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# *TITHONIA DIVERSIFOLIA* CROP ROTATION: AN EFFICIENT CULTURAL PRACTICE FOR CONTROL OF BURROWING (*RADOPHOLUS SIMILIS*) AND ROOT-LESION (*PRATYLENCHUS COFFEAE*) NEMATODES IN BANANA ORCHARDS IN CÔTE D'IVOIRE

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## ABSTRACT

The efficiency of *Tithonia diversifolia* on managing burrowing (*Radopholus similis)* and root-lesion (*Pratylenchus coffeae*) nematodes was examined under greenhouse and banana plantation conditions. During the greenhouse experiment, which lasted 16 weeks, 300 nematodes (150 *R. similis* + 150 *P. coffeae*) were inoculated in pots containing two-month-old burst young plants. Eighty pots were monitored and then removed at the rate of 5 pots per week so as to assess nematode development in *T. diversifolia* roots. The presence rates observed *in situ* with respect to the initial nematode inoculum were 25%, 20%, and less than 5% individuals, respectively, at 2; 6 and 7 weeks after inoculation. From 10 weeks until the end of the experiment, no presence (0 individual) could be observed *in situ*. In banana plantations; 1820 *T. diversifolia* / ha were transplanted at a rate of one cutting/banana corm. This fallow system, carried out in banana intercropping for six months, led to a thick canopy developed by *T. diversifolia* above ground with a significant production of root biomass in the soil despite the vicinity to infested banana corms. Monitoring of nematode infestations has highlighted a close relationship between the duration (X) of the fallow and the reduction in parasite pressure (Y). The regression curve $Y = 102.9X^{-3.37}$ fits this relationship with $R^2 = 0.82$. Nematode-free vitro plants implanted in the soil, 90% improved by fallow, made it possible to carry out two production cycles of bananas without nematicide application. The opportunity to involve *T. diversifolia* in the agrosystem for the production of "organic banana" as an alternative to the use of nematicide was discussed.

**Keywords**: *Radopholus similis, Pratylenchus coffeae*, banana orchards, *Tithonia diversifolia*, fallow.

## INTRODUCTION

Originating from Mexico and Central America, *Tithonia diversifolia* (Hemsl.) Gray, commonly known as Mexican sunflower, (Ipou *et al.*, 2009), has been introduced in various countries for its decorative appearance (Akobundu and Agyakwa, 1987). In Africa, *T. diversifolia* are found in several countries like: Cameroon, Kenya, Malawi, Nigeria, Côte d'Ivoire, South Africa, Tanzania, Uganda and Zambia (Duke, 1982). In Côte d'Ivoire, *T. diversifolia* (Asteraceae), *Chromolaena odorata* (Asteraceae), *Croton hirtus* (Euphorbiaceae), *Euphorbia heterophylla* (Euphorbiaceae) are examples of well-known invasive plants (Tiebre *et al.*, 2012) whose insertion happens to be one of the reasons for floristic biodiversity loss (Dutta *et al.*, 1986; Vitousek *et al.*, 1996).

For soil sanitization against endoparasitic nematodes (*R. similis* and *P. coffeae*), the establishment of non-host cover plants of those parasites in infested plots during an intercrop period is essential to generate good results by regulating the level of parasite pressure (Gowen *et al.*, 2005; Van der Veken *et al.*, 2008). Cover plants such as *Crotalaria* sp (Fabaceae), *Tagetes* sp (Asteraceae), *Mucuna* sp (Fabaceae) have often been reported as allelopathic plants with the ability to exude netamato-toxic substances (deshydrolizidine, alpha-thiernyl, L-3,4-dihydroxyphenylalanine, respectively) in soils and reduce nematode populations (Chitwood, 2002; Van der Veken *et al.*, 2008). Attempts to introduce these non-host plants into banana plantations in Côte d'Ivoire have had limited impact, due to local pedoclimatic conditions which are not very favorable for their development (Gnonhouri, 2017). In contrast, *Tithonia diversifolia* (Asteraceae), a non-native and invasive species in Côte d'Ivoire (Tiebre *et al.*, 2012) is also an allelopathic plant (Baruah *et al.*, 1994). It secretes toxic substances (Tagitinin C) capable of inhibiting the growth of other plant species (Baruah *et al.*, 1979; Tongma *et al.*, 1998). The establishment of this Asteraceae during the intercrop phase, could be beneficial in banana plantations because of its invasive behavior. The resulting monospecific fallow may: *i)* indirectly act on nematode populations by eliminating other weeds including those hosting banana nematodes (Quénéhervé *et al.*, 2006); *ii)* directly control nematodes if the non-host-plant status of this Asteraceae vis-à-vis *R. similis* and *P. coffeae* is proven. It is therefore necessary to better understand the links between *T. diversifolia*, nematodes (*R. similis* and *P. coffeae*) and banana orchards so as to better manage the integration of this non-native and invasive Asteraceae into a banana agrosystem. The aim of this work is to determine the host status of *Tithonia diversifolia* vis-à-vis *R. similis* and *P. coffeae* with a view to developing a cultural practice strategy that minimizes the use of nematicides in banana plantations.

## MATERIAL AND METHODS

**Assessment of *Tithonia diversifolia* host status in greenhouse:** The experimental studies on interaction between *Tithonia diversifolia* versus *Pratylenchus coffeae* and *Radopholus similis* were carried out at the Research Station of the National Center for Agronomic Research (CNRA) of Bimbresso in Côte d'Ivoire, West Africa in a greenhouse. Greenhouse conditions was adjusted. Humidity range was 80 - 90% and temperature 27 °C- 28 °C. *T. diversifolia* rods were taken from spontaneous fallow, carefully sliced into 15 to 20 cm long cuttings, and planted into 1.5 L plastic bag pots filled with substrate already steam-sterilized, and mixed with sand (1/3) and silt (2/3) (Messiaen and Lafon, 1970). Four weeks after nursery plantation, germinated seedlings of *T. diversifolia* were inoculated with *R. similis* and *P. coffeae* already cultured under laboratory conditions (Boisseau and Sarah, 2008), at the rate of 300 nematodes (150 *R. similis* and 150 *P. coffeae*) per seedling *with the help of* graduated pipette, into 3- 5 cm-deep within 0.5 cm radius around each seedling. This was further followed by daily watering. Among inoculated plants, 80 plants were uprooted, and 5 plants sample was observed after each seven day up to 112 days after inoculation. Nematodes penetration was observed by staining the roots with 3.5 $g.l^{-1}$ acid fuchsin (Bybd Jr *et al.*, 1983) and counted *in situ* under a stereomicroscope.

**Field studies on comparison of *T. diversifolia* controlled fallow in banana orchard:** This experiment consisted of four steps:

**Step 1: Chemical destruction of banana corms and follower suckers at intercrop phase:** In the BB5 square of the SAKJ plantation in Akressi (Southeastern Côte d'Ivoire) left as fallow for 6 months, the pseudo-stems of the mother plant and any follower suckers were chemically destroyed by a double glyphosate injection (Chabrier and Queneherve, 2003).

**Step 2: *Tithonia diversifolia* controlled fallow:** Two-month-old *T. diversifolia* seedlings raised in nursery were transplanted within less than 50 cm of the banana corms at the rate of one seedling per devitalized pseudo-stem. *T. diversifolia* and banana corms were left as fallow for six months with the following three benefits: *i) T. diversifolia* has the same density (1,820 seedlings/ha) as number of banana plants eradicated, *ii) T. diversifolia* take humidity and nutrients from decomposition of banana corms *iii) T. diversifolia* roots were in direct contact with a potential residual inoculum of nematodes in the rhizosphere of destroyed banana corms.

**Step 3: Diagnosis of the sanitization of *T. diversifolia* controlled fallow:** Soil sanitization against nematode infestations was monitored every two months with the biological test of fallow diagnosis (Lassoudière, 2007). Nematodes were extracted from the roots by double centrifugation technique (Coolen and d'Herde, 1972) and the populations were expressed as number of individuals / 100 g of root.

**Step 4: Replanting in vitro banana plants after the six months of fallow with *Tithonia diversifolia:*** The biomass of *Tithonia diversifolia* was removed from the experimental site to set up the test. Experiment was arranged into ''large'' and ''small'' plots with seven treatments. In ''large plots'' there were vitro banana plants (V) with two levels: V1 (plants treated at nursery with Nemastin©) and V2 (plants untreated at nursery). In ''small plots'' or elementary plots, with three levels: T0 (control, no nematicide), T1 (20 ml/plant of Nemastin©), T2 (30 g/plant of Nemathorin©). The previous six treatments (V1, V2) x (T0, T1, T2) were compared to the Reference (banana plots in continuous production, monitored from the 4th to 5th cycle with fostiazate ''Nemathorin ©10G'' applications at 30 g / plant every 4 months).

The seven treatments were four replications with 28 elementary plots and 2,520 plants. Each elementary plot (500 m$^2$) consisted of 90 bananas, including 36 border plants and 54 central plants useful for observations. Root samples for monitoring nematode populations were collected monthly. A composite sample of 500 g of roots from 10 plants / elementary plot was collected in the first 30 cm of the soil. The roots were chopped, carefully mixed and a 40 g sub-sample was processed with double centrifugation (Coolen and d'Herde, 1972). The yield parameters were measured individually in each useful plot at the end of each cycle (number of hands / bunches, grade of fingers, weight of bunches).

## RESULTS

In the greenhouse trial, weekly monitoring of *R. similis* and *P. coffeae* in the inoculated roots of *T. diversifolia* showed nematode population dynamics in two steps (Figure 1). During the first six weeks, 25% of the individuals of the initial inoculum entered the roots of *T. diversifolia.* After 10 weeks the juveniles from two species were found without ability to infect. *T. diversifolia* transplanted within a radius of 50 cm of the devitalized banana corm led to development of several stems (Figure 2) of this Asteraceae with a thick canopy formation above ground. In the soil, an important root biomass without necrosis was also developed by *T. diversifolia* (Figure 3) despite the vicinity of a potential inoculum from decomposed banana corms.

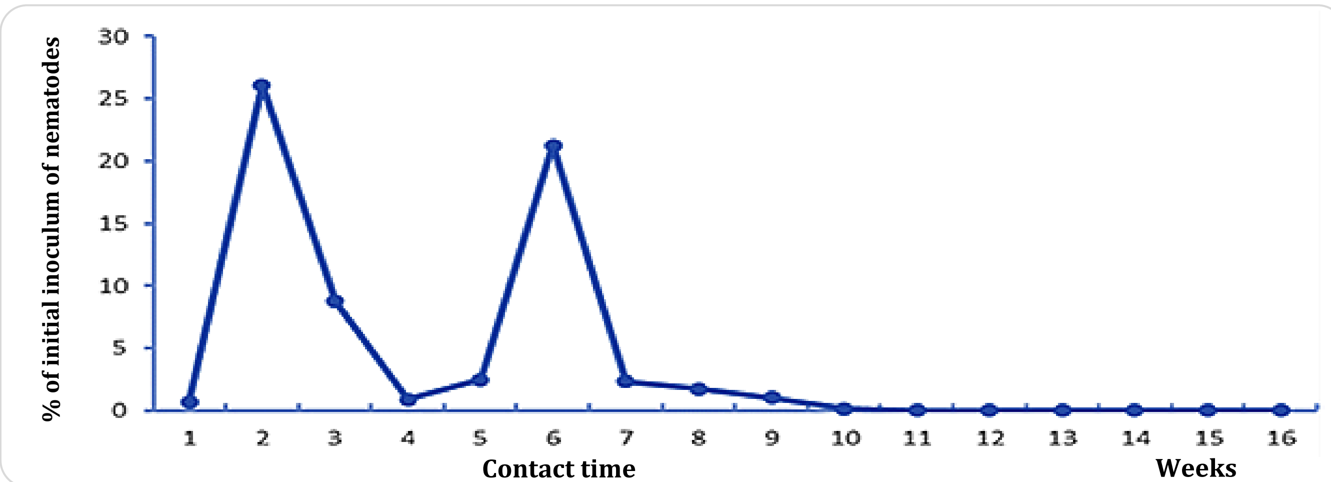



Figure 1. Penetration of nematodes in the roots of *T. diversifolia* inoculated with 300 juveniles (150 *Radopholus similis* + 150 *Pratylenchus coffeae*) during greenhouse experiment.

Figure 2. Several stems developed from a single cutting of *T. diversifolia* in six-month controlled fallow.

Figure 3. Important root biomass developed by *Tithonia diversifolia* despite the vicinity to nematode-infested corm.

During the six months fallow of *T. diversifolia,* monitoring of nematode infestations revealed a very good soil sanitization that fits the regression curve Y = $1012.9X^{-3.73}$ with $R^2$ = 0.82 (Figure 4). After the previous fallow of *T. diversifolia,* re-infestation of the experimental plots during the first cycle of banana tissue plants (vitro plants) production, showed very low levels (<300 individuals / 100 g) for both *R. similis* (Figure 5A) and *P. coffeae* (Figure 5B). Regarding the 2nd cycle, the very high level of infestations (10,001 <individuals/ 100 g < 20,000) of *R. similis* and *P. coffeae* (Figure 6) impacted only 10% of experimental plots. However, the threshold level of banana root infestations (1000 < individuals/ 100 g < 5000) was observed in 10% and 40% of experimental plots respectively for *R. similis* (Figure 6A) and *P. coffeae* (Figure 6B). This decrease in parasitic nematodes pressure in the plots previously planted with *T. diversifolia* resulted in a significant improvement in the number of hands/bunch and weight/bunch compared to the Reference (Table 1). From one cycle production to another, the average of the bunch weight rose from 23.97 kg in the first cycle to 25.48 kg in the second cycle compared to 16.35 kg for the Reference.

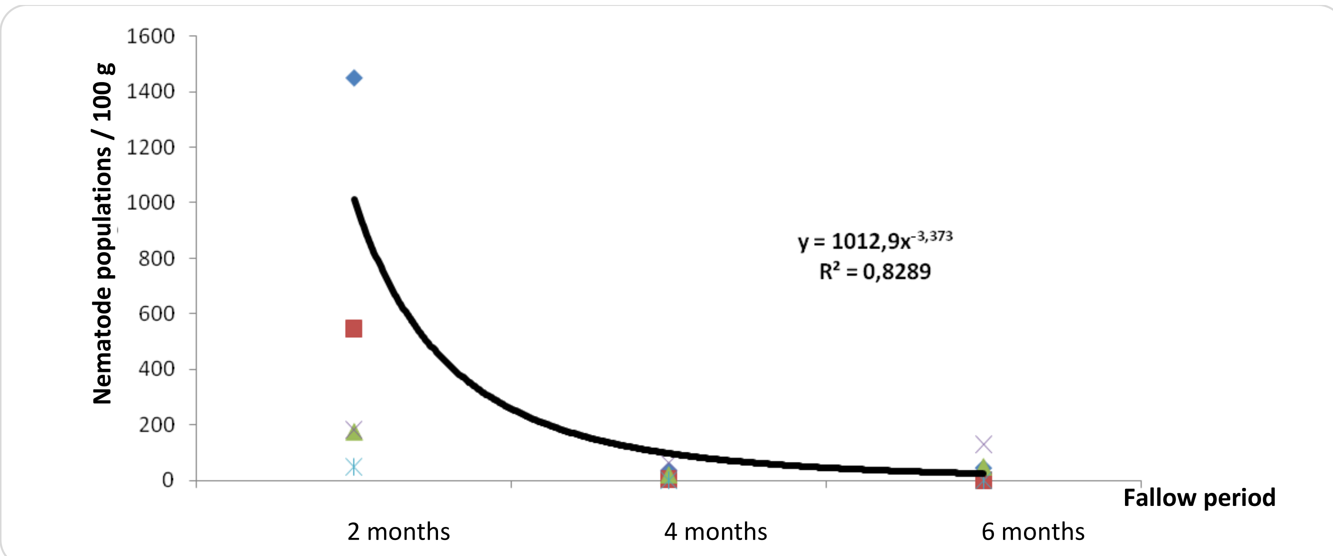



Figure 4. Regression curve of nematode populations in relation to the fallow time of *T. diversifolia* during banana intercropping.

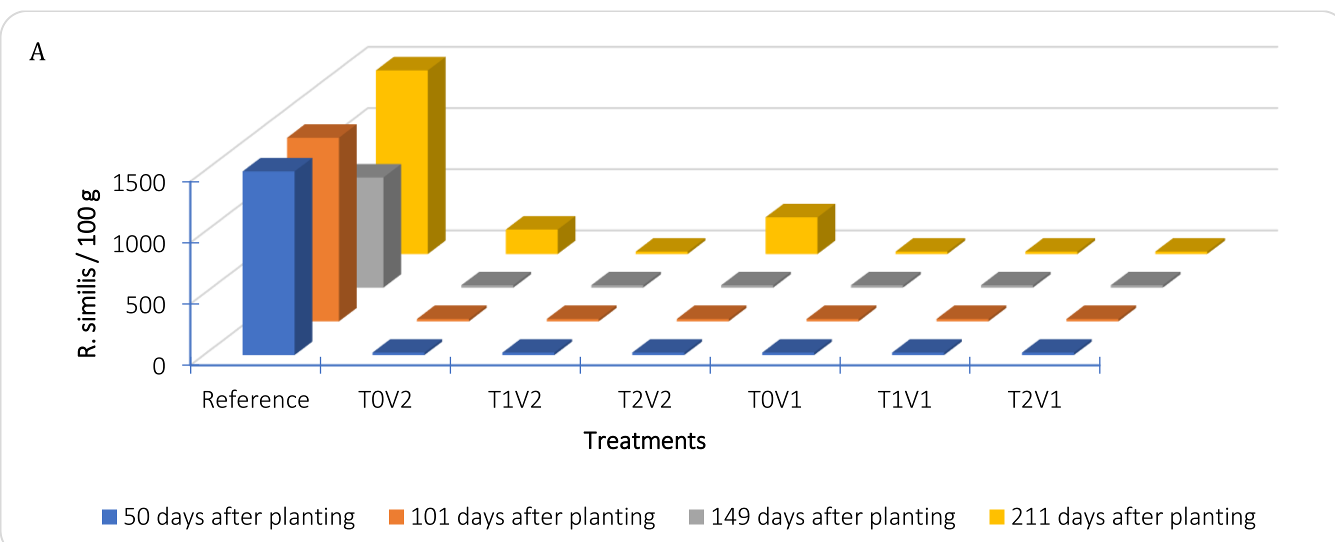



Figure 5 (A). Reinfestation of banana vitroplants by *Radopholus similis* during the first production cycle after the controlled fallow of *Tithonia diversifolia.*

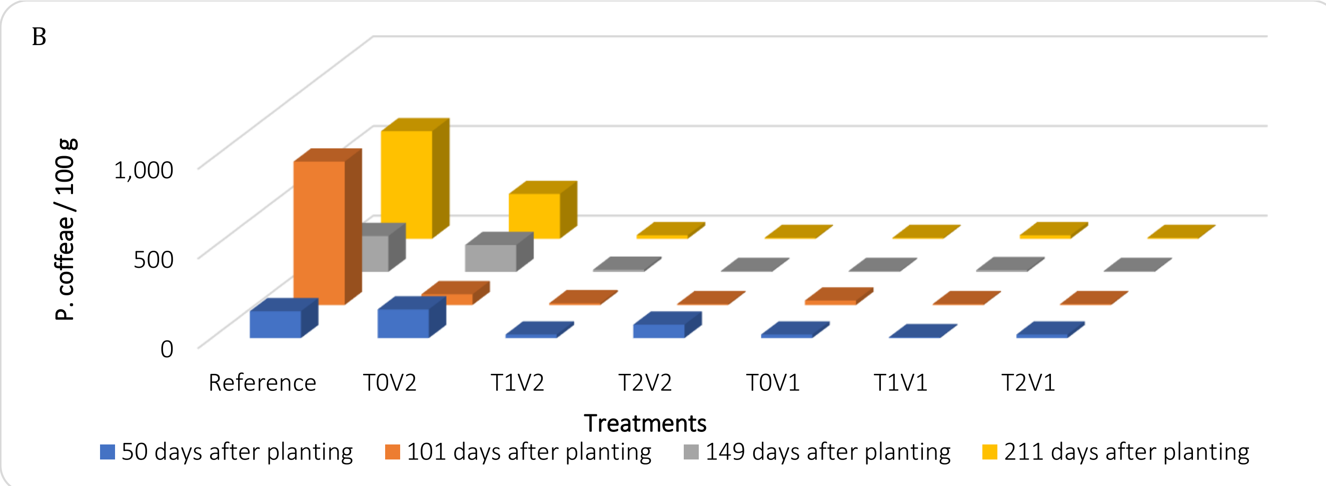
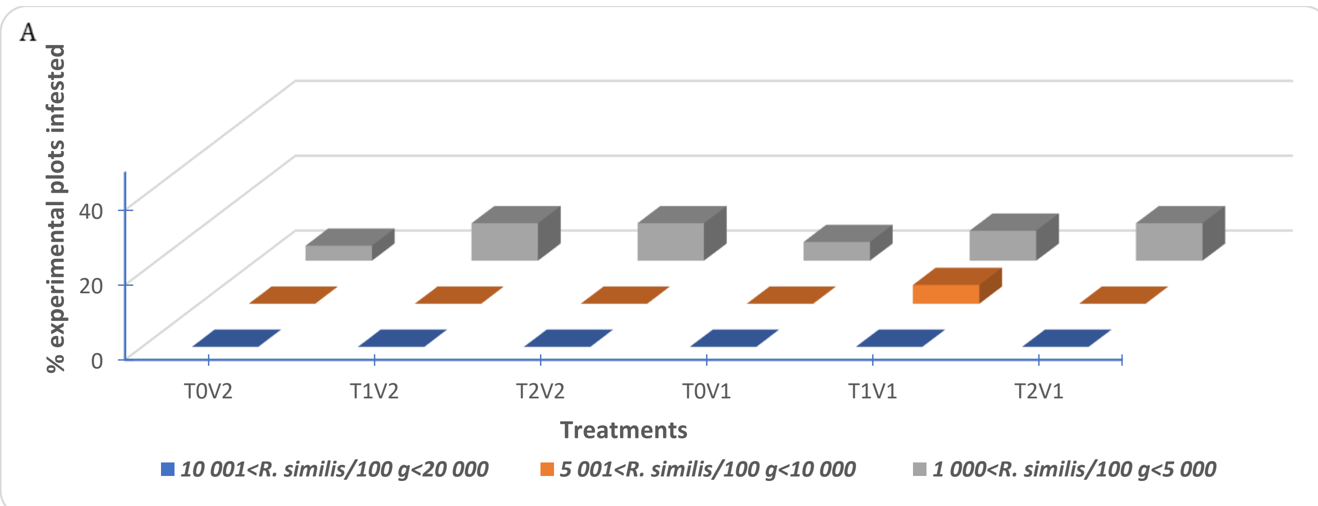



Figure 5 (B). Reinfestation of banana vitroplants by *Pratylenchus coffeae* during the first production cycle after the controlled fallow of *Tithonia diversifolia.*



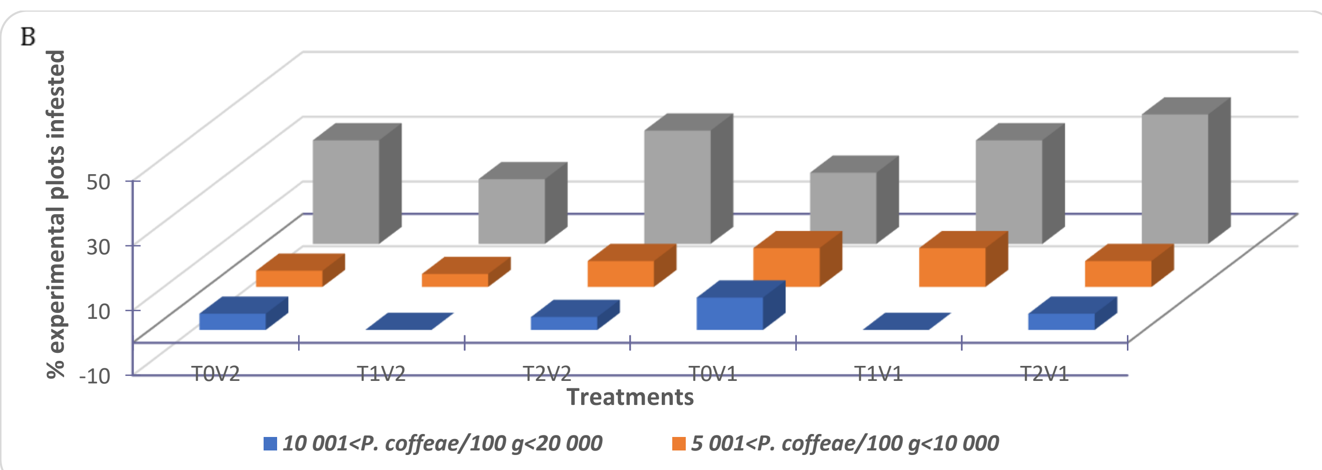




Figure 6. Percentage of experimental plots infested by *Radopholus similis* (A) and *Pratylenchus coffeae* (B) at the second banana vitroplants production after the controlled fallow of *Tithonia diversifolia.*

Table1. Effect of the previous six-month fallow of *Tithonia diversifolia* on the harvest parameters of a successive banana crop over two production cycles compared to a continuous banana production.

| Harvest parameters | Continuous banana production | Previous six-month fallow of *Tithonia diversifolia* | | | | | |
|---|---|---|---|---|---|---|---|
| | Reference¥ | T0V1 | T1V1 | T2V1 | T0V2 | T1V2 | T2V2 |
| *First cycle of production* | | | | | | | |
| Number of hands/bunches | 6a | 8b | 8,2b | 7,8b | 8,2b | 8b | 7,8b |
| Finger grade (cm) | 31,2a | 31,3a | 31,5a | 31,5a | 31,5a | 31,3a | 31,7a |
| Bunch weight (kg) | 16,5a | 24,3b | 23,7b | 24,3b | 23,7b | 23,5b | 24,3b |
| *Second cycle of production* | | | | | | | |
| Number of hands/bunches | 6a | 8,5b | 8,7b | 8b | 8,5b | 8,7b | 8,3b |
| Finger grade (cm) | 31,2a | 31,2b | 31,2b | 31,2b | 31,2b | 31,5b | 31,2b |
| Bunch weight (kg) | 16,2a | 25,8b | 25,2b | 25,9b | 25,2b | 25,5b | 25,3b |

¥ *:* Continuous banana production at 4th et 5th cycles

Letters in the same line correspond to homogeneous group (Newman-Keul's test P ≤ 0.05).

**T0V1**: vitro plants only treated at the nursery with bio-nematicide Nemastin©

**T1V1**: vitro plants treated at the nursery and during planting with bio-nematicide Nemastin©

**T2V1**: vitro plants treated with bio-nematicide Nemastin© at the nursery + chemical-nematicide Nemathorin© 10G during planting

**T0V2**: untreated vitro plants at the nursery and no nematicide applied during planting

**T1V2**: untreated vitro plants at the nursery + bio-nematicide Nemastin© applied during planting

**T2V2**: untreated vitroplants at the nursery + chemical-nematicide Nemathorin© 10G applied during planting

**Reference**: banana plots in continuous production, monitored from the 4th to 5th cycle with fostiazate applyings at 30 g/plant of Nemathorin© 10G every 4 months.

## DISCUSSION

During greenhouse trial, the presence rates observed *in situ* with respect to the initial nematode inoculum were 25%, 20%, and less than 5% individuals, respectively, at 2; 6 and 7 weeks after inoculation. This *in situ* low rates might be the maximum number of inoculated nematodes that can infest *T. diversifolia* roots. According to Kaplan and Keen (1980), the presence of penetration barriers for nematodes is not effective against the combined action of stylet penetration and enzyme release by nematodes. Interestingly, there was no development of nematodes (*R. similis* and *P. coffeae*) in *T. diversifolia* roots for 10 weeks after the first penetration. *R. similis* and *P. coffeae* could not multiply *in situ*, suggest the activation of a defense mechanism (Giebel, 1982) in *T. diversifolia* to protect itself against nematode parasites generally triggered (Kaplan and Davis, 1987; Fogain and Gowen, 1995; Sijmons *et al.*, 1994; Nicholson and Hammerschmidt, 1992). This process (Wuyts *et al.*, 2007) by release of phenolic compounds, flavonoids, terpenoids and lignification in attacked plant tissues to prevent any multiplication (Wilski *et al.*, 1970). It would be interesting to investigate this process of "acquired resilience" with subsequent biochemical analyzes to complete our encouraging results.

In the banana plantation trials, the evaluation of the control of soil infestations of *R. similis* and *P. coffeae* by *T. diversifolia* in fallow period, revealed a significant reduction in pest pressure. This result, confirming the observation of a defense mechanism developed in the greenhouse trial by *T. diversifolia*, corroborates the studies of Ternisien and Ganry (1990) on the non-host plant status of *Crotalaria juncea*, *Canavalia ensiformis* and "service plants" (Chauvin, 2015) that regulate nematode communities in a banana production system intercropping in Martinique. These cultural practices in this French West Indies have improved the productivity of banana plantations with a 60% reduction in nematicidal applications (Chabrier *et al.*, 2004; Quénéhervé *et al.*, 2006). Like legumes (*C. juncea* and *C. ensiformis*) and as the previously mentioned "service plants", *T. diversifolia* allowed to "break" the life cycle of *R. similis* and *P. coffeae* and has expanded the very narrow range of cover plants able to control *R. similis* (O'Bannon, 1977) and *P. coffeae* (Bridge, 1993) polyphagia.

For *T. diversifolia* integration in banana plants management technique, it would be interesting to adopt the fallow model with a density of 1,820 cuttings of *T. diversifolia* / ha. The vicinity of *T. diversifolia* with an infectious potential nematodes of devitalized banana corms, does not affect the root biomass of this Asteraceae. This beneficial effect of *T. diversifolia* on the nematode fauna is in addition to the improvement of the root drainage of this Asteraceae reported by Tié (2017) on the cultivation substrates (heavy and compact soil, clay soil) of banana orchards in Côte d'Ivoire. The positive effect of soil sanitization against nematodes led to a slow and lasting reinfestation of banana vitroplants during two production cycles; which corroborate the nematicide efficiency of powder residues (Odeyemi *et al.*, 2014) and the organic amendment (Atandi *et al.*, 2017; Osei *et al.*, 2011) of *T. diversifolia* on nematodes of other cultures.

The last ten years were marked by the development of ''organic banana'' (Lassoudière, 2007). In Côte d'Ivoire, this agrosystem represents about 3% of the areas intended for conventional production (Loeillet, 2019). With the growing trend of this crop, our results suggest that *T. diversifolia* crop rotation, during six-month fallow with 1,820 cuttings/ha could be an alternative solution to the use of nematicides. Because this agrosystem, as defined by the *Codex Alimentarius Commission*, is ''a global system of production management that avoids among other agricultural inputs, pesticides in order to optimize the health and productivity of interdependent communities of plants, animals and people" (Nadia, 2013). As the main strategies adopted to increase its resilience to stress, for example the constraint related to nematodes (*R. similis* and *P. coffeae*) as shown by our study, this agrosystem implements according to Nadia (2013): rotation, diversification and integration of cultures. In this perspective, our study has shown that *T. diversifolia* can 'break' the life cycle of *R. similis* and *P. coffeae* during banana intercropping and allow at least two production cycles with no application of nematicides. *T. diversifolia* proved as a non-host nematode plant suitable as alternate crop of banana in this production system which is environment friendly. The North of Côte d'Ivoire, which is infested with black Sigatoka disease (Traoré *et al.*, 2016), needs more for this type of production system without fungicides; and the nematodes (*R. similis*, and *P. coffea*) management without nematicides with intercropping of *T. diversifolia*.

## CONCLUSION

*T. diversifolia* turned out to be a ''trap'' plant for *R. similis* and *P. coffeae* can break the life cycle of these parasitic nematodes in six months' time period and ensured adequate soil sanitization in banana orchards at least for two seasons. These results are an opportunity to avoid the use of nematicides in banana orchards in general and in fast-growing ''organic banana'' production in particular. However, we recommend that further investigations should be carried out with biochemical analyses to better understand *Titonia diversifolia*'s "acquired resilience" vis-à-vis nematodes.

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