 6.** Classification of averages of leaf and pod sensitivity scores of both SCA6 and NA32 clones from the antagonist effect of *Trichoderma* isolates against *P. palmivora*.

| Effect of *Trichoderma* isolates on leaf disc stemming from SCA6* | | | | Effect of *Trichoderma* isolates on pods of NA32* | | | |
|---|---|---|---|---|---|---|---|
| **Isolate** | **Transformed average *** | **RC(%) *** | **Untransformed average*** | **Isolate** | **Transformed average *** | **RC(%) *** | **Untransformed average* (%)** |
| *T. harzianum* T36 | 0.000 a | - | 0.000 | *T.virens*T7 | 1.159 a | 10.61 | 83.98 |
| *T.virens*T58 | 0.000 ab | - | 0.000 | *T.harzianum*T40 | 1.114 ab | 11.04 | 80.55 |
| *T.virens*T56 | 0.075 ab | 142.48 | 0.006 | *T.virens*T19 | 1.085 ab | 11.34 | 78.20 |
| T.virensT19 | 0.077 ab | 138.49 | 0.006 | *T.virens*T8 | 1.085 ab | 11.34 | 78.20 |
| *T.virens*T33 | 0.080 ab | 134.24 | 0.006 | *T.asperellum*T54 | 1.05 abc | 11.71 | 75.24 |
| *T.virens*T10 | 0.089 ab | 119.86 | 0.008 | *T.virens*T13 | 1.045 abc | 11.77 | 74.81 |
| *T.virens*T41 | 0.091 ab | 117.88 | 0.008 | *T.spirale*T4 | 1.045 abc | 11.77 | 74.81 |
| *T.virens*T21 | 0.102 ab | 104.90 | 0.010 | *T.virens*T20 | 1,035 abc | 11.88 | 73.94 |
| *T.virens*T31 | 0.111 ab | 96.40 | 0.012 | *T.virens*T24 | 0.994 abcd | 12.37 | 70.26 |
| *T.harzianum*T40 | 0.112 ab | 95.54 | 0.013 | *T.virens*T25 | 0.86 abcde | 12.47 | 69.53 |
| *T.virens*T55 | 0.126 ab | 84.92 | 0.016 | *T.virens*T32 | 0.961 abcde | 12.80 | 67.20 |
| *T.virens*T7 | 0.134 ab | 79.85 | 0.018 | *T.virens*T15 | 0.55 abcde | 12.88 | 66.64 |
| *T.virens*T17 | 0.135 ab | 79.26 | 0.018 | *T.virens*T30 | 0.05 abcde | 13.59 | 61.85 |
| *T.virens*T9 | 0.146 ab | 73.29 | 0.021 | *T.virens*T45 | 0.91 abcdef | 13.80 | 60.48 |
| *T.virens*T25 | 0.171 abc | 62.57 | 0.029 | *T.virens*T31 | 0.81 abcdef | 13.96 | 59.50 |
| *T.harzianum*T44 | 0.185 abcd | 57.84 | 0.034 | *T.virens*T9 | 0.81 abcdef | 13.96 | 59.50 |
| *T.virens*T28 | 0.189 abcd | 56.61 | 0.036 | *T.virens*T29 | 0.8 abcdef | 19.77 | 59.40 |
| *T.virens*T15 | 0.190 abcd | 56.32 | 0.036 | *T.virens*T55 | 0.76 abcdef | 14.04 | 59.01 |
| *T.virens*T27 | 0.231abcde | 46.32 | 0.053 | *T.virens*T10 | 0.71 abcdef | 14.12 | 58.52 |
| *T.virens*T29 | 0.246abcdef | 43.50 | 0.061 | *T.virens*T17 | 0.49 abcdef | 14.49 | 56.34 |
| *T.virens*T24 | 0.259abcdef | 41.31 | 0.067 | *T.virens*T27 | 0.38 abcdef | 14.68 | 55.25 |
| *T.virens*T45 | 0.262abcdef | 40.84 | 0.069 | *T.virens*T6 | 0.26 abcdef | 14.89 | 54.06 |
| *T.spirale*T4 | 0.264abcdef | 40.53 | 0.070 | *T.spirale*T38 | 0.24 abcdef | 21.12 | 53.86 |
| *T.harzianum*T35 | 0.272abcdef | 39.34 | 0.074 | *T.virens*T16 | 0.18 abcdef | 15.04 | 5326 |
| *T.harzianum*T42 | 0.272abcdef | 39.34 | 0.074 | *T.harzianum*T44 | 0.8 abcdef | 15.38 | 51.46 |
| *T.virens*T8 | 0.275abcdef | 38.91 | 0.076 | *T.virens*T3 | 0.96 abcdef | 15.45 | 51.06 |
| *T.spirale*T34 | 0.299abcdefg | 35.79 | 0.089 | *T.harzianum*T36 | 0.82 abcdef | 15.73 | 49.66 |
| *T.virens*T2 | 0.307 abcdefg | 34.85 | 0.094 | *T.spirale*T46 | 0.61 abcdef | 16.16 | 47.56 |
| *T.virens*T20 | 0.326 abcdefg | 32.82 | 0.106 | *T.virens*T18 | 0.36 abcdef | 16.71 | 45.07 |
| *T.virens*T18 | 0.338 abcdefg | 31.66 | 0.114 | *T.virens*T28 | 0.32 abcdef | 16.80 | 44.67 |
| *T.virens*T16 | 0.342 abcdefg | 31.29 | 0.117 | *T.virens*T21 | 0.725abcdef | 16.97 | 43.97 |
| *T.asperellum*T5 | 0.459 bcdefgh | 23.31 | 0.211 | *T.harzianum*T42 | 0.718 bcdef | 17.13 | 43.28 |
| *T.virens*T12 | 0.527 cdefghi | 20.30 | 0.278 | *T.virens*T33 | 0,705 bcdef | 17.45 | 41.99 |
| *T.virens*T32 | 0.547 defghi | 19.56 | 0.299 | *T.virens*T2 | 0.701 bcdef | 17.55 | 41.60 |
| *T.virens*T30 | 0.565 efghi | 18.94 | 0.319 | *T.virens*T12 | 0.698 bcdef | 17.62 | 41.30 |
| *T.spirale*T46 | 0.583 efghi | 18.35 | 0.340 | *T.virens*T51 | 0,691 bcdef | 17.80 | 40.62 |
| *T.virens*T13 | 0.597 efghi | 17.92 | 0.356 | *T.spirale*T34 | 0.616 cdef | 19.97 | 33.38 |
| *T.asperellum*T54 | 0.605 fghi | 17.69 | 0.366 | *T.virens*T56 | 0.15 cdef | 20.00 | 33.29 |
| *T.virens*T3 | 0.655 ghij | 16.34 | 0.429 | *T.harzianum*T35 | 0.87 def | 20.95 | 30.68 |
| *T.spirale*T38 | 0.731 hij | 14.64 | 0.534 | *T.virens*T41 | 0.82 def | 21.13 | 30.22 |
| *T.virens*T6 | 0.760 hij | 14.08 | 0.578 | *T.spirale*T39 | 0.67 def | 21.69 | 28.85 |
| *T.virens*T51 | 0.829 ij | 12.91 | 0.687 | *T.asperellum*T5 | 0.56 ef | 22.12 | 27.86 |
| *T.virens*T26 | 0.956 jk | 11.19 | 0.914 | *T.virens*T26 | 0.458 f | 26.86 | 19.55 |

**Table 6.** contd.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *T.spirale*T39 | 1.582 m | 7.96 | 2.503 | *T.spirale*T39 | 1.496 o | 3.88 | 2.238 |
| Control | 1.624 m | 4.50 | 2.637 | Control | 1.538 o | 2.15 | 2.365 |

Transformed average*: Values followed by the same letter in a column are not significatively different according to Duncan's test at 5% threshold.
RC (%)*: Reliability coefficient in percentage.
Untransformed average*: Each value obtained squaring transformed average.
Effect of *Trichoderma* isolates on leaf disc proceeding from SCA6*: Because of the existence of distinct sensitivity of tested clones, *Trichoderma* isolates effect was compared for every group identified.
Effect of *Trichoderma* isolate on pod NA32*: Isolate T58 was not test for that, because unavailability of pods proceeded from manual pollinations.
Lack of control is attributable to its nil value, because in formula I(%) = (1 – Nn/No) x 100, No represents the average score of sensitivity at *P. palmivora* of pod control.

laboratory using bioassays led to selection of this efficient candidate for field trials. However, a positive correlation between *in vitro* assays and bioassays was observed.

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