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Summary

The teams involved in the current deliverable are CIRAD and PRI-WUR. As the reasserted objective for this deliverable was directly linked with the necessity to promote dissemination of scientific knowledge, we present here different scientific papers depicting short and mid- term innovations for reducing pesticides in banana cropping systems , as their potential risks for human health and the Environment. Some of these papers were recently released through international publishing, while others which are briefly mentioned are still under revision in scientific journals.

This deliverable is organized into four sections. The first section deals with a paper (paper 1) giving an overall view of alternative and innovative practical strategies for achieving pesticide reduction in bananas, through IPM approaches. This paper also underlines that in the last ten years, innovation in banana production systems combined with new regulations banning the most harmful pesticides, resulted in a drop by more than 60% in pesticide use expressed here as quantities of active ingredient by hectare or by ton of produced bananas (and not yet as TFI -Treatment Frequency Index-).

The second section depicts specific strategies implemented for controlling plant parasitic nematodes in bananas without having recourse to chemical nematicides. This section is structured by two papers (paper 2 and paper 3).

The third section presents the recent knowledge gained for potentially limiting dispersal of the black weevil *Cosmopolites sordidus* in banana fields, thus offering the opportunity not to permanently have recourse to insecticides. In particular, this section focuses on a paper (paper 4) describing a predictive model to analyse *C. sordidus* dispersal. Simulation should allow optimizing vegetation organization in banana fields in order to delay colonization and alleviate damage without a permanent recourse to insecticides.

In the fourth section, we depict various laboratory tools to i/ monitor in bananas the fungal resistance to the foliar pathogen *Mycosphaerella musicola* responsible for the well-known Yellow Sigatoka Disease (paper 5), or also to monitor resistance to the fruit pathogen *Colletotrichum musae* responsible for post-harvest damage (paper 6). ii/ assess diversity in *Mycosphaerella fijiensis* populations with VNTR markers (Variable Number Tandem Repeats, paper 7). Those fungi are the most damaging -and fungicide consuming sprayings- in banana agrosystems.

Many of the results presented here are often -but not exclusively- originating from the French West Indies. Nonetheless, they can validly be extended and adapted to any other banana producing countries willing to promote more sustainable banana cropping systems relying less on pesticides.

Most of the banana cropping strategies (sanitizing fallows against plant-parasitic nematodes, protective ditches ...), trapping of the black weevil, monitoring methods for fungal resistance in bananas are validated, and have begun to be applied at the field by growers and/or field extension officers. More recently released research tools with direct and short terms applications for pesticide reduction such as the model COSMOS for the black weevil, or VNTR markers for *Mycosphaerella* fungi require additional steps to be fully validated.

1. A review of short and mid-term possibilities to launch and consolidate IPM strategies in banana production systems : The case study of the French West Indies

The present review is based upon a paper (paper 1) making a census on the recent knowledge that was developed, and began to be applied in the fields of banana growers of the French West Indies willing to launch more sustainable cropping systems. It was of interest to focus on alternative and innovative cropping strategies for bananas in an insular and sensitive environment, seeing that many components of the proposed IPM strategies could be adopted or adapted in other growing banana countries.

Although residues of plant protection products in bananas generally match European MRLs (Maximum Residues Levels)[a], it is capital to minimize pesticide use and their risks as well for consumers in the importing countries, as for human populations and marine or terrestrial food webs in the producing zones, seeing they can potentially have adverse health and environmental effects)[b]. From which the necessity to give a particular attention to alternative or innovative management in banana production systems.

The proposed strategies integrate i/ well-fitted cultural practices including sanitizing fallows and use of nematode non-host cover crops; ii/ biological control means, among which are pheromone mass trapping against the black weevil, and biofungicides. iii/ forecasting systems and monitoring procedures against the fungal airborne *Mycosphaerella* leaf spot diseases (mainly *M. fijiensis* and *M. musicola*). iv/ recourse in the mid-term, to yet conventionally bred banana varieties showing resistance to the worldwide and threatening airborne Black Leaf Streak Disease (http://www.apsnet.org/education/feature/banana/) formerly named Black Sigatoka Disease.

[a](see for instance Hernandez-Borges et al., 2009. Analysis of pesticide residues in bananas harvested in the Canary Islands (Spain). Food chemistry, 113:313-319; Tixier et al, 2007. Pesticide residues in heterogeneous plant populations, a model-based approach applied to nematicides in banana (*Musa* spp). Journal of agricultural and food chemistry, 55:5204-2508).

[b](see for instance Coat al, 2006. Contamination of some aquatic species with the organochlorine pesticide Chlordecone in Martinique. Aquatic living resources, 19:181-187; Castillo et al., 2000. Pesticide residues in the aquatic environment of banana plantation areas in the North Atlantic zone of Costa-Rica. Environmental toxicology and chemistry, 8:1942-1950).

Paper 1:

Integrated Pest Management Approaches Developed in the French West Indies to Reduce Pesticide Use in Banana Production Systems

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Abstract

The monoculture of banana can have a serious detrimental impact on the environment as pesticide treatments can lead to surface and groundwater pollution. Different approaches based on IPM (integrated pest management) have been developed in the French West Indies to reduce the use of the pesticides in banana cultivation. These methods have been developed using management techniques that prevent the build-up of banana pathogens and also eliminate them, non-chemical techniques, such as cultural practices and biological control, and resistant cultivars. As a result, new crop management systems have led to a 65% decrease of pesticide use over the last 10 years. Major results of the research already undertaken and future research that is being considered to reduce pesticide use in banana plantations are reviewed.

INTRODUCTION

Dessert bananas grown for export, which amount to 16 million tonnes per year, form a large part of agricultural production in some tropical countries. They are usually cultivated in an intensive monoculture sustained by the application of large quantities of fertilisers and pesticides. The few cultivars used for production are susceptible to numerous pests and diseases. Genetic improvement is difficult, and resistant commercial export cultivars are not yet available. The present production system has a strong negative environmental impact, leading to reduced soil fertility, proliferation of soil pests, soil structure degradation, damage to the organic quality of the soil and the reduction of biodiversity. Increasingly strict regulations for pesticide use and the respect of 'good farming practices' are decided at public-authority level in Europe or imposed by retail chains anxious to meet consumer expectations. The development of new, less polluting cropping systems has become a major issue for producer countries and consumers. Recent results of work undertaken into the non-chemical control of banana pests and diseases in the French West Indies (FWI) are reported here.

IMPROVEMENTS TO NEMATODE CONTROL

Banana nematodes are frequently present in the roots in mixed species communities (Cofcewicz et al., 2005; Risède et al., 2004). Their overall effect on the crop results from interactions between the cropping system and environmental conditions (soil type, climate). Banana nematodes can cause production losses of more than 50% (Chabrier et al., 2005a; Gowen et al., 2005). Traditional control methods have long been based on the use of chemical nematocides, especially organophosphates and carbamates. Most of these substances are classified as toxic or highly toxic and are increasingly

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abandoned in favour of alternative methods that have a lower negative impact on human health and the environment.

In the mid-1980s, the precepts of preventive control "healthy plant material (from in vitro culture) in a healthy soil (obtained through rotation or fallow)" have been formulated. In the past decade, research has optimised cultural control through the rational use of nematode cleansing fallows and providing decision tools, such as biological tests to evaluate the quality of nematode cleansing in fallows, the occurrence of nematodes in tissue-culture plant nurseries or in the field (Chabrier and Quénéhervé, 2003). In addition, Cavendish (AAA genome) clones less susceptible to nematodes have been tested and validated. Based on the results of research, banana producers in the FWI have adopted the use of fallow and the practice of rotating banana with crops, such as pineapple or sugar cane, to control nematode infestations (Chabrier et al., 2005b). The adoption of these control methods has been more of a necessity because of French and European phytosanitary legislation, which has restricted the use of a number of registered nematocides. As a result, use of nematocides has decreased by more than 60% in the last 10 years (Chabrier and Quénéhervé, 2001; Chabrier et al., 2005b; Fig. 1).

Several innovations for cropping systems in the West Indies are ready for testing and incorporation in the crop management sequences already developed: new hybrid bananas that are resistant or tolerant to banana nematodes, and cover crops that are non-hosts for nematodes and that may be used in crop rotations or in association with banana (Quénéhervé et al., 2002). Ongoing research efforts include the identification of nematotoxic cover plants, the design of sustainable strategies for the use of banana hybrids resistant to nematodes and understanding the links between biological diversity in soils and the sustainability of cropping systems.

IMPROVEMENTS TO THE CONTROL OF MYCOSPHAERELLA LEAF SPOTS

Mycosphaerella leaf spot diseases are considered the most serious foliar diseases of banana and a major production constraint for dessert banana exports. Dessert banana production in the FWI - like everywhere else in the world - is based exclusively on banana cultivars in the Cavendish subgroup that are very susceptible to leaf spots. Control is essential if fruit is to be exported.

FWI banana plants are currently affected by Sigatoka leaf spot and are threatened by the introduction of black leaf streak, which is spreading in the Caribbean region. Currently, the only commercial control method is the frequent aerial spraying of fungicides. However, fungicides are harmful to the environment, and the causal fungi have an evolutionary potential to develop resistance against fungicides (de Lapeyre de Bellaire, 1990; de Lapeyre de Bellaire et al., 2009).

A reduction in fungicide usage for leaf spot control is a priority in the FWI because of the withdrawal of certain fungicides, the human health concerns of the local population and tourist industry, and the expected ban on aerial spraying in the near future.

Use of a Disease Forecasting System

The forecasting system is aimed at reducing the number of fungicide sprays per year while still achieving effective control of the diseases. This reduces the production costs, the pollutant load and the risk of appearance of resistance. The system is based on the use of mineral oil, which has a fungistatic effect, and a mixture of systemic fungicides (of five different chemical families). The treatment must be applied to an entire production area as leaf spot diseases are spread by wind (Ganry and Laville, 1983).

Forecasting systems perfected at the beginning of the 1970s for Sigatoka leaf spot (Ganry and Laville, 1983) and in the 1980s for black leaf streak (Fouré, 1988) have considerably reduced the number of fungicide spray applications per year, and thus production costs. Without a forecasting system, 25 and 50 sprays per year are required to control Sigatoka leaf spot and black leaf streak, respectively. Using the forecasting systems, spray applications to control Sigatoka leaf spot in the FWI has been reduced by 75%, which is amongst the lowest application rate in the world, and spray applications to

control black leaf streak in Cameroon have been reduced by more than 60% (de Lapeyre de Bellaire et al., 2009). Forecasting systems considerably reduce the environmental impact of chemical control of leaf spot diseases. Without a forecasting system for Sigatoka leaf spot, the quantities of active substance per hectare per year would amount to 20 to 40 kg compared to 0.7 to 1,5 kg with a forecasting system.

The use of forecasting systems may also slow the appearance of populations with resistance to certain systemic fungicides (Ganry and Laville, 1983). In the FWI, the forecasting system has been used to control Sigatoka leaf spot disease successfully for over 30 years, with an average of 6-8 fungicide spray applications sprays per year. In Cameroon where a forecasting system has been used, fungicide resistance to triazoles appeared in *M. fijiensis* populations only after more than 15 years as compared to 5-8 years in Latin America. The emergence of resistant strains in the conditions of Cameroon was due to insufficient logistics for spraying operations. With adequate disease management, fungicide resistance would probably have not emerged.

Research is now in progress on perfecting a new forecasting system using meteorological parameters that could trigger preventive treatments.

Resistant Cultivars

The use of banana cultivars with resistance to leaf spots is considered to be the most durable control method. A CIRAD breeding and selection programme was begun in the 1980s to produce dessert banana hybrids with resistance to leaf spot diseases. New cultivars developed are currently under evaluation. Large-scale cultivation of leaf spot-resistant banana cultivars will have several impacts. It will lead to a drastic reduction in fungicide spray applications and will also reduce the overall inoculum pressure in fields of susceptible cultivars grown in the same production area.

Knowledge of the fungal population structures and banana-pathogen interactions has also increased over the last 10 years (Rivas et al., 2004; Abadie et al., 2003). This helps in the choice of parents for breeding and has allowed the development of early tests for susceptibility to leaf spots. Strategies for the use of these new cultivars are now being tested.

IMPROVEMENTS IN THE CONTROL OF BANANA WEEVIL

Chemicals have historically been used to control the banana weevil (*Cosmopolites sordidus*). Highly toxic insecticides used earlier included organochlorides (chlordecone), organophosphorus insecticides and phenylpyrazole (Chabrier et al., 2005a). Past use of the persistent chlordecone has resulted in serious soil and water pollution problems today. The four groups of insecticides used worldwide today are nicotinoids, phenyl-pyrazol, carbamates and organophosphorus compounds. These are often toxic for humans and other non-target organisms.

In the FWI, a ban on cadusafos is planned, leaving Nemathorin® 10G, (which is primarily a nematocide and its insecticidal effect is a secondary feature (Chabrier et al., 2002)) as the only permitted product. In Martinique, the use of products has decreased over a 10-year period (1996 to 2006) from 130 tonnes to zero for insecticides only, and from 580 to 162 tonnes for combined insecticide/nematocide products, with no significant decrease in yields (Fig. 1). These results can be ascribed at least partly to the new cultural practices and control methods.

Pheromone Traps

The first traps using sordidines (aggregate pheromones of *C. sordidus*) were designed by Chemtica in 1997 (Alpizar et al., 1998). A study programme was launched to evaluate their efficiency as a mass-trapping tool and as a population indicator. Although mass trapping can be effective in slowing down infestation, when infestation is strong, traps only capture that part of the population that moves, which may be less than half of the total population. Many growers in heavily infested areas in Guadeloupe and Martinique have observed that when a plantation is destroyed using mechanical means,

the number of weevils captured increases three to five-fold, indicating that this proportion of the *C. sordidus* population remained sedentary when the banana plants were standing. In other words, the attraction of the banana plants competes with that of the traps. This control method also requires regular checks on the lure and good plantation management, as weed growth reduces trapping effectiveness and weevil incidence is greater on slow-growing plants. Mass trapping is less effective in controlling *C. sordidus* in the long term. Therefore, trapping should be complemented by the use of other methods.

Monitoring traps has helped to show how weevil populations behave in fallow fields after plants have been treated with glyphosate. Glyphosate destroys the burrowing nematode *Radopholus similis* effectively, but not *C. sordidus* (CIRAD, unpublished data). Numerous adults shelter in the corms of dead banana plants. When this material is destroyed mechanically, traps capture large numbers of weevils. This can be explained by the fact that, after the destruction of their habitat, practically all the adult weevils become mobile. Keeping traps in fallow fields would thus seem beneficial in reducing pest pressure. Mobile weevils would be destroyed instead of infesting neighbouring banana plantations.

Ongoing research efforts include studies of spatial arrangements of the cultures to reduce weevil development and to improve the efficiency of trapping.

Biological Control

The French National Institute for Agricultural Research (INRA) in Guadeloupe has bred entomopathogenic nematode strains that effectively control *C. sordidus* in the laboratory. In the field, the results were disappointing, because the nematodes are susceptible to nematocides and spread poorly. Sordidine traps can be used to attract weevils into a confined, limited space for inoculation by an entomopathogen rather than killing them. The trapped specimens can then be used as spreading agents. Pheromone traps to which entomopathogenic nematodes (*Steinernema carpocapsae*) were added weekly gave good results in Martinique in two series of trials in areas where pest pressure was particularly strong, on the condition that the general state of the plantations was good (Chabrier et al., 2002). Research to improve biological control methods is ongoing.

Prevention by Field Maintenance

To eliminate food and shelter for the weevil, corms and pseudostems of fallen or dead plants must be cut into sections and then split lengthwise to favour rapid decomposition. Mechanical destruction of the corms is necessary after chemical destruction of plants when fallows are initiated. Rotary spading machines break up dead corms much better than disc harrows. Field drainage should be installed as moist ground enhances the weevil development. Weeds should be controlled and the crop suitably fertilised to promote a vigorous crop.

IMPROVEMENTS IN THE CONTROL OF POST-HARVEST DISEASES

Two forms of anthracnose, both caused by *Colletotrichum musae,* produce storage diseases. Ripe-fruit (quiescent) anthracnose results in brown lesions on ripe fruits after they have left the ripening facility and rarely leads to commercial sanctions. Wound (non-quiescent) anthracnose results in large brown lesions on fingers damaged during harvesting or packing. The symptoms can be seen when the fruits are unpacked after sea transport, and serious commercial sanctions are applied (Jones, 2000).

Crown rot is another important disease. Here, a larger number of fungi, such as *C. musae*, *Fusarium* spp., *Verticillium* spp. and *Botryodiplodia*, are involved. The fungi spread from the cut surfaces resulting from dehanding at packing stations. Damage can be observed after sea transport, and this has serious commercial implications (Jones, 2000).

The fungi that cause post-harvest diseases are widespread in banana plantations. In the case of anthracnose, contamination by *C. musae* takes place mostly during the 1st month of flowering (de Lapeyre de Bellaire and Mourichon, 1997). Spores are spread by water and develop on banana material that is beginning to decompose, such as old leaves,

bracts and especially flowers (de Lapeyre de Bellaire et al., 2000). Control of infections must start in the field at the time the inflorescence emerges from the top of the pseudostem and continue through to the packing shed. Bunches can be contaminated by crown rot fungi at different stages of development, which complicates the implementation of control methods. However, washing banana clusters in contaminated water is probably the main cause of infection.

Post-harvest fungicide applications do not always give satisfactory control. It can be ineffective depending on the production zone and the time of the year (Chillet et al., 2000). In addition, resistance to fungicides has developed in the various fungi involved (de Lapeyre de Bellaire and Dubois, 1997). Furthermore, chemical treatments can lead to chemical residues in fruit, and the safe disposal of the fungicide preparations used in the packing stations is problematic. Interest in the development of other methods is thus growing.

Cultural Practices to Control Anthracnose

The work carried out in the FWI has shown that the use of the cultural practices alone enables anthracnose to be satisfactorily controlled. These practices involve the removal of dried flowers in the field combined with sanitary deleafing (de Lapeyre de Bellaire et al., 2000), early bunch bagging, harvesting bunches at an optimal development time (Mouen Bedimo et al., 2003), the use of good harvesting (Chillet et al., 2006) and packing practices aimed at limiting wounds and bruising, and the implementation of good sanitary practices in packing stations. The implementation of these control methods is more effective when the fruit, such as that grown in mountain areas, has low susceptibility to anthracnose (Chillet et al., 2007). This strategy makes it possible to export fruit from certain geographical locations without any chemical treatment (de Lapeyre de Bellaire et al., 2005).

Modified and Controlled Atmospheres to Control Crown Rot

Non-perforated plastic packaging makes it possible to create a modified atmosphere that is poor in O_2 and enriched in CO_2. This type of packaging has clear advantages over perforated or pre-cut packaging. It improves fruit keeping as long as the cutting date is correctly timed. In addition, it limits the development of crown rot without fungicide application. A reduction of 60 to 80% in crown rot at the green fruit stage was observed in export trials using 20 µm and 50 µm non-perforated bags in comparison with fruits packed in pre-cut bags (de Lapeyre de Bellaire et al., 2005). However, the beneficial effect of this packaging diminishes during ripening, especially for 50 µm film. The transport of fruit in controlled atmosphere enables an equivalent control of crown rot to that achieved with modified atmosphere. This system has advantages for ripeners as the packaging is perforated and does not have to be torn open before gassing. However, transport in controlled atmosphere alone does not provide commercially acceptable control in all situations after ripening. In addition, controlled atmosphere requires substantial changes in sea transport. Fruit would need to be in the hold and not in containers, and oxygen extraction systems would have to be installed.

Biofungicides to Control Crown Rot

Different essential oils have been evaluated for crown rot control, but none have provided effective control. They were also found to be extremely phytotoxic. A preparation of organic acids (lactic acid, citric acid, ascorbic acid, palmitic acid), glucose, mannose and tocopherols enabled the partial control of crown rot during controlled inoculation tests. Partial biocontrol of crown rot has also been achieved through the use of a yeast, *Pichia anomala* strain O (Lassois et al., 2008), and further improvement of this biocontrol is in progress. Methods that give better results remain to be developed, but these will certainly have to be combined with shipment in modified or controlled atmospheres.

CONCLUSIONS

The alternative control techniques developed by the research sector and by producers to control banana pathogens and pests, and increasingly restrictive regulations on agricultural chemical use in Europe have driven research that has led to a drastic reduction of the use of pesticides in Guadeloupe and Martinique in the last 10 years. Continuing this progress is essential for ensuring crop sustainability. The challenge is to develop production techniques that minimise chemical pollution and also maintain the economic viability of the banana industry. The next priority is the adoption of banana cultivars resistant to Mycosphaerella leaf spots, so that fungicide spray applications become unnecessary. A major challenge is the selection/development of new resistant cultivars which tolerate long-distance post-harvest handling while also being acceptable to consumers. The development of improved banana weevil control techniques is also a major issue for the future.

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Figures

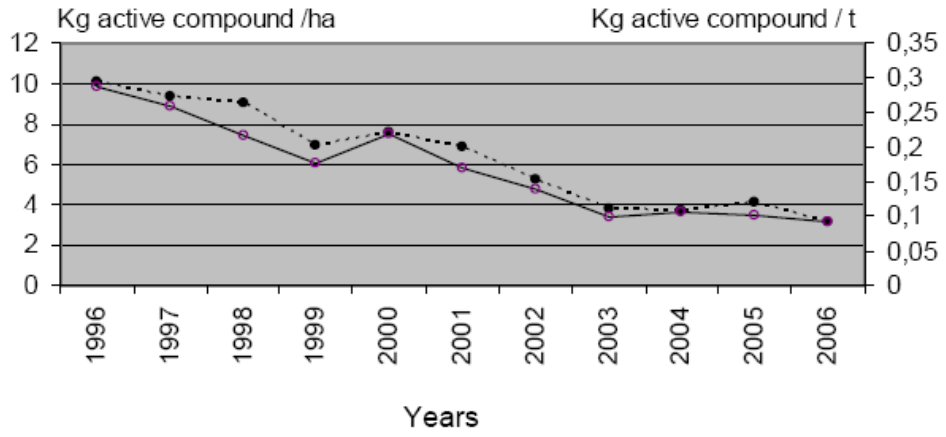

Fig. 1. Reduction in the use of nematocides and insecticides in Guadeloupe and Martinique banana production in the last 10 years expressed by cultivated area (○) or production level (●).

2. Agroecological methods to control banana pathogenic nematodes and sustain soil health without chemical nematicides

This section is mainly structured by two released papers focusing on specific practical strategies to control plant-parasitic nematodes in bananas without chemical nematicides.

The first paper (paper 2) reviews the recent and next coming practical strategies to control plant-parasitic nematodes in banana agrosystems of the French West-indies. It refers to an environmentally-friendly management of banana agrosystems that mainly includes: i/Crop sanitation (use of tissue culture derived banana plantlets, use of Cavendish lines less susceptible to parasitic nematodes); ii/Soil sanitation through sanitizing fallows and crop rotations, along with use of monitoring tools to assess sanitation; iii/Selection of nematode non-host cover crops; iv/promotion of conventionally bred banana cultivars that shows a partial resistance to black leaf Streak disease and Yellow Sigatoka disease.

The second paper (paper 3) provides an in deep analysis of the protective and useful role of water isolation ditches for yet nematode-sanitized plots. We showed in this paper that 50-80 cm deep ditches could efficiently prevent, at the field scale, the dispersion of the banana endoparasitic nematode *Radopholus similis*. Field infestation by this nematode could consequently be lessened, and delayed by more than 3 years.

At last, we also have to briefly mention a third paper not presented here because it is currently being reviewed by the scientific journal "*Fruits*" under the reference: Dorel M., Lakhia S., Pététin C., Bouamer S. Risède J.M. No-till banana planting on crop residues mulch - Effect on soil quality and crop functioning. This paper deals with the impact of soil tillage on i/Nematodes communities and other components of soil quality such as organic status, porosity, microbial biomass. ii/Functioning of the banana crop. It underlined that relative to conventional tillage, no-till banana planting improved soil quality and crop performance. Improving as well soil functioning as plant functioning, it could therefore allow to lessen the impact of soil pathogens and particularly that of parasitic nematodes, thus opening a possibility to restrict nematicide use. It is mentioned in this paper that the presented work was done in the framework of the NoE ENDURE.

Paper 2:

Recent and Up-Coming Strategies to Counter Plant-Parasitic Nematodes in Banana Cropping Systems of the French West Indies

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Keywords: fallow, integrated crop protection, *Musa*, non-host rotational crops, *Pratylenchus coffeae*, *Radopholus similis*, resistant banana hybrids, soil sanitation

Abstract

In the French West Indies, the productivity of export banana plantations is adversely affected by plant-parasitic nematodes (PPNs) including the endoparasitic species *Radopholus similis* (Pratylenchidae). In the last decades, control of PPNs was mainly based upon repeated applications of carbamate or organophosphate nematocides that are potentially toxic for human health and the environment. This paper describes a prophylaxis-based strategy, combining soil and plant sanitation that was developed in recent years to reduce dependence on chemical nematocides. Soil sanitation was implemented through a cleansing system based on glyphosate injection of banana plants before up-rooting. In addition, as a decision support tool, soil cleansing assessment biotests were developed to evaluate the effectiveness of the method before planting new banana crops. Crops were initiated using tissue culture-derived plants of 'Grande Naine' (AAA, Cavendish subgroup). In association with soil sanitation, this resulted in a reduction of 60% in nematocide use. The plant sanitation system is being modified by i) selecting *R. similis* non-host rotational crops, such as perennial soybean (*Neonotonia wightii*), siratro (*Macroptilium atropurpureum*) and forage grasses like *Digitaria decumbens* and *Brachiaria humidicola*, ii) exploiting the existing variation in susceptibility to *R. similis* within Cavendish clones, and iii) identifying *R. similis*-resistant or weakly susceptible clones among improved banana hybrids developed by the CIRAD breeding programme. New initiatives to further enhance the PPN control method are also discussed. They include the promotion of techniques designed for improving management of crop residues, the identification of nematotoxic plants showing allelopathic effects against *R. similis* and the benefits from the preservation of soil biodiversity in banana cropping systems. Most of these approaches are being carried out in the framework of a European Commission-funded network of excellence known as ENDURE.

INTRODUCTION

Plant-parasitic nematodes (PPNs) have long been recognised as being among the most detrimental soil-borne pathogens of banana (Gowen and Quénéhervé, 1990). They occur as communities that vary with geographic and climatic conditions, soil type and cropping sequences. The burrowing nematode *Radopholus similis* (Tylenchida, Pratylenchidae) is the most damaging member of these parasitic communities (Sarah, 2000). From the 1980s until recently, the application of chemical nematocides (mainly carbamates or organophosphates) was the main method of controlling PPNs in banana. However, their use has been increasingly restricted because of their hazardous effects on human health and on the environment. As an alternative, land was used as fallow in the French West Indies (FWI) (Ternisien and Melin, 1989). However, even combined with

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the use of plantlets derived from in vitro culture, recontamination occurred early in the first cropping cycle. Chabrier and Quénéhervé (2003) significantly improved the efficiency of the fallow period by injecting glyphosate into old banana plants to be destroyed before fallowing. Only 12% of the plants became infected in the following growing cycle of banana, compared with 76% with classical mechanical destruction. Nevertheless, in practice, as the duration needed for the fallow period to be fully efficient had not yet been defined, many attempts to satisfactorily complete fallows failed. At this time, growers were also asking for cover crops that could help to reduce populations of PPNs in the soil. Thus, there was an urgent need to design innovative cropping practices to control PPNs in banana that were also compatible with the need for sustainability.

The objectives of this paper were (i) to describe the prophylaxis-based strategy that was developed in the FWI in recent years to rationally reduce PPN populations in soils and (ii) to briefly present other approaches that are currently under study. How fallows coupled with the injection of banana plants with glyphosate were improved by a better knowledge of residual populations of *R. similis* in roots and soil after glyphosate injection is described firstly. How sanitation was implemented by selecting *R. similis* non-host rotational crops, exploiting variation in susceptibility to *R. similis* within Cavendish clones and identifying clones among improved banana hybrids that are resistant or less susceptible to PPNs is described secondly. Finally, approaches that are currently being developed in FWI to promote integrated crop protection against banana PPNs (Ferron and Deguine, 2005) are reviewed. Most of this work is being implemented in the framework of the European Network for Durable Exploitation of Crop Protection Strategies (ENDURE), a European Commission-funded network.

MATERIALS AND METHODS

All experiments described below were conducted in Guadeloupe. Similar experiments were also carried out in Martinique, with similar results.

Determination of Residual Populations of *Radopholus similis* in Roots and Soil after Glyphosate Injection

An old PPN-infested banana crop grown on an andosol soil (FAO-ISRIC-ISSS, 1998) was selected for the trial. Four glyphosate treatments injections were compared: T1: injection of 2 ml of commercial product (c.p.) titrating at 360 g/L into the pseudostem 1 m above the rhizome; T2: injection of 2 ml of c.p. directly in the rhizome at the base of the pseudostem; T3: injection of 5 ml of a mixture of 1/3 of c.p. + 2/3 of water into the pseudostem 1 m above the rhizome; T4: injection of 5 ml of a mixture of 1/3 of c.p. + 2/3 of water directly in the rhizome at the base of pseudostem. The trial was arranged in a randomised complete block with four replications, each comprising up to 80 banana plants. The dynamics of *R. similis* in roots were monitored every 15 or 30 days for 120 days. At this time, soil populations of PPNs were also extracted from soil samples taken near the rhizomes in rows (R) or 1.5 m from any rhizome in inter-rows (IR) and counted.

Biotests to Assess the Efficiency of Soil Cleansing

Biotests to evaluate soil cleansing in commercial banana plots were developed to check the efficiency of fallow periods already improved by glyphosate injections. Soil samples were taken from the commercial plots at various times to be analysed. One micropropagated plant of 'Grande Naine' (AAA genome, Cavendish subgroup), a cultivar that is susceptible to *R. similis* and other banana PPNs, was planted in each of 25 pots filled with 2 L of the soil taken for analysis. The banana plants were to act as nematode traps, and any residual PPNs in the soil would be expected to multiply in their roots. Potted plants were kept in a greenhouse (23-30°C and 80-90% relative humidity) for 60 days. PPNs were then extracted from the roots and counted, to calculate percentages of infested plants (number of plants with nematodes as percentage of total of 25 analysed plants per sampled plot).

The efficiency of fallow periods in commercial banana plots was monitored further by measuring PPNs in the roots of the new banana crop planted at the end of the fallow period using nematode-free, micropropagated plants. Composite root samples were taken from at least 10 plants in the 3rd month after planting and in the following months.

Extraction of PPNs from Root or Soil Samples

In all experiments, PPNs were extracted from roots using a centrifugal-flotation technique (Coolen and d'Herde, 1972) and from soil with a Seinhorst elutriator (Seinhorst, 1962).

Inoculation Experiments in the Greenhouse

Three series of experiments were carried out under greenhouse conditions (23-30°C and 80-90% relative humidity) to select *R. similis* non-host rotational crops/and or resistant banana cultivars. In each of the experiments, 'Grande Naine' was used as susceptible reference banana cultivar.

For the first series of experiments, seeds or cuttings of different tropical legumes, pasture grasses and diverse other plants (Table 1) were obtained from manufacturers or collected locally in fields. All plants were grown in 2-L plastic pots filled with a halloysitic soil until there was sufficient vegetative development for experimentation. Ten fully developed plants of each plant species were then inoculated with a native pathogenic population of *R. similis* (initial population Pi = 100 adults + juveniles).

In the second series of experiments, the susceptibility of 8 to 15 selected Cavendish lines (mainly mass selections of 'Grande Naine') to *R. similis* or *Pratylenchus coffeae* (Tylenchida, Pratylenchidae) were compared by inoculating micropropagated plants provided by the company VITROPIC. For each line, 10 plants were inoculated with either *R. similis* or *P. coffeae* (Pi = 400).

In the third series of experiments, the susceptibility to *R. similis* of a series of banana hybrids already identified as being partially resistant to both black leaf streak and Sigatoka leaf spot was assessed. Hybrids identified as 'Flhorban 925', 'Flhorban 926', 'Flhorban 927', 'Flhorban 928' and 'Flhorban 929' were provided by the banana-breeding program of CIRAD. For each hybrid, 10 micropropagated plants were inoculated with a population of *R. similis* (Pi = 200).

In these three series of experiments, plants were arranged in a completely randomised design. Nine weeks after the inoculation, PPNs were extracted from roots and counted. The susceptibility of plants to the inoculated PPNs was assessed by the ability of the initial inoculated population to reproduce. The multiplication rate mR = Pf/Pi (Pi: initial population of PPNs; Pf: final population of PPNs) was calculated at the end of the experiments. Variances were equalised by log(mR+1) transformations. Analysis of variance (ANOVA) was performed for transformed data. Mean values were separated at $P < 0.05$ using the Newman-Keuls test. When the conditions for an ANOVA were not satisfied, data were treated with the Kruskall-Wallis test and means further separated with the Mann-Whitney test.

Nematode Strains Used for Inoculation Experiments in the Greenhouse

The two pathogenic strains of *R. similis* and *P. coffeae* used in inoculation experiments were isolated from banana roots in Capesterre (Guadeloupe) and monoxenically reared on carrot discs (O'Bannon and Taylor, 1968; Pinochet et al., 1995). A nematode suspension containing 100, 200 or 400 individuals was poured onto the soil surface to inoculate plants.

RESULTS AND DISCUSSION

Residual Populations of *Radopholus similis* in Roots and Soil after Glyphosate Injection

Regardless of the treatment, *R. similis* populations in roots steadily declined following injection of glyphosate until they could not be measured after around 120 days (Fig. 1A). Similar results were obtained for other endoparasitic nematodes (data not shown). After 120 days, the roots were completely rotten due to the action of glyphosate, which is primarily an herbicide. In accordance with the biotrophic status of *R. similis* and other PPNs, it is not surprising that nematode populations decreased with increasing root decay.

In contrast, detectable populations of *R. similis* persisted in the soil in most of the treatments 120 days after plants were inoculated with glyphosate (Fig. 1B). At this time, residual populations of *R. similis* in soil were significantly higher in rows than those in inter-rows. These results suggest that rhizomes, which survive longer than roots, could be the main sources of *R. similis* inoculum in soil. These results also highlight the fact that fallow combined with an injection of glyphosate does not fully cleanse the soil of *R. similis* 4 months after injection of glyphosate. Replanting a new banana crop at this time and in following months thus still carries a risk. Since *R. similis* has no known survival form in soils, it is likely that a gradual decrease in its populations in soil may occur over time. In order to determine the appropriate length of time to cleanse the soil, biotests were developed to evaluate the quality of sanitation during fallow periods.

Biotests to Assess the Efficiency of Soil Cleansing

Biotests made at different times after the beginning of fallow periods allowed to monitor the progress of the soil cleansing of *R. similis* and to determine when sanitation is completed (no *R. similis* detected in soil samples) (Fig. 2A). Moreover, measurements of *R. similis* populations in the roots of a new banana crop planted when no more *R. similis* were detected by biotests showed that recontaminations only occurred at the end of the second growth cycle or the beginning of the third cycle (Fig. 2B). These results clearly illustrate the benefits gained from the sanitising procedure. Currently, in the framework of very restrictive laws on the use of pesticides, the implementation of fallow periods correctly monitored by such biotests and then replanted using only healthy micropropagated plants of 'Grande Naine' has resulted in a 60% reduction in the use of chemical nematocides in the FWI.

Selection of *Radopholus similis* Non-Host Rotational Crops

With a multiplication rate (mR) between 0 and 1, several plants did not allow *R. similis* to reproduce, thus behaving as non-hosts of this nematode (Table 1). In contrast, the highest build-up of *R. similis* populations occurred with the banana cultivar 'Grande Naine', which was used as a susceptible control.

We found that *Macroptilium atropurpureum*, *Crotalaria spectabilis, Digitaria decumbens*, *Panicum maximum,* and *Ananas comosus* were not susceptible to *R. similis* (Table 1), which is consistent with observations of other authors (Birchfield and Bistline, 1956; Colbran, 1963, 1964; Edwards and Wehunt, 1971; Rivas and Roman, 1985). With an mR close to 0, *Neonotonia wightii*, which is a wild perennial relative of *Glycine max*, was in our study also not susceptible to *R. similis*. This was unexpected as Huettel (1989) found fourteen cultivars of *Glycine max* to be a host of *R. similis*. The two cultivars of *Sorghum vulgare* screened were slightly susceptible to *R. similis* with an average mR of 1.5 to 2.75. The susceptibility of *S. vulgare* to *R. similis* is consistent with previous reports (Tarté et al., 1981; De Waele et al., 2006). In the literature, sugarcane is reported as either a host or non-host of *R. similis* (Edwards and Wehunt, 1971; Keetch, 1972; Rivas and Roman, 1985). In our study, two cultivars of sugarcane were found to be non-host plants for *R. similis* and one to be a host. It would be interesting to thoroughly investigate the host status of sugarcane by challenging different sugarcane cultivars with

various *R. similis* populations. Finally, this study may also be the first to report that *Brachiaria humidicola* and an *Impatiens* sp. are non-host plants to *R. similis*.

Selecting *R. similis* non-host plants that could be used as cover crops, not only to clear the soil of *R. similis* but also to replenish soil fertility, is a real alternative to fallow. Pineapple, sugarcane and the *Impatiens* sp. have already been introduced in banana cropping systems in the FWI. *Macroptilium atropurpureum*, *N. wightii*, *Brachiaria* and *Crotalaria* spp. are being considered as cover crops receiving increasing interest of growers. Given the fact that PPNs occur in multispecific communities, the susceptibility of these selected cover crops to PPNs other than *R. similis* will need to be considered.

Variation in Susceptibility of Selected Cavendish Lines to Nematodes

Although all tested Cavendish lines were susceptible to *R. similis*, they showed significant differences in their level of susceptibility (Fig. 3). 'Poyo' was the most susceptible of the tested lines (mR = 67), while the least susceptible lines were 'MA9827' (mR = 18), 'MB2' (mR = 32) and 'MA13' (mR = 34).

Multiplication rates were lower for *P. coffeae* than for *R. similis*, but variability of the susceptibility to the lesion nematode within the tested Cavendish lines was still detected (Fig. 4). 'Williams' (mR = 23.5) was found to be the most susceptible, while 'MB2' (mR = 9) showed the lowest susceptibility, along with 'Nord E1', 'L835', 'GB1' and 'Petite Naine'.

It is, of course, not surprising that Cavendish lines are susceptible to the PPNs tested in this study, since Cavendish cultivars have long been known to be suitable for reproduction of *R. similis* and *P. coffeae* (Gowen and Quénéhervé, 1990). Nevertheless, the differences in susceptibility measured in our experiments could be exploited commercially if producers of micropropagated Cavendish banana plants supplied growers with material less likely to increase pathogenic populations of nematodes.

Identification of Improved Banana Hybrids that are Resistant or Less Susceptible to *Radopholus similis*

All the banana hybrids tested had an mR value of less than 11 and displayed a level of susceptibility to *R. similis* that was significantly lower than that of the reference 'Grande Naine' (mR = 210) (Fig. 5). In particular, the hybrid 'Flhorban 924' had an mR of only 2.76, thus suggesting a status close to resistance. As all these hybrids have already been characterised as having partial resistance to both black leaf streak and Sigatoka leaf spot, they may have a promising future. However, more needs to be known about their agronomic qualities and commercial value before the potential contribution of these hybrids to a sustainable banana cropping system in the FWI is known.

Towards an Integrated Crop Protection Strategy against Plant-Parasitic Nematodes of Banana

A modular prophylaxis-based strategy against PPNs that integrates the results presented above is being developed in the FWI for banana cropping systems. It is based on three levels of interconnected modules.

The first level is composed of three modules devoted to soil and plant sanitation. It comprises i) the adoption of fallow periods, whose duration and quality of sanitation have proved satisfactory from biotests, ii) the rotation of banana crops with non-host plants to reduce *R. similis* populations in soils, and iii) the exclusive use of micropropagated banana planting material.

The second level includes two modules that are operational but not fully adjusted: i) the selection of PPN-resistant or tolerant cultivars among banana hybrids already characterised as partially resistant to both black leaf streak and Sigatoka leaf spot. This module is built on a technological platform designed to increase capacity for selecting banana hybrids and evaluating their agronomic performance and commercial value. It takes place in the framework of a workplan that has the objective to design more sustainable banana cropping systems, which reflects also the commencing ENDURE

network of excellence designed to reduce pesticide inputs in agriculture, promote alternative technologies and develop a unified knowledge of pest organisms in terms of ecology, epidemiology and genetics;

ii) the formulation of mathematical models to gain a better understanding of epidemic process, population growth of PPNs and crop losses. As an initial step, Tixier et al. (2006) have developed a predictive model that simulates the population dynamics of *R. similis* and *P. coffeae*. This model enables a reduction in the use of nematocides through a better knowledge of the population growth of PPNs.

The third level comprises three pre-modules that will evolve into new modules to further complete the backbone provided by modules of levels one and two. The first pre-module concerns the management of crop residues (mainly fragments of PPN-infected rhizomes) in banana fallows. The survival of PPNs in decaying banana crop residues and the influence of the localisation of such residues in the soil profile are being studied in relation to the overall infectious potential of soil. The second pre-module is the further identification and selection of nematotoxic plants that are suitable for crop rotations or integration into banana cropping systems. The allelopathic properties and biological effects of extracts from such plants on banana PPNs are being studied. The third pre-module is devoted to the development of a better understanding of links between soil biological diversity and overall soil functioning. This ongoing work, which is also being completed within the ENDURE network, is mainly aimed at studying whether soil biological diversity would favour the regulation of PPNs.

CONCLUSION

It is anticipated that the overall approach outlined in this paper will lead to an integrated crop protection strategy against PPNs in banana cropping systems.

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Tables

Table 1. Mean multiplication rates of *Radopholus similis* on different plant species and cultivars 9 weeks after inoculation with 100 nematodes per pot.

Common name	Botanical name	Multiplication rate mR=Pf/Pi
Siratro	*Macroptilium atropurpureum*	0.47 d
Perennial soybean 'Cooper'	*Neonotonia wightii*	0.02 d
Crotalaria	*Crotalaria spectabilis*	0.10 d
Sugarcane 'B 8008'	*Saccharum officinarum*	0.12 d
Sugarcane 'B69566'	*Saccharum officinarum*	0.10 d
Sugarcane 'R570'	*Saccharum officinarum*	6.88 b
Guinea grass	*Panicum maximum*	0.67 d
Pangola grass	*Digitaria decumbens*	0.00 d
Creeping signal grass	*Brachiaria humidicola*	0.10 d
Sorghum 'Supersile'	*Sorghum vulgare*	2.75 bc
Sorghum 'Sorgi'	*Sorghum vulgare*	1.50 c
Banana 'Grande Naine'	*Musa*, AAA, subgroup Cavendish	82.80 a
Pineapple 'Cayenne Lisse'	*Ananas comosus*	0.03 d
Impatiens	*Impatiens* sp.	0.10 d

Pf - final population; Pi - initial population; mR followed by the same letter are not significantly different at $P = 0.05$.

Figures

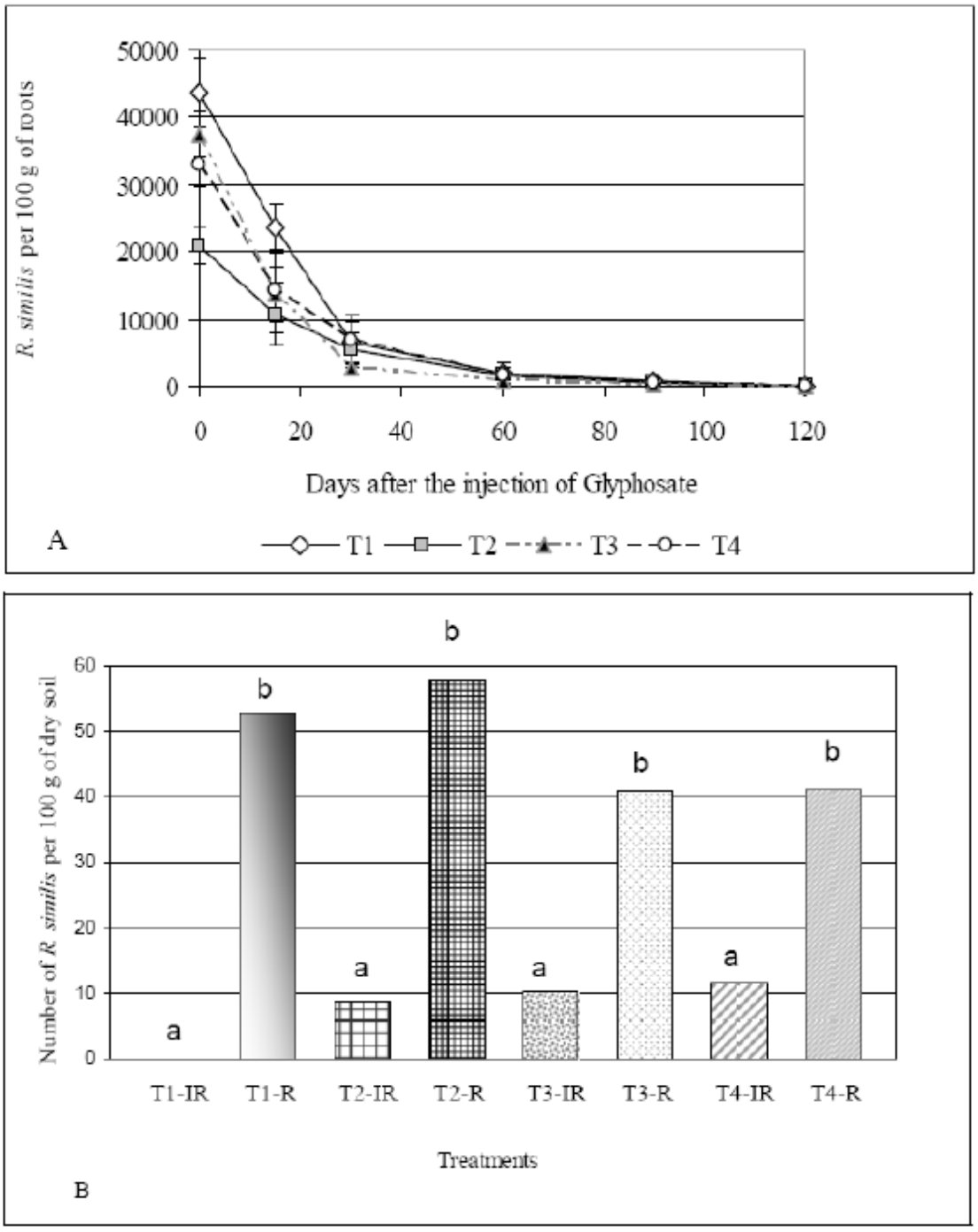
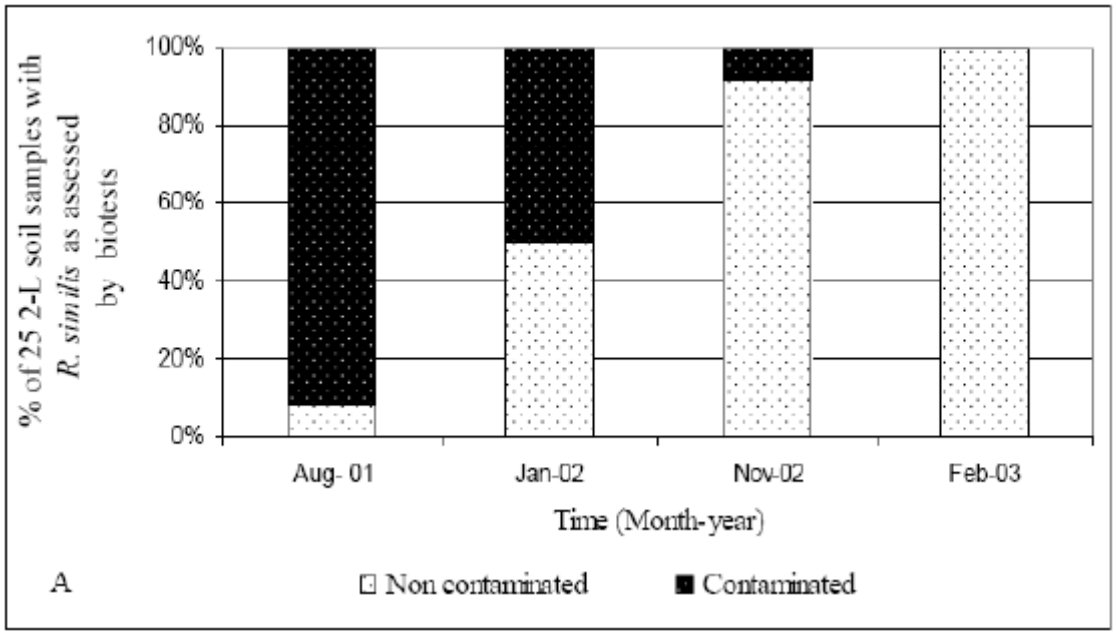


Fig. 1. *Radopholus similis* populations in banana roots and soil following the injection of banana plants with glyphosate. T1 to T4 are modalities and doses for glyphosate injection (see materials and methods). A: Numbers of *R. similis* in roots from 0 to 120 days after injection, B: Numbers of *R. similis* in soil in inter-rows (IR) and in rows (R) 120 days after injection.



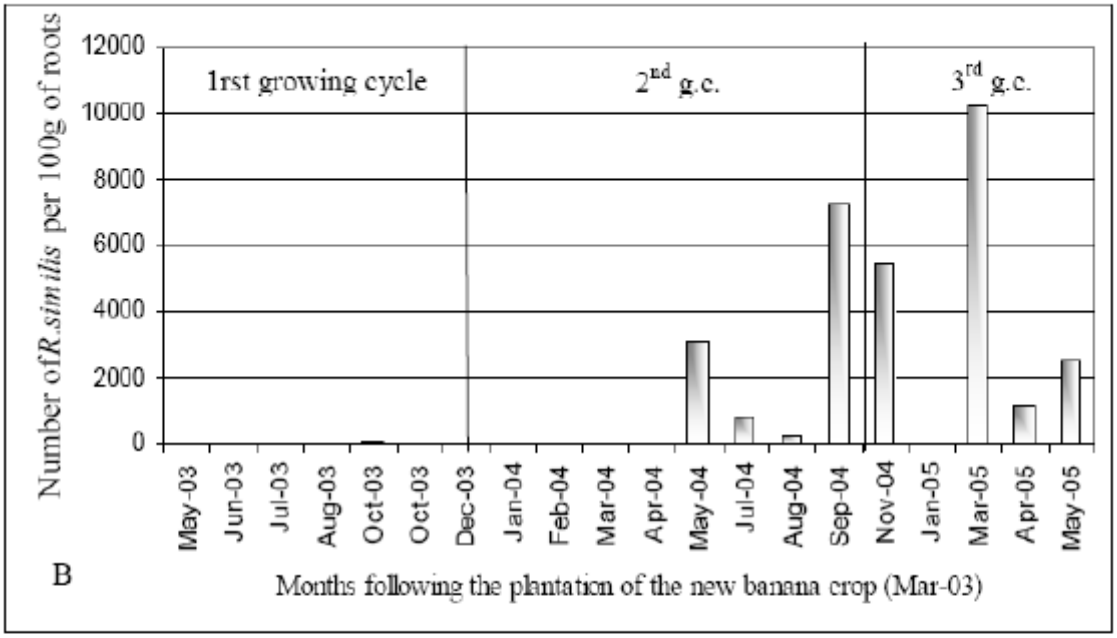


Fig. 2. Effect of a fallow period between banana crops on the population of plant-parasitic nematodes A: Levels of nematodes in plantation soil during the fallow period as assessed by biotests. B: Number of *R. similis* in roots sampled at intervals after the planting of a nematode-free banana crop after the fallow period.

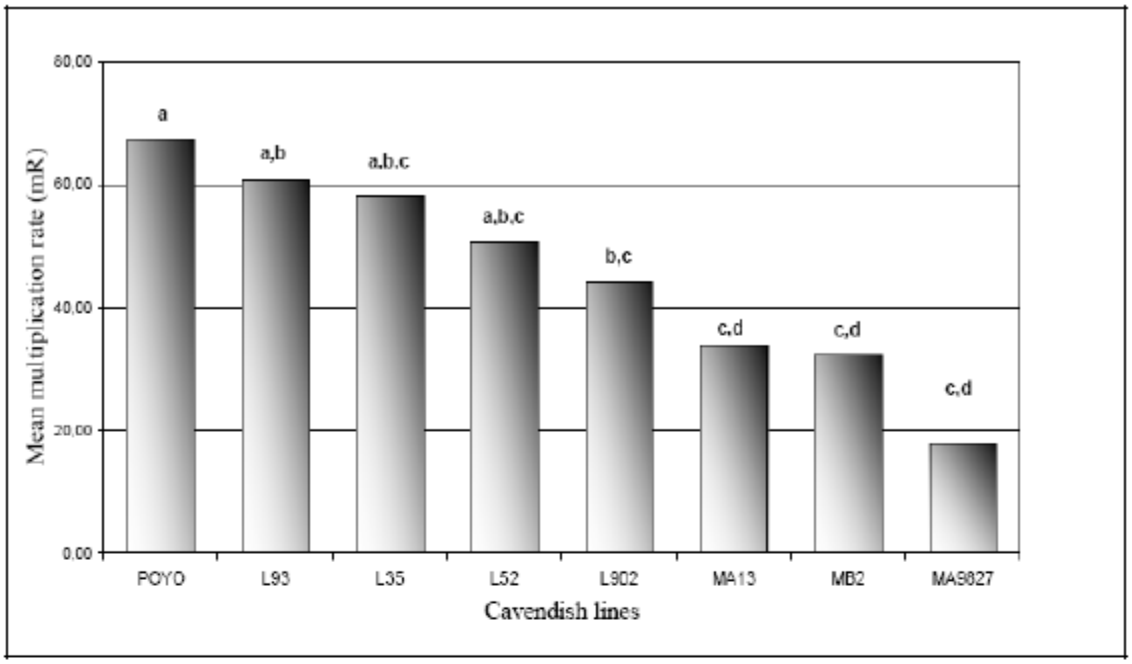


Fig. 3. Mean multiplication rates of *R. similis* on selected lines of Cavendish banana 9 weeks after inoculation with 400 nematodes per pot. Bars with the same letter are not significantly different at $P = 0.05$.

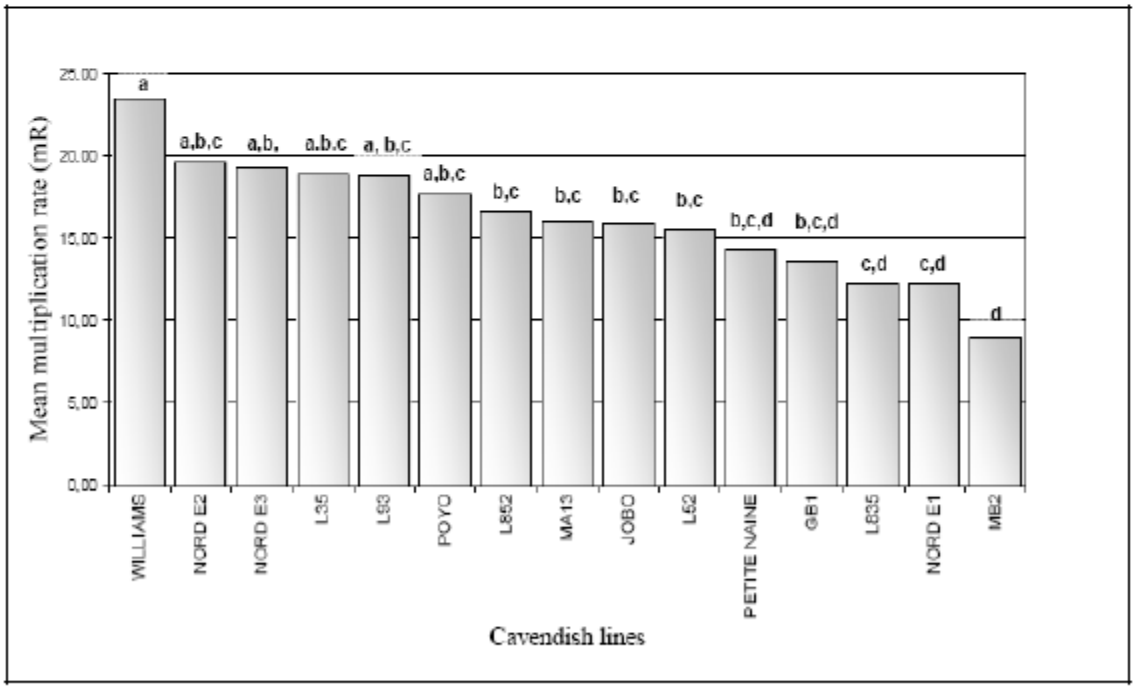


Fig. 4. Mean multiplication rates of *P. coffeae* on selected lines of Cavendish banana 9 weeks after inoculation with 400 nematodes per pot. Bars with the same letter are not significantly different at $P = 0.05$.

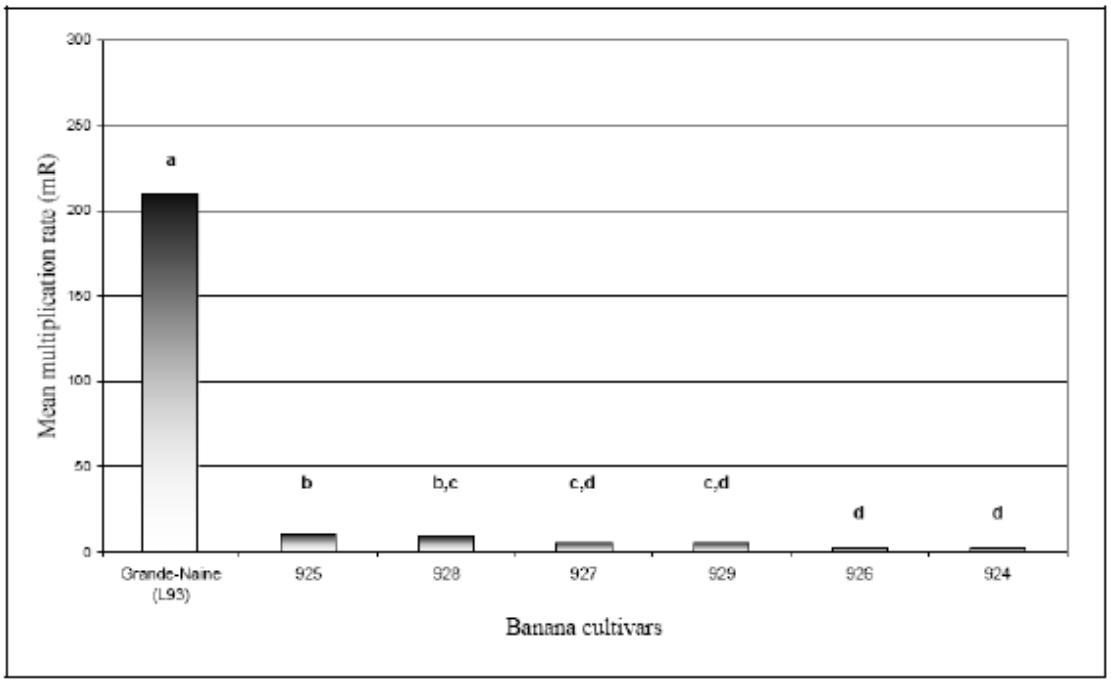


Fig. 5. Mean multiplication rate of *R. similis* on banana hybrids partially resistant to black leaf streak and Sigatoka leaf spot 9 weeks after inoculation with 200 nematodes per pot. Bars with the same letter are not significantly different at $P = 0.05$.

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Preventing nematodes from spreading: A case study with *Radopholus similis* (Cobb) Thorne in a banana field

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ABSTRACT

During the last decade, new crop systems have been developed in the French West Indies to avoid repeated applications of nematicides in banana fields. These combine fallow or rotation crops and nematode-free *in vitro* plants. In many fields, however, after 2–4 years, the burrowing nematodes *Radopholus similis* progressively re-infest banana fields, leading growers to re-apply nematicides. Among different hypotheses for re-infestation, we studied the possibility that nematodes were disseminated by runoff water. The study was conducted in an experimental field on plots that were defined by ditches or marked with flags and weeded or not, prior to replanting with *in vitro* plants. Results showed that 50–80 cm deep ditches efficiently prevent *R. similis* dissemination and that dispersion by water runoff is the major route of contamination. In contrast, weed management during the fallow period had little influence.

1. Introduction

The burrowing nematode *Radopholus similis* is a major pest of banana worldwide (Gowen et al., 2005; Quénéhervé, 2008). In large commercial plantations of banana, nematode control is still based on the application of two to four nematicide treatments per year. An alternative cropping system has therefore been developed in Martinique (French West Indies), during the last 15 years that is based on the cleanup of lands contaminated by plant-parasitic nematodes prior to planting. This cleanup is done through either a fallow period or an appropriate crop rotation, and then nematode-free banana *in vitro* plants are planted. Consequently, growers may cultivate bananas for 2–3 years without nematicide application in banana fields free of the burrowing nematode (Chabrier et al., 2005b). After a 2–4-year period, some banana fields are re-infested with *R. similis*, leading to reduction in both yield and plantation longevity. Previous studies (Zem, 1983; Rivas and Roman, 1985; Quénéhervé et al., 2006) showed that several weeds may act as transitional hosts of *R. similis*. Another hypothesis, that nematodes are disseminated by runoff water, was selected for this study. Numerous authors have considered that water dissemination can be a major factor in their spread (Faulkner and Bolander, 1970a, b; Bur and Robinson, 2004; Robinson, 2004). Furthermore, DuCharme (1955) and Loos (1961) observed *R. similis* in drainage water.

In this study, we present the spatial monitoring of the recontamination of a banana field by *R. similis*. Experimental plots were set up to evaluate, plant by plant, the respective influence of (i) weeds as nematode reservoirs and (ii) runoff water circulation on the successive nematode infestations, and consequently on the following nematode-free period.

2. Materials and methods

This study was performed in a 1 ha field near the Northern Atlantic coast of Martinique (61°02′11″ West, 14°48′14″North). The field was on a steep slope (38%), but such slopes are often found in banana fields in the Windward Islands and French West Indies. The soil is a nitisol, developed on volcanic andesitic ashes; these soils are 71% clay and the major clay mineral is halloysite, which forms sand-size particles with organic compounds. In such peculiar soils, the percolation of water is similar to that in sandy soil. Khamsouk (2001) showed that at soil capacity, water conductivity varies between 50 and 60 mm h^{-1} and that this nitisol contains numerous pores of 30–300 μm, which are ideal for nematode movement (Wallace, 1958). On such bare soil at field capacity, 10–20 mm of water precipitation is needed to observe runoff with a rainfall intensity of 30–60 mm h^{-1}.

At the beginning of the study, up to 32,000 individuals of *R. similis* per 100 g of fresh banana roots were found after

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extraction by the centrifugation–flotation method (Coolen and d'Herde, 1972). This highly infested field was split into five parts; upstream, a band of 20 m width was preserved with *R. similis*-infested banana plants, while downstream, four plots designated 11, 12, 21 and 22 with surface areas between 685 and 1227 m^2 were marked out (Fig. 1).

All banana plants in these four plots were destroyed by two successive glyphosate injections (Chabrier and Quénéhervé, 2003). Plots 11 and 12 were surrounded by ditches, 50–80 cm deep, to isolate them from any water runoff or flooding from adjoining plots. Plots 21 and 22 were delimitated only by flags, so that runoff and leached water could pass freely from the nematode-infested upper band.

After 3 months, the four experimental plots were ploughed and natural vegetation was allowed to grow freely on plots 11 and 21 ("weedy fallow") whereas glyphosate was applied (1 080 g ha^{-1}) on plots 12 and 22 each time a weed reached the early flowering stage ("mulched fallow"). Every 4 weeks, a floristic inventory was taken.

Thirteen months later, nematode-free *in vitro* plants of the widely grown but nematode susceptible Cavendish banana cv. "Grande Naine" were planted. Nematode infestations were evaluated at the flowering period of the first cycle (25 weeks after planting) to the third cycle (96 weeks after planting) of production. At each flowering, five root samples (about 20 cm long) were collected from the base of each corm of each banana plant where *R. similis* densities are usually the highest (Quénéhervé, 1990; Araya and De Waele, 2005).

The presence or absence of *R. similis* was assessed on every banana plant from each plot using a qualitative method of nematode extraction with hydrogen peroxide on an aliquot of 5 g root per plant (Gowen and Edmunds, 1973). Following this assessment, positive samples were combined in groups of five to 10 to reduce the number of composite samples to be extracted for quantitative results in the mist chamber (Seinhorst, 1950). We analysed 710 samples by hydrogen peroxide maceration and 82 (1st cycle) to 89 (3rd cycle) samples by mist chamber incubation.

Yield parameters were measured individually at flowering or at the end of each cycle (dates of flowering and harvest, numbers of hands and fingers per bunch, bunch weight and proportion of harvested plants). These data were used to calculate an annual raw yield indicator, expressed in tonnes ha^{-1} $year^{-1}$ and calculated according to the following formula:

ARY = plant density × proportion of harvested plants × average bunch weight × (365 days $year^{-1}$/duration in days of production cycle).

Weather data were also collected at a station 250 m from the experimental field. On bare nitisol, rainfall of 60 mm h^{-1} intensity (strong shower) needs at least 12 min for runoff to begin on fresh bare soil (water potential of −10 kPa), and almost 20 min on dry soil (water potential of −100 kPa). For this reason, we may consider that water leaching may occur only when rainfall exceeds 10–20 mm, depending to the initial soil moisture.

3. Results

3.1. Evolution of the infested plants from first to third cycles

During the fallow period, all plots were covered by weeds after 5 weeks. Creeper weeds (*Mikania micrantha* HBK and *Ipomea tilliacea* (Willd.) Choisy) dominated first, but there then developed many clumps of Poaceae (*Echinochloa colona* (L.) Link, *Eleusine indica* (L.) Gaertn, *Chloris radiata* (L.) Sw., *Paspalum fasciculatum* Willd., *Leptochloa filiformis* Beauv. and *Sorghum halepense* (L.) Pers.) and Cyperaceae (*Cyperus* spp.). Also present were some *Phenax soneratii* (Poir.) Wedd., *Euphorbia heterophylla* (L.) Kl. and Garcke, *Vernonia cinerea* (L.) Less, *Amaranthus dubius* Mart. and *Solanum torvum* Sw.

On plots 12 and 22, flora was destroyed three times by glyphosate application. On plots 11 and 21, Poaceae first replaced the creepers and were then replaced by *P. soneratii*, *E. heterophylla* and *S. torvum* after the second month. Throughout the fallow period, both plots were completely covered by weeds.

The rainfall data collected close to the study field are presented in Table 1. The study began during a rather dry year; rainfall of more than 20 mm that might have generated water runoff on bare soil occurred only 25 times in 459 days. But the second and third cycles occurred during wetter years, with more than 40 rainfalls exceeding 20 mm per year. On bare soil, rainfall that may generate leaching occurred approximately once every 8 days.

Fig. 2 shows the location of infested plants at the first flowering period. On plot 11, surrounded by ditches and not

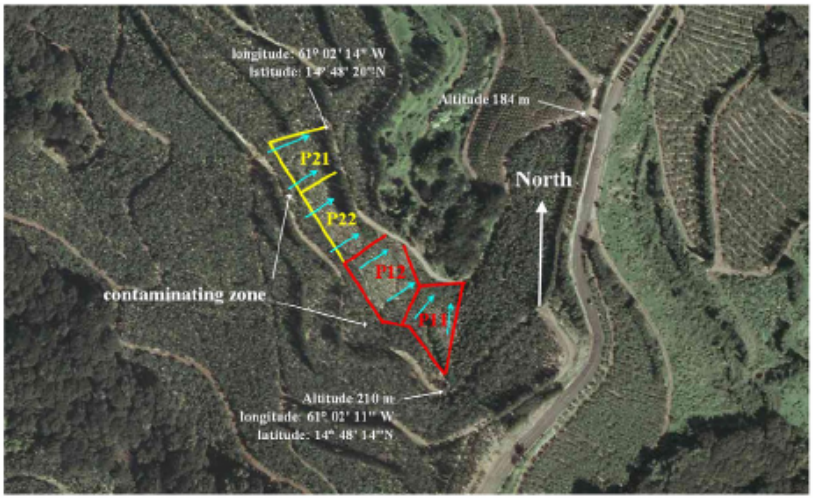

Fig. 1. Schematic map of the fields. P11 to P22: plot 11 to plot 22. Red line: plot border with ditches; yellow line: plot border without ditches. Blue arrows give the slope sense.

weeded during the fallow period, five isolated mats were found infested, and five plants formed a group around plant 1150. At that place, the *in vitro* plant had died early and had been replaced by a ratoon sucker coming from an adjacent *R. similis*-infested field. This plant was destroyed at the end of the first cycle and replaced by an *in vitro* plant at the end of the second cycle. On plot 12, surrounded by ditches and weeded during the fallow period, six isolated plants were infested, most of them close to the northern ditch. On plot 22, with no ditches but weeded during the fallow period, 27 plants were infested; all were close to the upper border of the plot or to the northern ditch. On plot 21, also with no ditches but not weeded during the fallow period, 15 plants were infested; 12 of them were close to the upper border of the plot.

Fig. 3 shows the location of infested plants at the third flowering, slightly less than 2 years after planting. Only 10 plants were infested on plot 11 (only ditches). With the exception of the mats of the infested pocket downstream, former plant 1150, infested plants seemed to be randomly distributed. On plot 12 (ditches and weeded), nine plants were infested; five of them, highly infected, were close to the southern ditch, whereas the other four, much less infected, were randomly distributed. On plots 21 and 22 (no ditch), the upper border was totally contaminated and the distribution of infested plants seemed to follow lines from the top to downstream.

Fig. 4 summarizes the percentage of infected mats of each plot. On plots 11 and 12 surrounded by ditches, these percentages stayed very low (from 3.6% to 4.5%), whereas on plots 21 and 22, not surrounded by ditches, the percentages of infested plants were three times higher at first flowering and then increased dramatically (up to 45.5% and 41.8%).

In nematology, density and frequency provide different information about nematode infestations. In this study, the frequency was more important than density; in order to combine these data, we used a modified prominence value index (Beals, 1960; Quénéhervé and Ferris, 1990). This index, calculated as $P = \text{density} \times \text{frequency}^{1/2}$, used geometric means of *R. similis* concentrations in roots of infected plants. Geometric means were used because the *R. similis* distribution did not follow a normal law, and arithmetic means are not relevant. Depending on the plot, aggregative or even discrete distribution was observed (Figs. 2 and 3). In such cases, the modified prominence value index using geometric means is a better descriptor of the abundance of nematode communities. At the end of the first cycle, indexes were already 23 times lower on ditched plots and were equivalent on formerly weeded and mulched fallow (Table 2). After the third cycle, density increased even more dramatically than frequency in the plot not surrounded by a ditch and weeded during the fallow period.

Table 1
Weather data collected during the study at Bellevue's Meteorological station, 250 m from the experimental field

	Number of days	Cumulated rainfall (mm)	Number of days with rainfall	
			>10 mm	>20 mm
Year				
2002	365	1974	53	24
2003	365	2112	56	29
2004	366	3167	80	41
2005	365	3178	133	42
Period				
Fallow	459	2168	57	25
From planting to 1st flowering (nematode sampling)	178	1003	28	15
From 1st flowering to 3rd flowering (nematode sampling)	494	4463	111	62

3.2. Incidence of nematode dispersal on horticultural results

During this 3-year experiment (Table 3), only a few differences in yield parameters appeared among the treatments. Plots 12 and 21 produced bigger bunches with more fruits and a higher proportions of bunches were harvested (90.1% and 89.1%) on plot 12 at the end of the second and third cycles of production. As a result, yields were higher on plot 12. Conversely, plot 22 produced lower yields.

As few plants were infected at the end of the first and second cycles of production, and as *R. similis* damage usually tends to

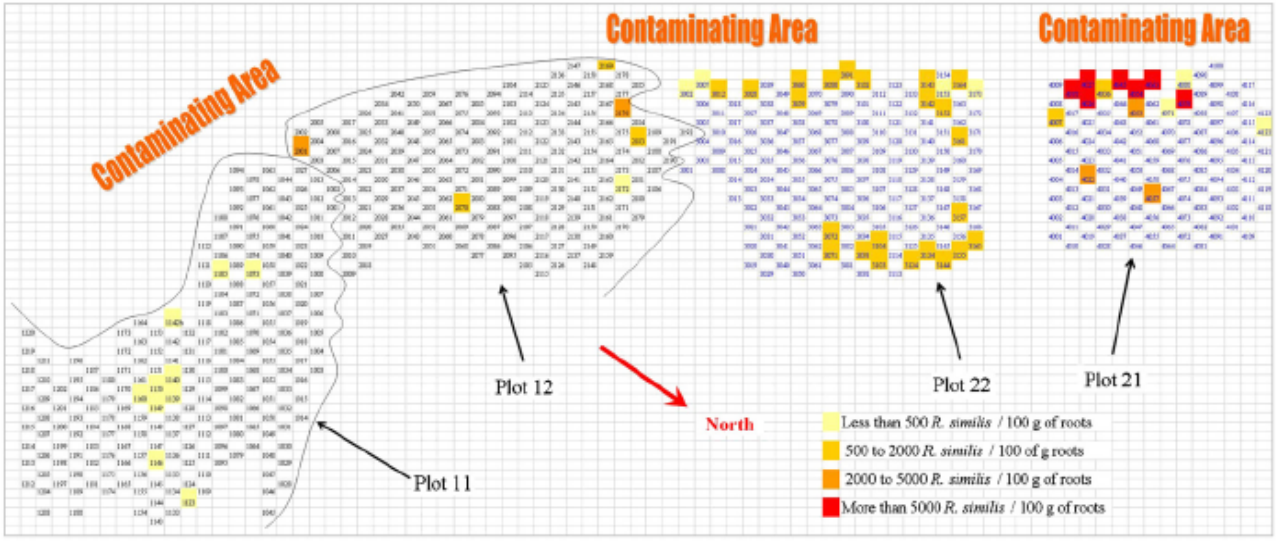

Fig. 2. Location of the infested (coloured) and uninfested plants at first flowering, 5.5–6 months after planting. Each number corresponds to a mat. Continuous lines represent ditches in the field.

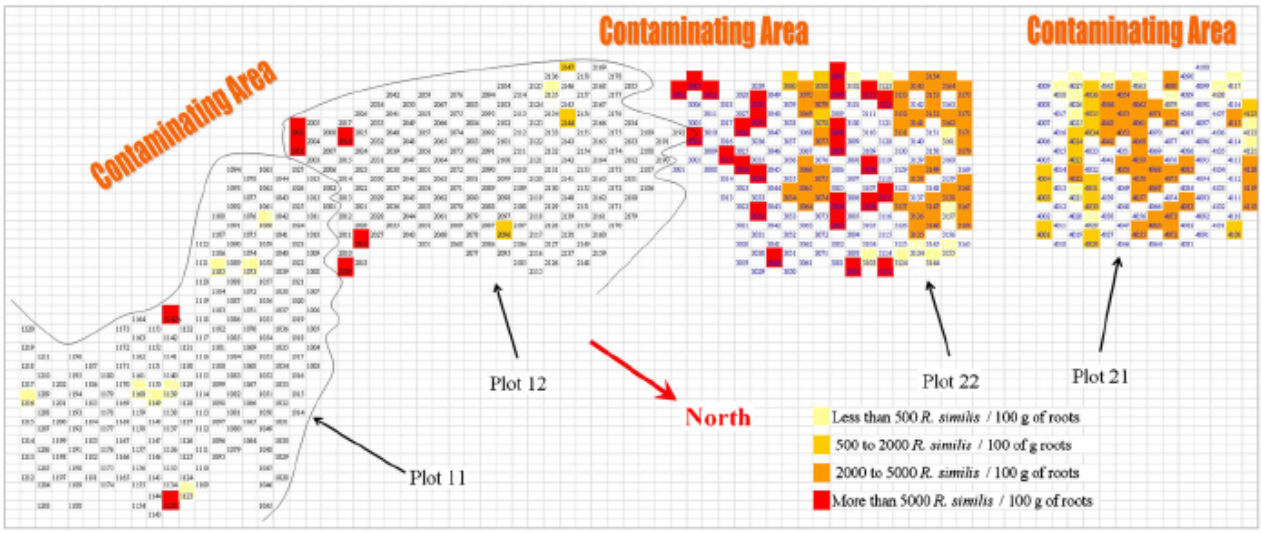

Fig. 3. Location of the infested (coloured) and uninfested plants at third flowering, 22–22.5 months after planting. Each number corresponds to a mat. Continuous lines represent ditches in the field.

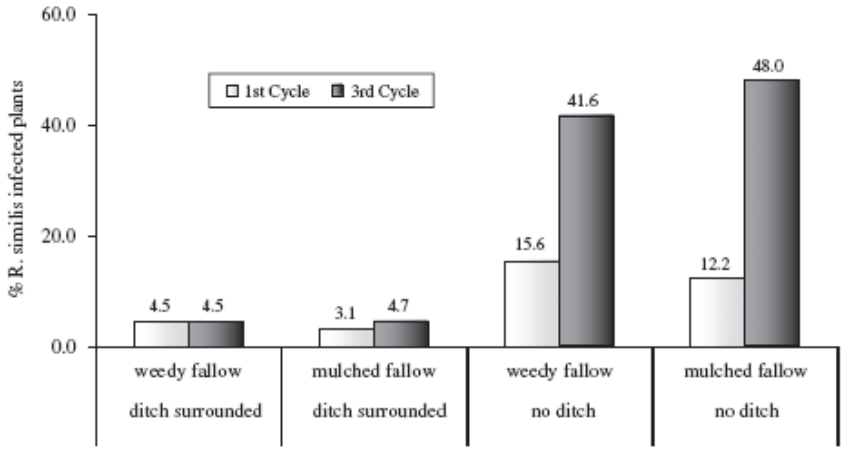

Fig. 4. Percentage of plants infected by *R. similis* after the first and third flowering.

Table 2
Evolution of prominence value index of plots and percentage of plants infected by *R. similis* from the first to the third flowering

Plot	Cycle	11	12	21	22
		Ditch surrounded, weedy fallow	Ditch surrounded, mulched fallow	No ditch, weedy fallow	No ditch, mulched fallow
Infected plants (%)	1st	4.5	3.1	15.6	12.2
	3rd	4.5	4.7	41.6	48.0
Prominence value index	1st	239	348	5748	7658
	3rd	320	4208	44 135	13 501

This index was calculated following the formula: PV = (number of infested plant/number of plants)$^{1/2}$ × (geometric mean of *R. similis* population in infested plants).

cumulate with time (Quénéhervé, 1993), results in Table 4 are limited to those obtained during the third cycle of production. For the most part, few differences were observed (Table 4). The gross yield reduction caused by *R. similis* reached only about 4% of the average yield for this last production cycle.

Table 3
Effect of fallow management and ditch protection on the yield of a banana field over three successive production cycles

Plot Number of plants	11 221	12 192	21 123	22 173
First cycle of production				
Interval planting–flowering (days)	185.1 ± 0.3	184.3 ± 0.3	183.8 ± 0.5	188.9 ± 0.4
Number of hands/bunch	7.5 ± 0.1	7.8 ± 0.1	8.1 ± 0.2	8.1 ± 0.1
Number of fingers/bunch	145.1 ± 3.4	155.9 ± 3.6	161.1 ± 3.9	162.4 ± 3.3
Duration of the cycle (days)	272.8 ± 2.3	280.2 ± 1.7	275.9 ± 2.8	283.0 ± 1.8
Bunch weight (kg)	28.2 ± 0.6	31.3 ± 0.7	29.4 ± 0.8	29.7 ± 0.8
% Harvested plants	85.0	80.2	84.6	77.5
Gross yield ($t\,ha^{-1}\,year^{-1}$)	57.7	58.9	59.3	53.3
Second cycle of production				
Interval previous harvest–flowering (days)	164.4 ± 2.8	161.2 ± 2.1	160.7 ± 3.6	168.2 ± 2.3
Number of hands/bunch	8.7 ± 0.2	9.1 ± 0.2	9.7 ± 0.3	8.9 ± 0.2
Number of fingers/bunch	184.8 ± 6.1	193.0 ± 5.1	211.4 ± 8.4	186.1 ± 6.4
Duration of the cycle (days)	244.5 ± 2.8	241.3 ± 2.4	242.6 ± 3.3	249.1 ± 2.8
Bunch weight (kg)	42.5 ± 1.3	44.0 ± 1.0	44.1 ± 1.7	41.7 ± 1.4
% Harvested plants	86.8	90.1	82.9	78.0
Gross yield ($t\,ha^{-1}\,year^{-1}$)	99.1	108.0	99.0	85.8
Third cycle of production				
Interval previous harvest–flowering (days)	161.6 ± 4.0	161.4 ± 2.4	157.1 ± 4.7	169.2 ± 4.9
Number of hands/bunch	8.2 ± 0.2	8.3 ± 0.2	9.1 ± 0.2	8.4 ± 0.2
Number of fingers/bunch	171.3 ± 5.5	174.5 ± 5.3	193.8 ± 7.6	175.5 ± 5.2
Duration of the cycle (days)	249.4 ± 2.5	250.6 ± 1.8	247.5 ± 2.9	253.0 ± 2.7
Bunch weight (kg)	42.5 ± 1.2	41.9 ± 1.1	46.5 ± 1.9	41.2 ± 1.4
% Harvested plants	74.1	89.1	70.7	68.8
Gross yield ($t\,ha^{-1}\,year^{-1}$)	82.9	97.9	87.3	73.5

11: ditch surrounded, weedy fallow; 12: ditch surrounded, mulched fallow; 22: no ditch, mulched fallow; 21: no ditch, weedy fallow. Confidence intervals were calculated with a 5% error.

Table 4
Effect of *R. similis* infestation on banana plants: average yield parameters collected on infected and uninfected mats

Plot	11	12	21	22	Average loss (%)
Number of plants					
Infected	10	9	56	72	
Uninfected	211	183	67	101	
Interval previous harvest–flowering (days)					
Infected	158.8 ± 20.8	151.0 ± 11.2	161.4 ± 8.2	165.2 ± 7.9	−1.7 ± 1.2
Uninfected	161.8 ± 4.1	161.8 ± 2.5	158.8 ± 5.7	171.7 ± 6.2	
Plant height					
Infected	3.16 ± 0.18	3.14 ± 0.19	3.26 ± 0.08	3.20 ± 0.08	
Uninfected	3.2 ± 0.04	3.13 ± 0.04	3.19 ± 0.06	3.26 ± 0.06	
Plant circumference					
Infected	77.1 ± 6.8	78.4 ± 3.5	79.1 ± 2.0	74.0 ± 1.9	
Uninfected	75.3 ± 0.9	75.2 ± 1.1	77.5 ± 1.7	74.9 ± 1.4	
Number of hands/bunch					
Infected	7.4 ± 2.2	7.7 ± 2.0	8.1 ± 0.9	6.3 ± 0.9	−13.1 ± 8.0
Uninfected	8.2 ± 0.2	8.2 ± 0.2	9.0 ± 0.3	8.4 ± 0.2	
Number of fingers/bunch					
Infected	179.0 ± 23.1	185.5 ± 22.2	195.8 ± 12.6	174.2 ± 8.7	2.9 ± 3.3
Uninfected	170.9 ± 5.5	174.0 ± 5.4	192.3 ± 9.5	176.2 ± 6.5	
Duration of the third cycle (days)					
Infected	249.5 ± 11.6	243.3 ± 9.8	244.6 ± 5.0	250.3 ± 4.9	−1.7 ± 1.2
Uninfected	249.4 ± 2.5	250.9 ± 1.9	249.7 ± 3.2	254.8 ± 3.0	
Bunch weight (kg)					
Infected	38.6 ± 5.4	42.9 ± 4.8	45.7 ± 2.9	41.0 ± 2.5	−2.7 ± 4.8
Uninfected	42.6 ± 1.2	41.9 ± 1.1	47.1 ± 2.6	41.3 ± 1.6	
% Harvested plants					
Infected	88.9	77.8	67.9	66.7	−2.7 ± 13
Uninfected	75.4	89.6	72.9	71.7	
Gross yield ($t\,ha^{-1}\,year^{-1}$)					
Infected	90.4	90.1	83.5	71.7	−3.97 ± 7.1
Uninfected	84.5	98.3	90.2	76.4	

11: ditch surrounded, weedy fallow; 12: ditch surrounded, mulched fallow; 22: no ditch, mulched fallow; 21: no ditch, weedy fallow. Average loss = (1–R) *100, with R: ratio between measures on infected and uninfected plants.

4. Discussion

During the fallow period, several host plants of *R. similis* were observed: among them, *E. colona*, *P. fasciculatum*, *E. indica*, *P. soneratii* and *S. torvum* are reported as good hosts of *R. similis* (Quénéhervé et al., 2006). It was rather surprising that only a few differences appeared between weedy and mulched fallow. This is probably the result of a very efficient previous fallow period that destroyed almost all *R. similis* of the previous banana plants.

In contrast, the presence of ditches had a very important effect on nematode dissemination. Plants at the upper part of plots without ditches 21 and 22 immediately downstream from the infested area were highly likely to be contaminated. On plot 22, it also seems that a spot of *R. similis* had remained at the lowest extremity of the plot. In contrast, on plots 11 and 12, if we discount downstream plant 1150, the few contaminated plants seemed to be randomly distributed.

Fourteen months later, little had occurred on plot 11: we found *R. similis* in only three new plants (among 210 previously uninfested). In plot 21, contamination spots developed upstream to downstream, following lines of planting, which, in that plot, were disposed parallel to the main slope. In plot 22, spots developed not only in the slope direction, but also along diagonal lines (see for example the line formed by plant 3015–3024–3034), which, in the field, corresponded to a major water runoff pathway. It also seems that some contamination spots grew in all directions, perhaps because *R. similis* was disseminated by root contact. O'Bannon and Tomerlin (1969) showed that *R. similis* can rapidly move on roots of *Solanum nigrum* and thereby migrate 21 cm per month. At such a speed, it may theoretically spread from one banana plant to another in 11 months, as the average distance between banana plants was 2.3 m. However, in our study, *R. similis* spread a lot faster than previously described. In plots without ditches, *R. similis* migrated up to 14 m in 17 months, corresponding to five or six distances between two plants.

On the whole, these results suggest that although several spreading phenomena may occur simultaneously, spread by runoff was probably the major way of dissemination. Numerous authors consider that nematodes, including *R. similis*, can be disseminated by runoff water (Bur and Robinson, 2004). Duncan et al. (1990), however, observed that in a Florida citrus orchard, *R. similis* was unable to disseminate through root-free soils. In Florida, bare soil acts as barriers that are wide enough to avoid water runoff and thus nematode dispersion. Conversely, in our experiment, runoff water crossed plots without ditches up to 25 times per cycle of production.

Paradoxically, we found in small-scale studies, that dissemination by runoff water affected only a marginal portion of the *R. similis* population (Chabrier, unpublished data). This phenomenon apparently requires many conditions: soil has to be close to water saturation, nematode population has to be close to the soil surface and rain intensity has to be high enough. But, as banana plants concentrate up to 30 times the water flows that run along their stem (Bassette and Bussière, 2004), and as *R. similis* populations are concentrated near the corm (Quénéhervé, 1990; Araya and De Waele, 2005) and thus the plant base, these conditions occur more often than we would expect. Moreover, although nematode numbers from a single square metre may be very low, in the field, water flow may collect nematodes from areas of several hundreds of square metres, thereby disseminating populations large enough to infest banana plants downstream. When ditches are present, they can prevent contamination from an entire plot upstream and also large runoff inside the plot, so that little, if any, water dissemination occurs. This is probably why we observed hardly any dissemination inside plots 11 and 12.

However, at flowering of the third cycle, although the *R. similis* distribution in the northern half of plot 12 looked like those observed overall in plot 12, four mats were extensively attacked near the border of the eastern ditch. It is possible that these plants had some roots in contact with the ditch wall and thus were likely to be contaminated by some nematodes transported and decanted in the ditch.

Despite these differences in nematode infestation, there was little impact on yield indicators. In the French West Indies, the main damage caused by *R. similis* is by plants toppling over (Blake, 1972; Chabrier et al., 2005a) and thus nematode damage is also linked to other phenomena, especially wind during fruit fill. As no high winds (more than 60 km h^{-1}) occurred between flowering and harvest of the second and third cycles, few bunches were lost.

5. Conclusion

Ditches efficiently isolated field sectors and thus protected banana plants from *R. similis* infestation. However, other studies (Faulkner and Bolander, 1970a,b; Waliullah, 1989; Tapia et al., 2007) showed that irrigation canals and ditches may also drain nematodes and disseminate them. In the eastern border of plot 12, this dissemination occurred but was limited to four plants. Depending on their depth and orientation, ditches may thereby favour or prevent dissemination.

What is more, in this study, the main *R. similis* dissemination process clearly involved leaching. Even though *R. similis* leaching appears as a secondary phenomenon at the 0.1 m scale (Chabrier, unpublished data), runoff water is an efficient dissemination process on a field scale. As a result, although ditches are efficient in preventing *R. similis* dissemination, they are likely to be even more efficient with nematodes such as *Scutellonema cavenessi*, whose behaviour favours their leaching (Cadet et al., 2002).

In this study, prevention was efficient enough to delay field infestation by more than 3 years. By combining efficient fallows with appropriate set-up and management, *in vitro* plant planting and ditches to prevent recontamination, it is now possible to manage an intensive banana field without any nematicide.

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3. Dispersal analysis of the black weevil *Cosmopolites sordidus* in banana agrosystems

This section mainly relies upon a paper (Paper 4) focusing on the development of a spatialized model for analyzing, at the field scale, the dispersal of the banana black weevil (*Cosmopolites sordidus*). This COSMOS model allows testing as well planting patterns of banana mats, as the spatial heterogeneity of plant stages. Simulation takes into account the local movement of individuals (mainly females since males do not cause damage, and pupae and larvae are unable to disperse themselves in the banana fields), egg laying of females, banana plants infestation by larvae, along with the main features of insect and host plant development. The COSMOS model also allows to test the spatial localization of pitfall traps with pheromone (Sordidin) in banana fields, and to optimize their density (number per hectare). This model thus appears has a true innovating tool to rationalize vegetation organization in banana agrosystems in order to delay colonization of plots by *C. sordidus* and to lessen its level of damage, thus anticipating and avoiding the need for a permanent recourse to insecticides.

In addition, we also have to briefly mention a second paper, not presented here because it is still under press, after its very recent acceptation for publication in the scientific journal *Agricultural and Forest Entomology*. This paper is referenced: Rhino, B., Dorel, M, Tixier, P., and Risède, JM, 2009. Effects of fallows on population dynamics of *Cosmopolites sordidus*, toward integrated of banana fields with pheromone mass trapping. It focuses on the relevancy of mass trapping in fallows with pitfall traps and pheromone, to improve sanitation of banana agrosystems towards the black weevil *C. sordidus*. This paper mentioned that the presented work was done in the framework of the NoE ENDURE.

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COSMOS, a spatially explicit model to simulate the epidemiology of *Cosmopolites sordidus* in banana fields

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ABSTRACT

A stochastic individual-based model called COSMOS was developed to simulate the epidemiology of banana weevil *Cosmopolites sordidus*, a major pest of banana fields. The model is based on simple rules of local movement of adults, egg laying of females, development and mortality, and infestation of larvae inside the banana plants. The biological parameters were estimated from the literature, and the model was validated at the small-plot scale. Simulated and observed distributions of attacks were similar except for five plots out of 18, using a Kolmogorov–Smirnov test. These exceptions may be explained by variation in predation of eggs and measurement error. An exhaustive sensitivity analysis using the Morris method showed that predation rate of eggs, demographic parameters of adults and mortality rate of larvae were the most influential parameters. COSMOS was therefore used to test different spatial arrangements of banana plants on the epidemiology of *C. sordidus*. Planting bananas in groups increased the time required to colonise plots but also the percentage of banana plants with severe attacks. Spatial heterogeneity of banana stages had no effect on time required to colonise plots but increased the mean level of attacks. Our model helps explain key factors of population dynamics and the epidemiology of this tropical pest.

1. Introduction

Understanding the epidemiology of pests is of special importance for better management (Zadoks and Schein, 1979; Madden, 2006). The spatial component of epidemiology is a crucial element in the spread of damages from a localised inoculum or when pest dispersal is limited (Winkler and Heinken, 2007). Fecundity, mortality, and dispersal are the driving forces of insect epidemiology (Schowalter, 2006, p. 137). Pests can disperse heterogeneously (Lopes et al., 2007). The dispersal behaviour of mobile stages between each host plant contributes greatly to explaining variations of local densities of the species (Coombs and Rodriguez, 2007). In tropical and subtropical regions, where populations of plants and pests are not synchronised by severe winters, all stages of most insects are present simultaneously (Godfray and Hassell, 1987). In these conditions, all stages should be considered simultaneously to understand the distribution and abundance of organisms in the field. This approach is different from the ones in temperate regions, which focus on a particular part of the life cycle of insects that is considered as a key point of spatial patterning and demography, such as attacks or dispersal behaviour of adults (e.g. Cain, 1985; Brewster et al., 1997), egg laying of females (Zu Dohna, 2006), or post-embryonic stages (egg or larva) (e.g. Johnson et al., 2007).

In this work, we took as case study the banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae) (Germar. 1825), a major pest of banana cropping systems. Larvae bore into the corm of banana plants and damage the points of insertion of primary roots, leading to plant snapping and toppling (Montellano, 1954; Gold et al., 2001). *C. sordidus* can contaminate new banana plantations through infested planting material or by means of adults that have survived since the last banana planting, because it has a long development time and life span, a low mortality rate, and is able to survive without food for extended periods (2–6 months) in moist environments (Gold et al., 2001). Adult weevils, which have limited dispersal abilities, can also invade new plantations from nearby plantations or from fallows when heavily infested banana plots are transformed into fallows (Gold et al., 2001). Banana plant stages may be heterogeneous in a plot, because plants are successively replaced (as many as 50 times) by suckers emerging at irregular intervals from a lateral shoot of the mother plant (Turner, 1994). This spatial heterogeneity of banana plant stages is likely to

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influence weevil population dynamics because of the influence of banana stage on female egg laying (Cuillé, 1950; Vilardebo, 1973). Based on these characteristics, we chose (i) a spatially explicit approach to understand how local movements influence the spatial distribution and damages of this pest in relation to its habitat and (ii) an individual-based modeling (IBM) approach to help explain observed population patterns (Winkler and Heinken, 2007), considering that different behaviours at the individual level can lead to the emergence of population-level properties (Grimm and Railsback, 2005). Modeling was considered as a good means to implement these approaches and an IBM was chosen as the modeling framework.

In this paper, we present the COSMOS model, aimed at simulating the spatial epidemiology of *C. sordidus* in the long-term by describing its population dynamics and the resulting infestation of host plants. The model considers all insect stages simultaneously and assumes there are individual variations in behaviour according to each developmental stage. We hypothesised that the distribution of *C. sordidus* populations and attacks in banana fields can be modelled according to epidemiological rules identified at an individual level and calibrated from the literature, with a model that is less parameter-demanding than most IBMs. The COSMOS model, like many IBMs, aims at bridging the gap between individual behavioural ecology and population dynamics (De Angelis and Gross, 1992). We validated COSMOS by comparing model outputs with field data, which is rarely done with most IBMs (Alderman and Hinsley, 2007; Charnell, 2008). Then, because sensitivity analyses are key steps of the modelling processes (Parry et al., 2006; Arrignon et al., 2007), we first conducted an exhaustive sensitivity analysis using the Morris method (Morris, 1991) to identify the most influential parameters in our model. In a second step, these parameters were studied in detail on an extended range of variation, including extreme values. Finally, we used COSMOS to test how planting patterns and the spatial heterogeneity of plant stages, resulting from the variability of sucker appearance over cropping cycles, could modify the time necessary to colonise the whole plot and the level of damage during three cropping cycles, when the initial weevil population was distributed along one side of the plantation.

2. Model description and parameterisation

2.1. General features of the COSMOS model

The COSMOS model is a stochastic IBM that runs on a daily time step. It simulates the local movement and egg laying of females in the field, infestation of larvae in banana plants, and the main features of insect and host plant development (Fig. 1). According to the model, individual *C. sordidus* disperse in a field that is represented by a grid with one banana plant per cell (grid area ranged between 144 and 441 m^2). Plants pass through three distinct stages until harvest: maiden sucker, preflowering, post-flowering. Just before flowering, a new sucker of the mother plant is selected that grows simultaneously in the same cell. The time lag between two consecutive harvests, corresponding to a cropping cycle, is about 200 days (see Tixier et al. (2004) for details on banana cropping cycles).

C. sordidus females lay eggs on banana plants, and larvae issued from these eggs bore into the corm of the plants. The stage duration of juveniles and the phenologic stages of banana plants are temperature-dependent. In the COSMOS model, each *C. sordidus* is an autonomous individual that has a set of rules for egg laying and movement behaviour, depending on the plant stage at the insect's current position. Males do not cause damage, and no data are available on the influence of mating on egg laying. Therefore, males were excluded from the model.

A rule is an algorithm specified by the modeller to define a behaviour of individuals (Grimm and Railsback, 2005). The platform used to develop the model was the CORMAS (Common-pool Resource and Multi-Agents System) software (Bousquet et al. (1998); see http://cormas.cirad.fr), which is based on the Smalltalk object-oriented language (Visual Works 7.5, Cincom Softwares). The architecture of the model was developed in accordance with Ginot et al. (2002). Table 1 presents all the model parameters described below and their estimated values.

2.2. Dispersion

Eggs, larvae, and pupae cannot disperse between banana plants, and adults disperse slowly by crawling (Gold et al., 2001). Although the banana weevil has functional wings, most observers have reported that the weevil seldom, if ever, flies (Gold et al., 2001). In banana fields planted in monoculture (1500–2200 plants/ha, with standard planting distances of 2.4 m × 2.4 m), individuals do not search for food in a large area; their behaviour rather corresponds to an area-restricted search response type (Morris and Kareiva, 1991). The proportion of individuals that disperse to a given banana plant can be estimated as a negative exponential function of the distance to the plant (Schowalter, 2006). Adjusting the data of Delattre (1980) and Gold et al. (2001) to such a function, the probability (P) each time step of an adult moving to a given banana plant at distance d (in m) is the following (Eq. (1)):

$$P = 0.06\, e^{-0.62d} \quad (1)$$

2.3. Egg laying and longevity of adults

Once inseminated, *C. sordidus* females can stay gravid for 15 months without renewed mating (Cuillé, 1950; Treverrow et al., 1992). Authors disagree on the possible effect of age on egg laying (Gold et al., 2001). Yet it is agreed that egg laying depends mainly on two processes. First, egg laying probability and fecundity increase over banana phenologic stages (Cuillé, 1950; Vilardebo, 1973); the maximal probability of egg laying and fecundity occurs at the post-flowering stage, see Table 1 (Koppenhofer, 1993; Abera-Kalibata et al., 1999). Second, egg laying activity declines when the number of adults per plant increases (Cuillé, 1950; Koppenhofer, 1993; Abera-Kalibata et al., 1999).

In our model, mating and the effect of age on egg laying are not considered. Egg laying occurs for each female once a week, according to the period found in the literature (Koppenhofer, 1993), and follows a binomial distribution with a probability depending on the stage of the host plant (flowering, preflowering and maiden sucker) as estimated by Abera-Kalibata et al. (1999). If conditions for egg laying are fulfilled, the fecundity of each female is assumed to be Poisson-distributed (in accordance with Hilker et al. (2006)), with parameter equal to 2.7 if the adult density exceeds a given threshold (DE, Table 1) and 0.8 otherwise.

The maximal lifespan of adult of *C. sordidus* was estimated to be 748 days (Froggatt, 1925; Gold et al., 2001). The mortality rate of adults is often considered as constant during their lifespan (Godfray and Hassell, 1989; Berec, 2002; Potting et al., 2005). To our knowledge, no data are available on the predation rate of *C. sordidus* adults in the field.

Following Bousquet et al. (2001), MR was calculated assuming a discrete decreasing process, as a function of the maximum lifespan (ML, in days, Table 1; Eq. (2)). We assumed a high mortality rate (0.99) of adults from emergence to the maximum lifespan and a constant daily mortality (MR). The shape of the survival schedule exponentially decreases in those conditions and is convex (Carey,

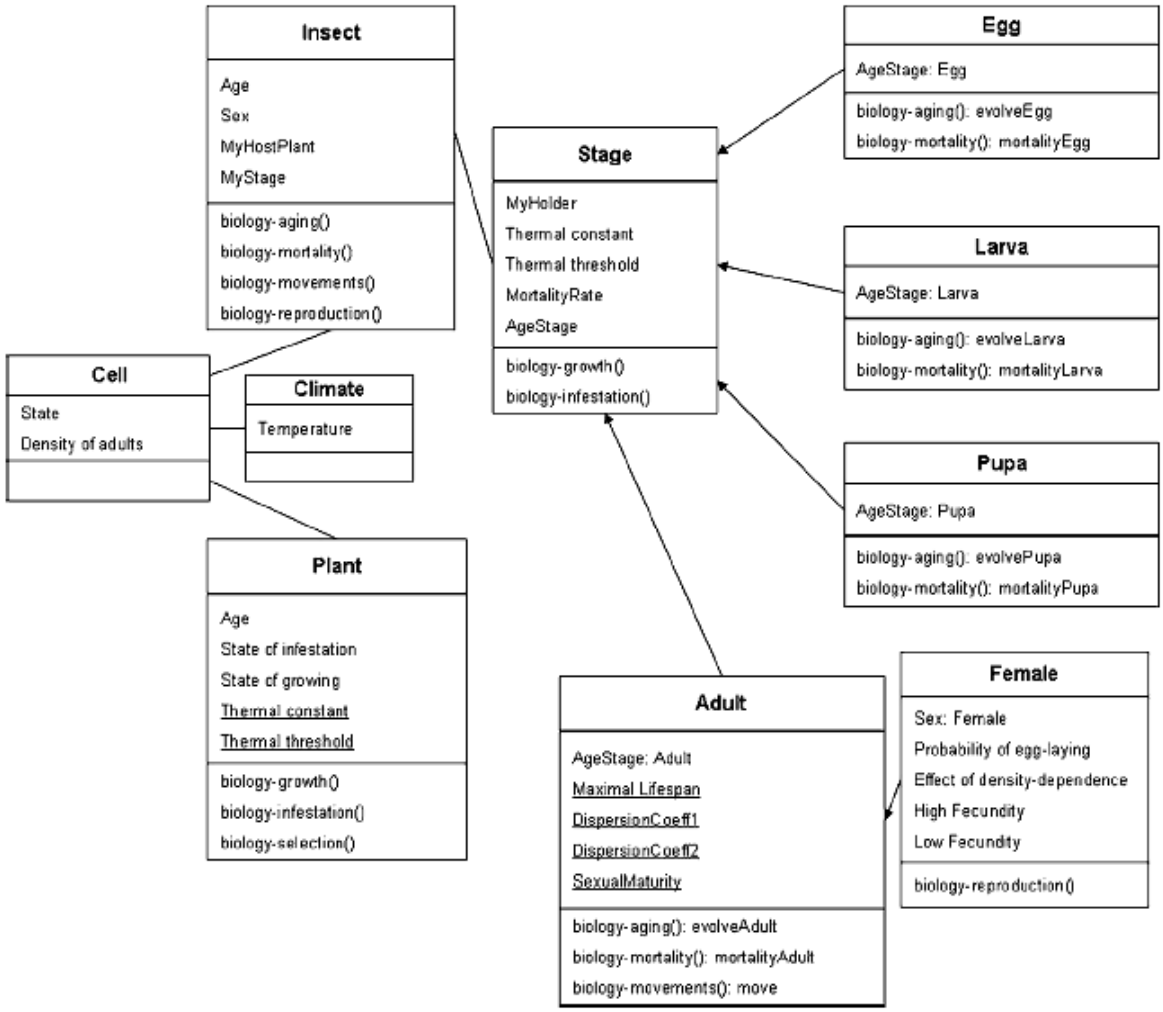

Fig. 1. Static structure of the spatially explicit model COSMOS in Unified Modeling Language (UML). Each box contains the name of a class in the first part, its key attributes in the second part, and the rules in the third part. For example, an individual of class Adult moves according to the rule biology-movements ( ) and the key attributes DispersionCoeff1 and DispersionCoeff2. Class attributes are shared for all the individuals of the class (underlined names) and instance attributes have a specific value for each instance (non-underlined names). Arrows between boxes signify inheritance, and simple links signify association. For example, an individual of class Adult inherits from class Stage and is associated with class Insect.

2001):

$$MR = 1 - (0.01)^{1/ML} \quad (2)$$

2.4. Development and mortality of immature stages

The development of *C. sordidus* is driven by temperature (Kiggundu et al., 2003a). Eggs, larvae, and pupae have different intrinsic mortality rates; larvae are the most susceptible stage (Traore et al., 1993, 1996; Kiggundu et al., 2003b). However, eggs laid on the surface of the corm are exposed to additional mortality by predators, e.g. ants (Koppenhofer, 1993; Abera-Kalibata et al., 2007, 2008). Mortality rates of immatures and additional mortality resulting from predators are shown in Table 1.

In the model, the physiological age for each juvenile stage i increases each day, at a rate determined by the difference between the daily temperature and a thermal threshold corresponding to stage i. Daily temperature was calculated as the mean between minimum and maximum temperature. Table 1 presents the thermal constants, i.e. the number of degree-days above the thermal threshold required to complete development from stage i to the $i+1$th stage. Mortality at stage i follows a binomial distribution based on a constant mortality rate, because the literature gives only cumulative mortality rates at the end of each stage.

2.5. Development of banana plants

The thermal threshold for banana-plant development was estimated to be 14 °C (Ganry, 1980), and the duration in degree-days of each stage from planting to harvesting was determined by Abera-Kalibata (1997) and Tixier et al. (2004) (Table 1). In the COSMOS model, flowering rate follows a normal distribution (mean = 2350 degree-days; σ = 200 degree-days), adapted from Tixier et al. (2004). The sucker of the following cycle is selected after 2180 degree-days (Tixier et al., 2004).

2.6. Infestation of banana plants

Damage resulting from adult *C. sordidus* feeding is negligible compared to that resulting from larvae (Gold et al., 2001). When

Table 1
Model parameters, their values and ranges for sensitivity analyses, and corresponding references.

Description	Code	Value	Range used for the first sensitivity analysis	References
Egg				
Thermal constant to reach next stage (degree-days)	TCE	89	80.1–97.9	Gold et al. (2001)
Thermal threshold for development (°C)	TTE	12	10.8–13.2	Gold et al. (2001)
Mortality rate for eggs	MRE	0.11	0.09–0.12	Kiggundu et al. (2003a,b)
Proportion of eggs removed by predators	PE	0.6	0.33–0.68	Koppenhofer (1993) and Abera-Kalibata et al. (2008)
Larva				
Thermal constant to reach next stage (degree-days)	TCL	537.9	484.1–591.7	Traore et al. (1996)
Thermal threshold for development (°C)	TTL	8.8	7.9–9.7	Traore et al. (1996)
Mortality rate for larvae	MRL	0.48	0.32–0.64	Kiggundu et al. (2003a,b)
Diameter of gallery (in cm)	G	1	0.8–1.2	Montellano (1954) and Sponagel et al. (1995)
Pupa				
Thermal constant to reach next stage (degree-days)	TCP	120.7	108.6–132.8	Traore et al. (1996)
Thermal threshold for development (°C)	TTP	10.1	9.09–11.11	Traore et al. (1996)
Mortality rate for pupae	MRP	0.18	0.095–0.265	Traore et al. (1996)
Adult				
Sex-ratio (male:female)	–	1:1	–	Gold et al. (2001)
Sexual maturity for females after emergence (days)	SM	34.5	33–36	Cuillé (1950)
Probability of egg-laying on maiden sucker compared to flowered plants	OPMS	0.11	0.08–0.13	Estimated from Abera-Kalibata (1997)
Probability of egg-laying on preflowered plants compared to flowered plants	OPPF	0.41	0.39–0.46	Estimated from Abera-Kalibata (1997)
Number of adults per week necessary for density-dependent effect on fecundity	DE	20	10–33	Abera-Kalibata (1997)
Number of eggs per week per female without density-dependent effect	FH	2.7	1.7–3.2	Koppenhofer (1993)
Number of eggs per week per female with density-dependent effect	FL	0.8	0.6–1.1	Koppenhofer (1993)
Proportion of individuals moving 2 m per time step (%)	DC1	1.4	1.5–6.6	Delattre (1980)
Proportion of individuals moving 4 m per time step (%)	DC2	0.3	0.0–3.0	Delattre (1980)
Maximum lifespan of adult (days)	ML	748	520–900	Estimated from Froggatt (1925)
Banana plant				
Interval planting–maiden sucker (degree-days)	–	800	–	Estimated from Abera-Kalibata (1997)
Interval planting–preflowering (degree-days)		1600		Estimated from Abera-Kalibata (1997)
Interval planting–post-flowering (degree-days)		2350		Tixier et al. (2004)
Standard deviation for flowering rate (degree-days)		200		Adapted from Tixier et al. (2004)
Appearance of first sucker (degree-days)		2180		Tixier et al. (2004)
Interval planting–harvesting (degree-days)		3250		Tixier et al. (2004)
Thermal threshold (°C)		14		Ganry (1980)
Maximal circumference of plant at harvesting (cm)		60		

larvae are ready to pupate, they burrow toward the outer surface of the corm (Froggatt, 1925). The attacked circumference (*AC*), measured at the outer surface of the corm of each banana plant, is a common indicator of damage; it is assumed to be proportional to the number of galleries bored by the larvae. When the whole circumference of the corm is attacked, eggs and larvae die because of resource limitation (Koppenhofer and Seshu Reddy, 1994).

In the model, at each time step, the attacked circumference (*AC*) is estimated as the total number of larvae that have reached emergence multiplied by the mean diameter of a gallery (i.e. 1 cm according to Montellano (1954) and Sponagel et al. (1995)). The maximum value of *AC* is equal to the maximum circumference of the banana plant at harvest.

3. Materials and methods

3.1. Field data

Damages of *C. sordidus* on banana plants were measured on 18 plots during two cropping cycles at the CIRAD experimental station,

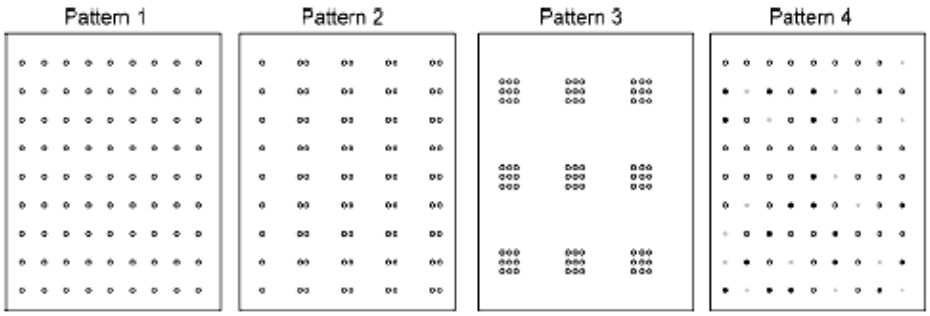

Fig. 2. Spatial arrangements of banana plants used in simulations: regular planting (1), double row planting (2), patch planting (3), regular planting with heterogeneity of banana stages (4). Color gradation figures from white to black the different banana stages from the youngest to the oldest, respectively. Planting density is 1750 plants/ha everywhere.

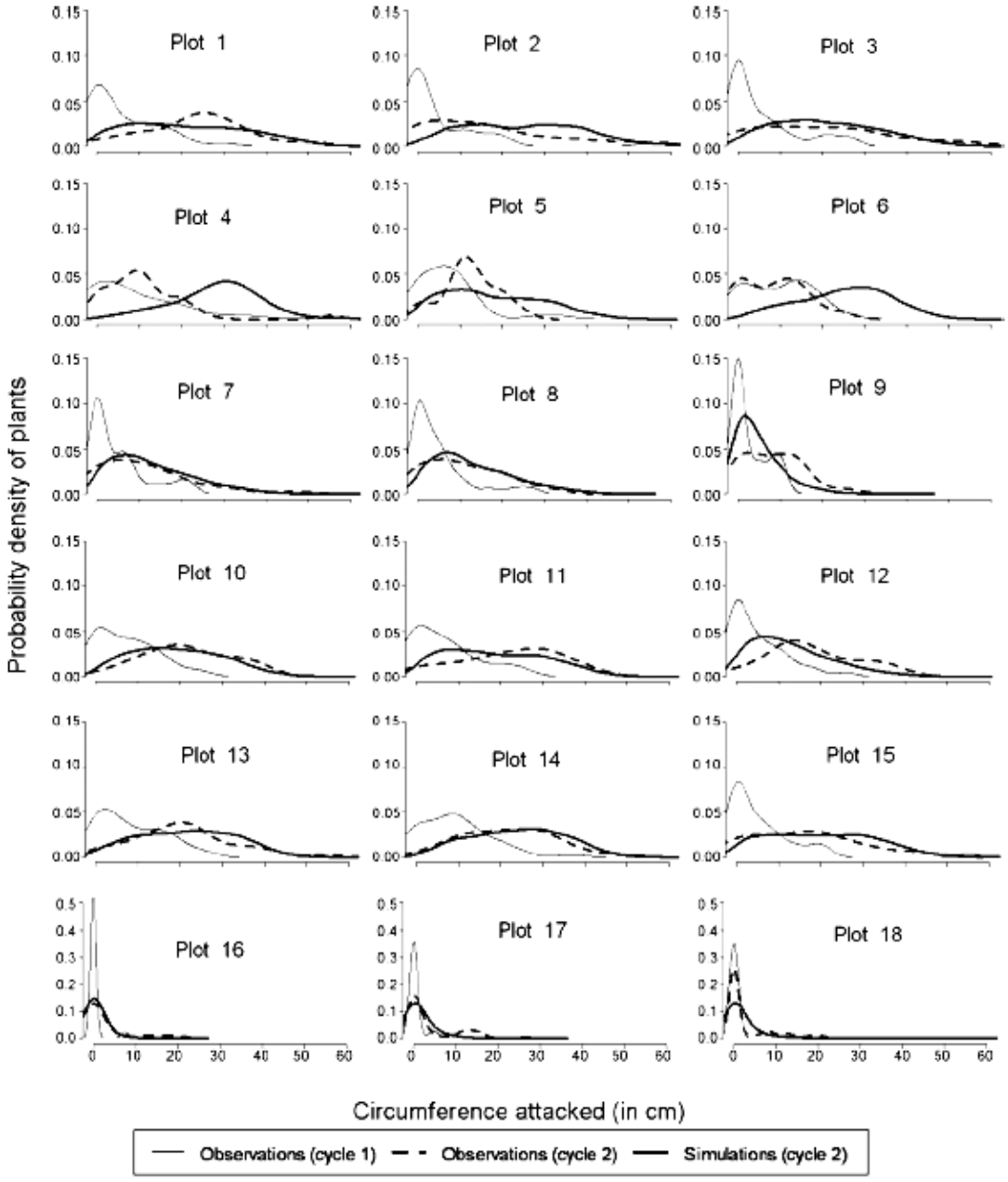

Fig. 3. Observed and simulated distribution of banana damages in 18 plots infested by *Cosmopolites sordidus* in Guadeloupe. Distributions are depicted by probability densities. Simulated probability densities were obtained over 100 runs for each plot. The solid thin line represents the distribution of attacks at the end of the first cycle (initialization). Bold lines represent the observed (dotted) and simulated (solid) distributions. Note that *y*-scale is different for plots 16–17–18.

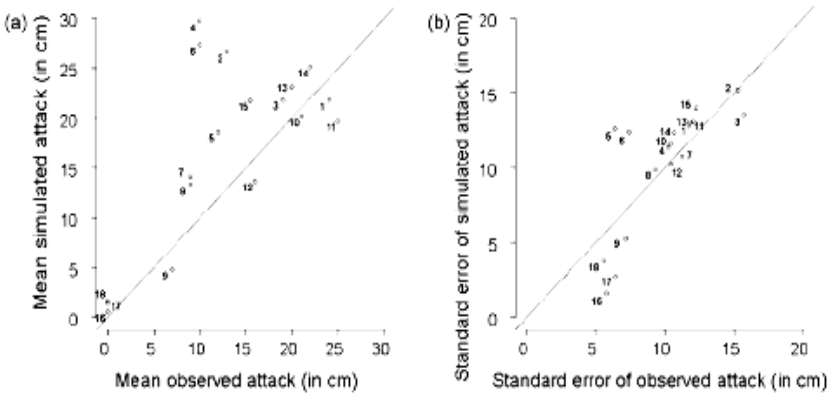

Fig. 4. Comparison of observed vs. simulated (a) mean and (b) standard deviation of distributions for each plot. Solid line indicates a perfect fit between observation and simulation. Numbers correspond to plot numbers.

Neufchâteau, Guadeloupe (French West Indies, 16°15′N, 61°32′W, altitude 250 m) between 1990 and 1995. The plots contained 30–42 banana plants (2174 plants/ha, *Musa* spp., AAA group cv. Cavendish Grande Naine) and were separated by a row without plants. Initial inoculums of *C. sordidus* arrived from previous banana crops. At each harvest, damages caused by larvae inside the corm were evaluated on each banana plant by removing 10 cm of topsoil around the corm and a band of tissue 7 cm wide and 0.5 cm deep across the corm at its widest point. The circumference of the corm with galleries was measured using a tape measure.

3.2. Simulation procedures

3.2.1. Model validation

The simulation area was a 15 × 15 to 18 × 21 cell grid (cell dimension: 0.8 m × 0.8 m), according to the number of banana plants in each field. Each banana plant belonged to one cell and was separated from other plants by two empty cells. Simulations were run over 200 days, corresponding to the period between two consecutive harvests. Model inputs consisted of daily mean temperature from a five-year dataset and of initial populations (see below). Because of the model stochasticity, we performed 100 replicates for each situation and averaged the results.

For each of the 18 plots used for model validation, the model was initialised using populations of individuals distributed in the plot, estimated according to the attacks recorded at the end of the first cycle for each plant, i.e. the attacked circumference (*AC*). For this estimation, we first established a relation to calculate the number of adults per plant from *AC* using data from a capture-recapture study performed in a banana field in Neufchâteau (1996–1997). In this study, populations had been trapped using pseudostem traps (Gold et al., 2002), and *AC* had been measured for each banana plant. The ratio of the abundance of *C. sordidus* adults (square-root-transformed to stabilise the variance) to *AC* was 0.22 ± 0.07 ($F = 18.85$; $P < 0.01$; df = 50). Having calculated the number of adults at the end of the first cropping cycle in each cell of the 18 grids by

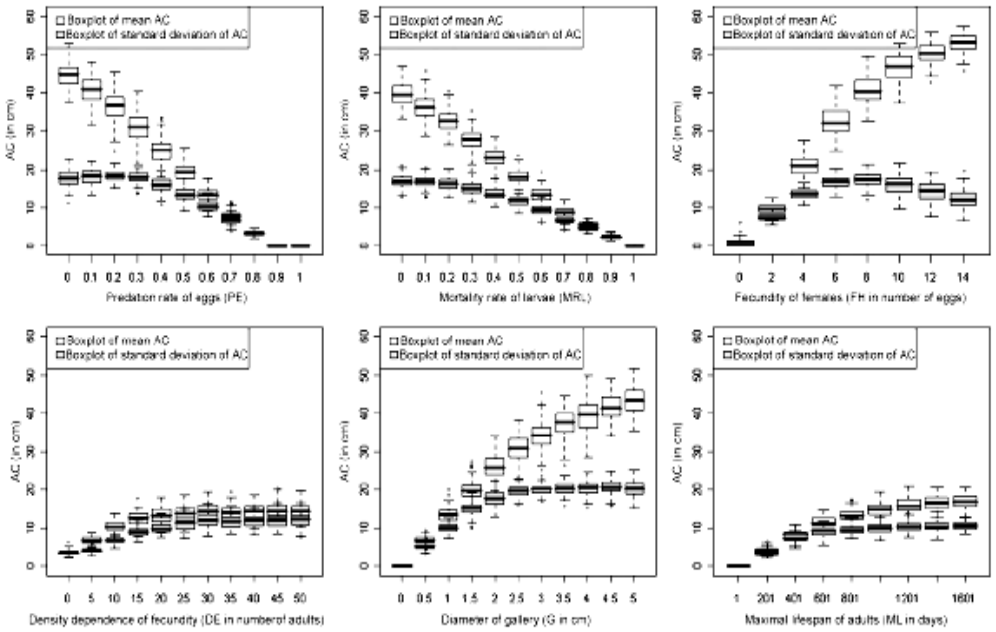

Fig. 5. Analyses of the COSMOS model sensitivity to the most influent insect biological parameters, focusing on two main parameters of the distribution of attacks on plot 8: mean (white boxes), standard deviation (grey boxes). A range of values was tested for each parameter, the other parameters being held constant. The output of 100 runs was computed in a boxplot. Each boxplot contains the lower whisker, the lower hinge (first quartile), the median, the upper hinge (third quartile) and the extreme of the upper whisker. The whiskers extend to the most extreme data point that is no more than 1.5 times the interquartile range from the box.

using this ratio, we set the population age structure, using ratios of 0.24, 0.48, 0.10, and 0.18 for egg, larvae, pupae, and adults, respectively (Koppenhofer, 1993). Within each stage the age was considered to follow a uniform distribution. Then, the model simulated the epidemiology of *C. sordidus* during the second cropping cycle.

3.2.2. Simulation of spatial arrangements of banana plants

We simulated different spatial arrangements of banana plants thought to have an effect on the time necessary for *C. sordidus* to colonise a plot and to cause damage. First, we simulated three planting patterns with synchronous banana stages (Fig. 2): (1) regular planting (2.4 m × 2.4 m), (2) double row planting (0.8 or 4 m × 2.4 m) and (3) patches of nine banana plants (5.6 m × 5.6 m between each patch). The age of banana plants at initialisation was 1 month. Then, we simulated a regular planting pattern with asynchronous banana plant stages (4; Fig. 2), i.e with different stages of plants in the same plot at the same time. In pattern 4, plant stages were randomly set from 1 month (planting) to 9 months (harvest); this situation is representative of old banana plots, which are unsynchronised because of the common practice of repeated sucker selection (Tixier et al., 2004; Lassoudière, 2007). For all patterns, 81 banana plants were distributed over a grid of 27 × 27 cells with a cell size of 0.8 m × 0.8 m, yielding a planting density of 1750 plants/ha.

At initialisation, different populations of adults of random age were equally distributed over the first column of the grid, representing the beginning of a rapid invasion due to putting an infested plot in fallow near the tested grid. For each pattern, we computed the time necessary for at least one adult to reach the column on the opposite side of the grid, the time-series of the mean intensity of attacks of each plant (*AC*), and the time-series of the percentage of plants with severe attacks (more than 20 cm of *AC*) over the entire period of simulation (600 days). Boundaries of grid were closed. Top and bottom edges represented a barrier; left edge the source of contamination that is unidirectional. As simulation stopped when one adult reached the last column of the grid, effect of right edge is absent. This experimental design allowed low edge effects, based on an infestation from one side to the other.

3.3. Sensitivity analyses

In a first step, we used the Morris method (Morris, 1991; Cariboni et al., 2007; see Appendix A) to discriminate the model parameters having the highest influence on the variability of mean and standard deviation of attacks, on four plots with different level of attacks (plots 8, 9, 10 and 16). Two ranges of parameter values were defined for this analysis, the first one corresponding to the uncertainty of estimates according to the literature, the other equally proportioned from −20 to 20% of the value in Table 1. Parameters equally discriminated using the two ranges were considered as the most influential.

In a second step, the parameters that were the most influential according to the first discrimination were tested one by one using a simple sensitivity analysis, the other parameter values being held constant. The model outputs were as before the variability of mean and standard deviation of attacks. For each parameter, different ranges of values were set, from 0 to 1 for biological rates and from 0 to an extreme value empirically defined (when model outputs no longer responded to parameter variations) for the other parameters. For each parameter value, 100 simulations were performed and the results arranged as boxplots showing the quartiles of the output distribution (Arrignon et al., 2007). For all the sensitivity analyses, plot 8 was chosen as representative of the studied plots, after examination of the first simulations (data not shown).

3.4. Statistical methods

For each plot used for model validation, smoothed distributions of the simulated attacks were plotted using 100 replicates of each simulation and compared with observations; this smoothing method is issued from Sheater and Jones (1991). Plotting smoothed distributions instead of histograms allow a better comparison between simulations and observations. The average distribution of the simulated attacks was compared to the observated attacks for each plot using the Kolmogorov–Smirnov (ks) test (Mellin et al., 2006). If the value of the probability associated to the ks test is greater than the level of significance (commonly 0.05), the null hypothesis of conformity (similar distributions) cannot be rejected. For each plot, the simulated mean and standard deviation of the distribution of attacks were compared to the observations over 100 replicates. The mean difference between observation and simulation was calculated using the root mean squared error (RMSE (Wallach and Goffinet, 1989)).

All statistical analyses were performed with the R software (R Development Core Team, 2008) using basic packages: "lattice" (for plotting the distributions of attacks using the kernel density estimate) and "sensitivity" (for sensitivity analysis using the Morris method).

4. Results

4.1. Model validation

Fig. 3 shows a good agreement between observed and simulated smoothed distributions of attacks for most plots. However, observed and simulated distributions were different for plots 2, 4, 6, 9, and 17 according to the Kolmogorov–Smirnov test ($P < 0.05$). For plots 2, 4, and 6, the model overestimated the frequency of high levels of attacks while it underestimated low levels of attacks (Fig. 4). For these plots, mean observed and simulated attack circumference (*AC*) were 10–15 and 25–30 cm, respectively. For plots 9 and 17, the model could not simulate the bimodal distribution of observed attacks. The model predicted well when the level of attacks at initialisation was relatively low (e.g. on plots 16 and 18, where the mean observed *AC* was 0 and 2 cm, respectively); and relatively high (plots 1, 11, 14, where the observed *AC* was 23–25 cm). The RMSE between the observed and simulated mean *AC* of the 18 plots was 7.7 cm; it improved when excluding plots 2, 4, and 6 (3.7 cm). The RMSE of the standard deviation was 2.6 cm for the 18 plots.

4.2. Sensitivity analysis

The Morris method showed that six parameters had a major influence on mean and standard deviation of the distribution of attacks: *DE*, *FH*, *ML*, *MRL*, *PE* (demographic parameters), and *G* (diameter of gallery; Appendix A, Table 1). Since the six parameters were similarly highlighted for the four tested plots, only the results for plot 8 were showed in Appendix A. *PE*, *MRL*, *G* and *FH* had a greater influence than *DE* and *ML*. On an extended range of variation, the increase in *PE* and *MRL* linearly decreased the mean level of attacks. The influence of *FH* (female fecundity) on the standard deviation of attacks decreased for more than eight eggs per week. For increasing values of *FH*, *DE*, *G*, and *ML*, the mean values of attacks increased linearly and then plateaued (Fig. 5).

4.3. Simulated effect of spatial arrangements of banana plants

The time necessary to cross the field was considerably higher for pattern 3 than for the other patterns, while a shorter time was found for pattern 4 (Table 2). This result remained the same when

Table 2
Time to cross the plot and mean level of attacks at 300 and 500 time steps for the four spatial arrangements of banana plants illustrated in Fig. 2 (initial population: 50 adults).

	Time to cross the plot (in days) ± SE	Mean *AC* at 300 days (in cm) ± SE	Mean AC at 500 days (in cm) ± SE
Pattern 1	184 ± 8	2.44 ± 0.08	6.89 ± 0.3
Pattern 2	197 ± 8	2.41 ± 0.09	7.03 ± 0.2
Pattern 3	395 ± 14	2.55 ± 0.08	7.47 ± 0.3
Pattern 4	170 ± 7	6.22 ± 0.16	16.24 ± 0.46

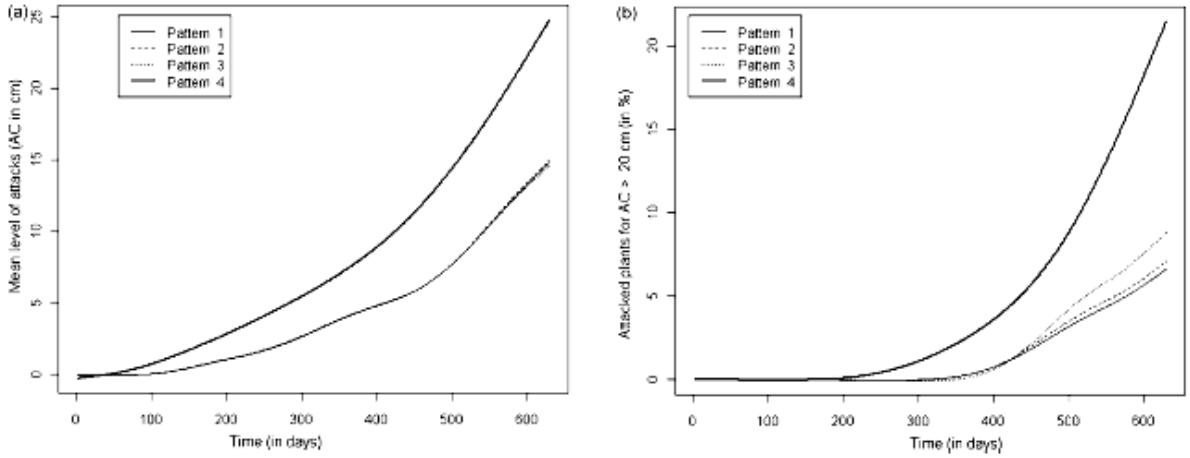

Fig. 6. Evolution of the mean intensity of attacks (a) and percentage of severe attacks (AC greater than 20 cm) (b), resulting from *C. sordidus* infestation of a plot. Initialization of the model was done for 50 individuals at one side of the plot, figuring a massive infestation from a neighboring field.

the initial population varied; times were reduced by half when the population varied from 50 to 400 individuals (data not shown). Mean levels of attacks at 300 days (harvest of first cycle) and 500 days (harvest of second cycle) were similar for planting patterns 1, 2 and 3 and twice as high for pattern 4.

Fig. 6 shows that the mean level of attacks increased to a higher level for pattern 4 than for patterns 1, 2, and 3. For patterns 1, 2, and 3, we observed a small inflexion of the mean level of attacks between 300–350 days and 500–550 days after planting (Fig. 6a). The percentage of plants with severe attacks (*AC* > 20 cm) increased faster for planting pattern 4, followed by patterns 3, 2, and 1 (Fig. 6b).

5. Discussion and conclusion

The individual-based COSMOS model accurately predicted the distributions of attacks of *C. sordidus* on banana plants for 13 out of 18 plots. This quality of prediction is attested for a large range of initial levels of attacks. The RMSE value of mean attacked circumference (7.7 cm) may be the consequence of overestimation of three validation plots and/or measurement error in the field. Furthermore, in the tested range of attacks, the standard deviation of the attacks in the plot was well maintained. Measurement error in the field could be due to an overlapping of some galleries in the same plant or to the presence of some galleries above or below the observation area. For three validation plots out of 18, the model overestimated the mean level of attacks. This overestimation could be explained by a greater predation of eggs in these plots than is accounted for by the model, predation of eggs being a major parameter according to the results of the sensitivity analyses. A highly variable density of *Pheidole* spp., a possible predator of *C. sordidus* eggs, was found among sites in a field trial in Uganda, ranging from 3.1 to 38.4 individuals per trap (Abera-Kalibata et al., 2008). Based on a recent survey in French West Indies, it seems that several species of ants are present in banana fields, including *Pheidole* spp. (Duyck, P.-F., pers. com.).

COSMOS compiles almost all of the existing knowledge about the biology of *C. sordidus*, benefiting from many experimental studies (Gold et al., 2001 and references therein). Nevertheless, our sensitivity analyses highlight the importance of better specifying key biological parameters to improve predictions, such as egg predation, adult mortality, and density-dependent effects. The level of egg predation is a key factor but is variable (Abera-Kalibata et al., 2008), which calls for further investigations. As explained by Carey (2001), little is known about mortality and longevity of insects, whereas they are fundamental epidemiological processes. The effect of density dependency of egg laying is also an influential parameter (Cuillé, 1950; Koppenhofer, 1993; Abera-Kalibata et al., 1999), but further studies should explore the whole range in which density dependency is established. It is also important to fill the lack of available data on predation rate of adults in the field, following the example of Sutherst et al. (2000) on ticks. For that purpose, field and laboratory experiments are currently conducted in French West Indies to identify the main predators of *C. sordidus*, and quantify their predation rates (Duyck, P.-F., pers. com.).

Our simulations on the effect of different spatial arrangements of banana plants on the epidemiology of *C. sordidus* show that planting in patches with a large distance between patches should limit the time necessary for the pest to colonise a new field. Indeed, in this case, only a small proportion of individuals is able to invade new patches. In contrast, the simulations indicate that the severity of attacks may increase when banana plants are planted in patches. Potting et al. (2005) in a modeling study on herbivores, found the same result, with a higher level of damages in patches than in rows. The pattern 3 figures patches with high concentration of plants. The hypothesis of resource concentration has been studied by Levine and Wetzler (1996). They have tested with an individual-based model the effect of planting decisions on attack frequencies by herbivorous insect pests, and they concluded that probability of host plant attack emerged partly as function of density of plants within patches. Furthermore, they estimate that probability of attack is function of radial distance

detection of host by insect. In COSMOS, radial detection is defined by weak dispersal abilities of adults, as defined by literature. These weak abilities contribute also to increase intensity of attacks inside patches. Planting banana regularly or in double rows resulted in similar simulated colonisation time and intensity of attacks. This is probably because in the case of double row spacing, the slow spreading of *C. sordidus* in large interstices between rows was compensated by fast spreading in small interstices inside rows. Unsynchronised banana plantation decreased the time of colonization of the plot by *C. sordidus* and increased the severity of attacks. In this pattern, at every time step, *C. sordidus* can find stages of banana plant suitable for egg laying. In contrast for the other patterns, the inflexion of mean *AC* observed at $t = 300–350$ days and $t = 500–550$ days may be explained by the lack of stages of banana plant suitable for egg laying after harvest. For management purposes at the landscape scale, farmers should avoid transforming a heavily infested field into fallow close to an unsynchronised field free of *C. sordidus*. At the field scale, planting in patches would limit the time of colonisation but after two or three cropping cycles, attacks might be severe. Such a strategy might be suitable for cropping systems with a limited number of cropping cycles. For cropping systems with more cropping cycles, regular and double row planting patterns of plantation would be more suitable.

The choice of the model type is governed by both spatial characteristics of habitat and insect traits. In a spatial insect model figuring infestation of melon by aphids, Lopes et al. (Lopes et al., 2009) introduced space implicitly because they consider local movement as negligible. In that model, populations of aphids are described by partial differential equations, figuring the continuous development of populations. In our case, weak dispersal abilities of *C. sordidus* have required to introduce space explicitly. Populations of *C. sordidus* are described at individual level because of its discontinuous development and the presence of all stages with different behaviour at the same time. These results show that COSMOS is an interesting tool to design planting schemes for the control of banana weevil. IBM models have rarely been used for such purposes on pests. Generally, they have dealt with spatial heterogeneity as a means of controlling pests by simulating the incorporation of non-attractant crops in the field (Potting et al., 2005; Choi et al., 2006).

The basic principles of the epidemiology of *C. sordidus* were successfully integrated in the COSMOS model. Further steps in developing this model should consist of integrating more management practices able to influence the epidemiology of this pest and to contribute to Integrated Pest Management (Huffaker and Gutierrez, 1999, p. 682), such as the use of resistant varieties, traps, and biological control agents, as suggested by Gold et al. (2001). This could be done by designing a sub-model that accounts for trapping. For this, existing algorithms (Byers, 1993; Branco et al., 2006) may be adapted to COSMOS. Furthermore, to design IPM schemes at the farm scale, the next step will be to upscale the model to a group of fields and to account for interfaces between fields.

The COSMOS model, by capturing the population trend of a tropical pest, is a powerful tool to analyse population processes of this pest in various management conditions. COSMOS can be seen as a 'virtual laboratory' (Charnell, 2008) for studying different agricultural practices that can influence the epidemiology of a pest. Emergence of population spatial properties from individual biology is the main driver of our study, as we consider that these practices will influence the individual behaviour of pests. In that way, IBMs can be applied to several pests, for which the spatial heterogeneity of agricultural practices influences biological parameters of individuals.

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Appendix A.

Twenty biological parameters of the insect (k "factors", according to the Morris method) were tested, considering their possible influence on the variability of mean and standard deviation of attacks. Each tested range was divided into four levels, corresponding to the resolution (Δ) at which the factor was examined. Following the method, for each factor, one of the four possible levels was randomly chosen, leading to a first sample. A first sensitivity run was done on this sample that consisted of 100 replicates of a one cropping cycle (200 time steps) simulation (see Section 3.2); the results were further averaged over the 100 replicates. Starting from the first factor sample, similar sensitivity runs were performed by considering successively each factor and increasing (or decreasing) its value by the quantity Δ. The combination of these $(k+1)$ sensitivity runs is called a trajectory and has to be repeated r times, thus leading to $r(k+1)$ sensitivity runs.

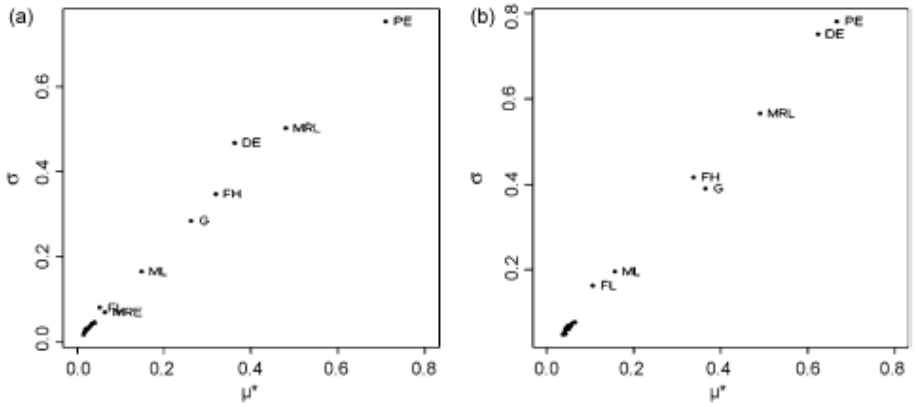

Fig. A.1. Sensitivity analysis on mean (a) and standard deviation (b) of attacks of *C. sordidus*. For each parameter, the tested range was defined according to the uncertainty of estimates according to published experimental studies.

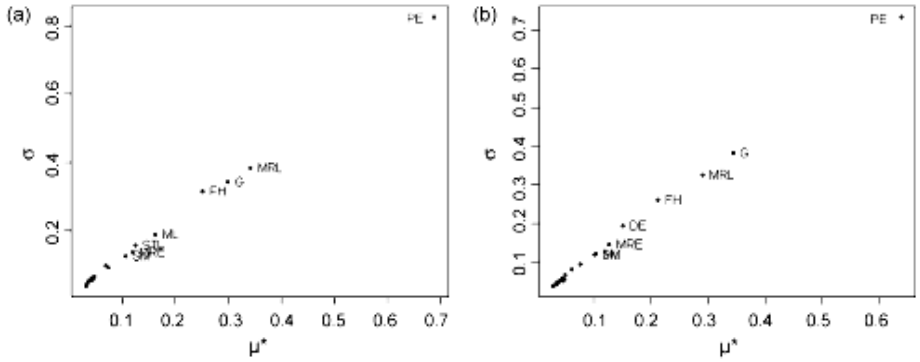

Fig. A.2. Sensitivity analysis on mean (a) and standard deviation (b) of attacks of *C. sordidus*. For each parameter, the tested range was equally proportioned (−20, −10, 10, 20% of the value in Table 1).

The elementary effect (EE_j) of a parameter θ on a trajectory j was calculated as:

$$EE_{i,j}(\theta) = \frac{y_j(\theta + e_i\Delta) - y_j(\theta)}{\Delta} \quad (A.1)$$

with $e_i = \pm 1$ and y_j the model output, here the mean or variance of attacks in the plot.

Thus, we generated a design experiment of 20 levels of parameters on 30 trajectories, which corresponded to a series of 630 sensitivity runs. The mean μ and the standard deviation σ of the absolute values of the elementary effects over the trajectories were used as sensitivity measures to ascertain the importance of the factors. A large μ indicates a large overall influence of the parameter and a large σ implies a dependency of the parameter on the value of the other parameters through non-linear or interaction effects (Figs. A.1 and A.2).

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4. Laboratory tools to monitor fungal resistance in bananas or genetic diversity in *Mycosphaerella* diseases

In this section, laboratory tools designed to monitor fungicide resistance in *Mycosphaerella musicola* or *Colletotrichum musae* populations, are described. Also, new VNTR markers to assess genetic diversity in *Mycosphaerella fijiensis* are also presented.

A first paper (paper 5) presents a newly adapted and performing methodology to monitor sensitivity of the ascomycetous fungi *Mycosphaerella musicola*. This airborne fungi is responsible of one of the most damaging and fungicide-consuming foliar diseases of bananas. Seeing that on the last years, shifts in sensitivity of *M. musicola* to different systemic fungicides led to a worsening of resistance problems, and consequently to an increased use of contact fungicides, it was critical to have efficient tests to adequately monitor these resistances. A major problem was that *M. musicola* ascospores are morphologically similar to those of other *Mycosphaerella* species in bananas, including non-pathogenic species. Among many other advantages, the method presented in paper 5 allows to correctly diagnose fungicide resistance in *M. musicola* populations by addressing conidia of the anamorph *Pseudocercospora musae*.

A second paper (paper 6) similarly illustrates a methodology designed to assess fungicide resistance in populations of the fruit pathogen *Colletotrichum musae*, *a fungi* responsible for consistent post-harvest damage in bananas.

Finally, the *M. fijiensis* genome sequence was used to develop variable number of tandem repeat markers (VNTRs) to study the genetic diversity in natural populations of *M. fijiensis* in Costa Rica (paper 7, under submission).

Paper 5:

A laboratory method to evaluate *Pseudocercospora musae*'s (teleomorph: *Mycosphaerella musicola*) sensitivity to fungicides

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A laboratory method to evaluate *Pseudocercospora musae*'s sensitivity to fungicides.

Abstract — Introduction. This protocol aims at detecting and evaluating sensitivity shifts in Pseudocercospora musae (teleomorph: *Mycosphaerella musicola*) populations towards fungicides that are currently sprayed to control Sigatoka disease of banana. The principle, key advantages, starting plant material, time required and expected results are presented. **Materials and methods**. Necessary laboratory materials, and details of the seven steps of the protocol achieved during four days of experiments are described. **Results**. The protocol results in the observation of conidial germination of *P. musae* (pattern or germ tube elongation) according to the sensitivity to fungicides.

France (Guadeloupe) / *Musa* sp. / disease control / methods / laboratory equipment / *Pseudocercospora musae* / pesticide resistance

Une méthode de laboratoire pour évaluer la sensibilité de *Pseudocercospora musae aux* fongicides.

Résumé — Introduction. Le protocole vise à détecter et évaluer des variations de sensibilité dans des populations de *Pseudocercospora musae* (téléomorphe : Mycosphaerella musicola) vis-à-vis de fongicides qui sont utilisés pour contrôler la maladie de Sigatoka chez le bananier. Le principe, les principaux avantages, le matériel végétal de départ, le temps nécessaire et les résultats attendus de la méthode sont présentés. **Matériel et méthodes**. Le matériel de laboratoire nécessaire et le détail des sept étapes du protocole réalisées sur quatre jours d'expérimentation sont décrits. **Résultats**. Le protocole aboutit à l'observation de la germination de conidies (aspect ou croissance du tube germinatif) de *P. musae* selon la sensibilité aux fongicides.

France (Guadeloupe) / *Musa* sp. / contrôle de maladies / méthode / matériel de laboratoire / *Pseudocercospora musae* / résistance aux pesticides

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1. Introduction

Application

This protocol aims at detecting and evaluating sensitivity shifts in *Pseudocercospora musae* (teleomorph: *Mycosphaerella musicola*) populations towards fungicides that are currently sprayed to control Sigatoka disease of banana.

Principle

Heavy conidiogenesis is induced on individual banana leaf spots. Conidia within a same lesion are considered genetically similar since they issue from a single strain. These conidia are transferred to different agar plates amended or not with a specific fungicide concentration. Conidial germination (or subsequent germ tube growth) on the different media is evaluated to determine *in vitro* sensitivity of each strain. The sensitivity of a conidial population from a fungicide-sprayed area is compared with the sensitivity of a wild conidial population originating from an unsprayed area.

Key advantages

This method is derived from that described by Cronshaw [1]. Other methods focus on

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ascospores and are based on a hyphal or colony growth test [2]. However, key advantages of the described method include:

– in contrast with ascospore methods, no risks of confusion with other morphologically similar *Mycosphaerella* species that are present on banana leaves;

– for a given strain, it is possible to carry out simultaneous tests on different media amended with different fungicides. This is particularly important to study cross-sensitivity among several fungicides or to analyse conidial germination on several fungicide concentrations;

– this method is easier to carry out than methods based on colony radial growth, since radial growth is very slow for this pathogen.

However, conidia observation is time-consuming and requires well-trained technicians.

Starting material

The method requires banana leaves bearing individual stage 4 lesions of Sigatoka disease.

Time estimation for one test

Four days are necessary before observing germination or germ tube elongation.

– Day 1:
• leaf sampling: 1 h (transportation to field not included),
• leaf preparation: 45 min,
• agar plate preparation: 1 h preparation, 3 h autoclave, 1 h fungicide stock solutions and addition, and pouring media into petri dishes.
– Day 2: selection of sporulating leaf lesions under a stereomicroscope and transfer of conidia to agar plates: 3–4 h.

– Day 4: germination or germ tube elongation evaluation: (1 to 5) h according to the fungicide [evaluation is faster for antimitotics than for Sterol Biosynthesis Inhibitor (SBI)]

Expected results

We obtain (a) for antimitotic products, the percentage of resistant strains in the population analysed; (b) for SBI fungicides, variation and distribution of the fungicide sensitivity of the isolates, within the population analysed, according to their level of hyphal growth reduction.

2. Materials and methods

Laboratory materials

The protocol requires: plastic bags (30 cm × 40 cm) and clean paper for incubation; agar, sterile plastic petri dishes (90 mm), glassware for media preparation, distilled water, autoclave, fungicide preparations with known concentration, ethanol, 0.45-µm filters, micropipettes; stereomicroscope, glass needles; light microscope.

Protocol

Day 1

• Step 1
Leaf sampling: collect, within the same banana plot, at least 15 leaves (from 15 different plants) bearing numerous Sigatoka disease lesions at the stage 4 according to Brun's scale [3].

Note: selected sample leaves should also bear young active stages of the disease, to ensure that fungal sporulation will not be affected by the last fungicide sprays. For this reason, sampling should not be done less than 4 weeks after the last fungicide application.

• Step 2
Incubation of samples to induce conidial production:
– cut large [(10–20)-cm] leaf portions bearing stage 4 lesions and group all the pieces from the same leaf in a plastic bag humidified with a wet clean paper,
– incubate bags at 22–25 °C for 10–16 h.

• Step 3
Agar plate preparation:

– prepare 90-mm agar plates (30 g agar·L^{-1}),
– prepare fungicide-amended agar plates (30 g agar·L^{-1} + the necessary amount of the fungicide to reach the correct concentration).

Note: fungicides should be added after autoclaving the agar. Stock solutions are prepared in order to add a volume of 500–

1000 µL of fungicide solution for 1 L of medium. If the fungicide is miscible in alcohol, fungicide solution can be added to the medium directly. If the fungicide is miscible in water, sterilise the solution through filtration (0.45 µm) before adding the fungicide to the medium. Commonly used concentrations are: benomyl (5 mg·L^{-1}), propiconazole and most triazoles (0.1 mg·L^{-1}). Agar plates amended with fungicide should not be conserved for more than 1 week.

Day 2

• Step 4
Selection of sporulating lesions: select, under a stereomicroscope, lesions bearing large quantities of conidia.

Note: conidial production should be heavy and distributed on the whole surface of the selected spots in order to facilitate the transfer to the agar plates. Select four lesions per leaf, so that the total sample size is 60 strains per plot.

• Step 5
Transfer of conidia to agar plates:

– draw with a felt-tip pen 16 diametrical lines on the back of each plate. Number the 32 sectors thus obtained,
– using a glass needle, pick conidia off a selected sporulating spot and gently streak them over a marked sector of the non-amended agar plate (control). Repeat this, from the same lesion, for the successive fungicide-amended plates.

Note: in order to facilitate the observations, for the different fungicide and concentration studies, always streak the same strain on the same corresponding sector of the plates (same number),

– incubate plates for 48 h at 25 °C.

Day 4

• Step 6
Microscopic observations:

– after 48 h, observe sectors of the different plates under a light microscope,
– for antimitotic products, conidia with distorted germ tubes are considered as susceptible; conidia with normal germ tubes are considered as resistant (*figure 1*),

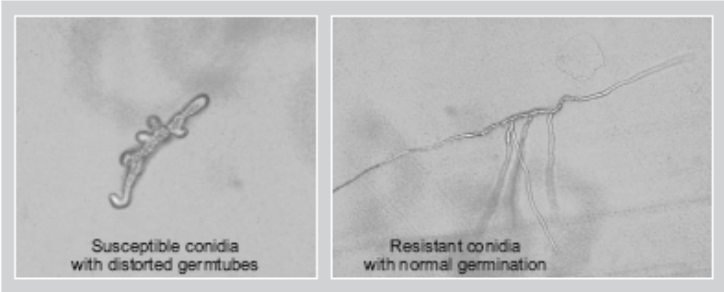

Figure 1.
Example of *in vitro* germination patterns observed on conidia of *Pseudocercospora musae* exposed to a concentration of 5 mg benomyl·L^{-1}.

– for SBI products, measure the germ tube length of conidia with a micrometer on control (Lc) and fungicide-amended medium (Lf).

• Step 7
Data analysis:

– for antimitotic products, calculate the percentage of resistant strains in the whole sample (60 strains),

– for SBI products, calculate the growth inhibition (GI) as {GI = [1 – (Lf / Lc)] × 100},

– for each fungicide concentration, determine the distribution of sensitivity of each strain using the following growth inhibition classes [0–10[, [10–20[, [20–30[, [30–40[, [40–50[, [50–60[, [60–70[, [70–80[, [80–90[, [90–100]. Calculate the frequency of strains in each growth inhibition class and make a graphic representation of this distribution (*figure 2, 3*). Compare this distribution with that obtained for the wild conidial population originating from the unsprayed

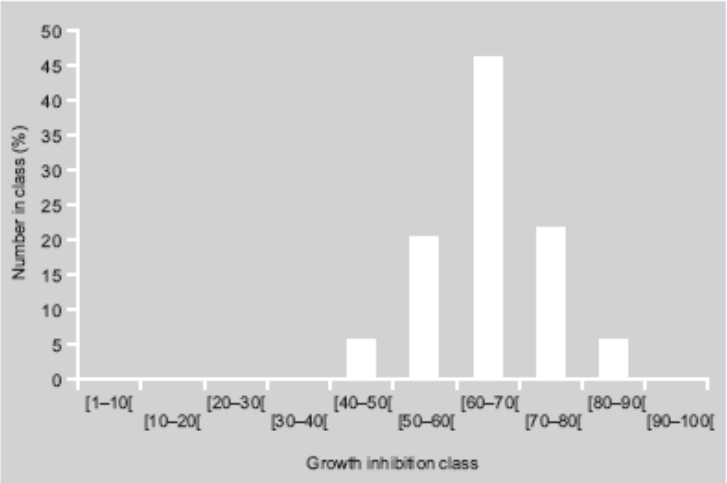

Figure 2.
Example of growth inhibition distribution obtained in a sensitivity test conducted on a conidial population of *Pseudocercospora musae* sampled in an untreated area, for a concentration of 0.1 mg propiconazole·L^{-1}.

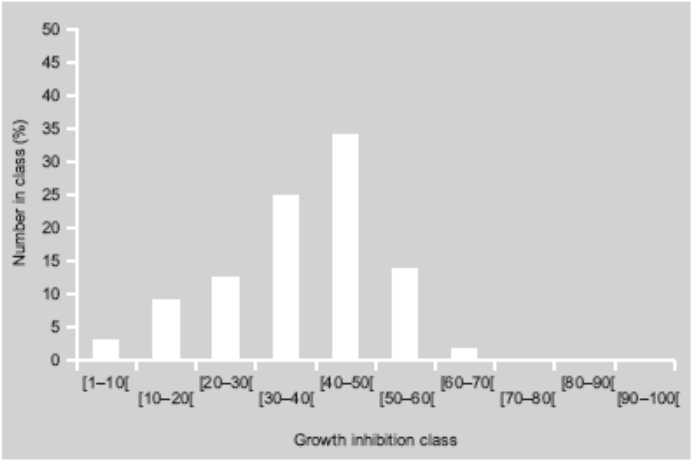

Figure 3.
Example of growth inhibition distribution obtained in a sensitivity test conducted on a conidial population of *Pseudocercospora musae* sampled in a treated area, for a concentration of 0.1 mg propiconazole·L^{-1}.

reference area. Particularly, calculate (NRC), the percentage of strains distributed in classes not represented in the untreated area population (% of strains with a GI < 50%), as well as the mean growth inhibition (MGI). A statistical analysis of the distribution of the two populations will show whether the distribution in the fungal population in contact with the fungicide is different from that of the reference population.

Troubleshooting

Two main problems can occur:

(a) There is little sporulation on lesions: time between the last fungicide application and leaf sampling is too short.

Solution: take care when sampling that active lesions (stages 2–3) are still present on the leaf.

(b) No conidia or too few conidia are present on the sectors when viewing under the microscope. This can result from:

– an incorrect selection of the spots: conidia should cover the whole surface of the spot,

– all conidia were transferred onto the same plate.

Solutions: do not cover all the spots with the glass needle for the same transfer onto one sector; select larger lesions.

3. Typical results obtained

The protocol results in the observation of conidial germination of *P. musae* according to the sentivity fongicides (*figures 1 to 3*).

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Paper 6:

A laboratory method to evaluate the sensitivity of *Colletotrichum musae* to postharvest fungicides

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A laboratory method to evaluate *Colletotrichum musae*'s sensitivity to thiabendazole and SBI fungicides.

Abstract — Introduction. This protocol aims at detecting and evaluating sensitivity shifts in *Colletotrichum musae* populations towards fungicides that are currently used for the control of postharvest diseases of bananas. The principle, key advantages, starting plant material, time required and expected results are presented. **Materials and methods.** The necessary laboratory materials and details of the twelve steps of the protocol for banana sampling and anthracnose lesion development, the agar plate preparation, single-spore isolation, germination test, and data analysis are described. Possible troubleshooting is mentioned. We obtain (a) for antimitotic products, the percentage of resistant strains in the population analysed; (b) for Sterol Biosynthesis Inhibitor (SBI) fungicides, the variation and distribution of the isolates' sensitivity within the population analysed according to their level of radial growth reduction by the fungicide.

France (Guadeloupe) / *Musa* sp. / chemical control / methods / *Colletotrichum musae* / resistance to chemicals

Une méthode de laboratoire pour évaluer la sensibilité de *Colletotrichum musae* aux fongicides utilisés en traitement après récolte.

Résumé — Introduction. Ce protocole vise à détecter et évaluer des variations de sensibilité dans des populations de *Colletotrichum musae* vis-à-vis des fongicides actuellement utilisés pour le contrôle des maladies de conservation des bananes. Le principe, les principaux avantages, le matériel végétal de départ, le temps nécessaire et les résultats attendus de la méthode sont présentés. **Matériel et méthodes.** Le matériel de laboratoire nécessaire, et le détail des douze étapes du protocole réalisé pour l'échantillonnage des bananes et le développement des lésions dues à l'anthracnose, la préparation des boites de milieu gélosé (agar), les isolements monospores, les tests de germination et l'analyse des données sont décrits. De possibles problèmes sont évoqués. Nous obtenons (a) pour les produits antimitotiques, le pourcentage de souches résistantes dans la population analysée ; (b) pour des fongicides inhibiteurs de la biosynthèse des stérols (SBI), la variation et la distribution de la sensibilité des isolats dans la population analysée en fonction de la réduction de leur croissance radiale, imputable au fongicide.

France (Guadeloupe) / *Musa* sp. / lutte chimique / méthode / *Colletotrichum musae* / résistance aux produits chimiques

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1. Introduction

Application

This protocol aims at detecting and evaluating sensitivity shifts in *Colletotrichum musae* populations towards fungicides that are currently used to control postharvest diseases of bananas.

Principle

Heavy conidiogenesis is induced on anthracnose lesions of fruits, in order to make

single-spore isolations. For each strain, the radial growth of the colony is compared on fungicide-amended and non-amended media. The sensitivity of strains from sprayed areas is compared with the sensitivity of reference wild strains that have never been exposed to the fungicide. This method has already been described by de Lapeyre de Bellaire and Dubois in 1997 [1].

Key advantage

Other methods used for the monitoring of resistance in fungal populations focus on germination tests. However, for this pathogen it is more practical and more reliable to use a mycelial growth test, since the radial growth of the colony is very fast (7–8 cm in 5 d at 25 °C). Thus, it is easier and more comfortable to measure this radial growth with a ruler than to measure the hyphal growth with a micrometer, especially because this hypha is not straight but mostly curved. Nevertheless, this method requires making single-spore isolations.

Starting material

The protocol requires banana fruit, as mentioned in the materials and methods part below.

Time estimation

The time necessary to achieve the method is as follows:

– banana sampling: 30 min (transportation to the field is not included),

– banana ripening for anthracnose lesion development: 10–20 d,

– agar plate preparation: 1-h preparation, 3-h autoclave, 1 h for fungicide stock solution preparation and pouring media into petri dishes,

– single-spore isolation: 3 h for 30 strains,

– transfer of strains to fungicide-amended and control media: 45 min (30 strains, 1 control and 1 fungicide concentration),

– incubation of plates: 3 d,

– radial growth evaluation: 15 min (30 strains, 1 control and 1 fungicide concentration),

– data processing: 30 min to 1 h.

Expected results

We obtain (a) for antimitotic products, the percentage of resistant strains in the population analysed; (b) for SBI (Sterol Biosynthesis Inhibitor) fungicides, the variation and distribution of the isolates' sensitivity within the population analysed according to their level of radial growth reduction by the fungicide (EC50).

2. Materials and methods

2.1. Laboratory materials

The protocol requires: boxes and perforated plastic bags for fruit conservation, a controlled environment cabinet regulated at 25 °C, agar, sterile plastic petri dishes (90 mm in diameter), an autoclave, fungicide preparations with known concentration, ethanol, chloramphenicol, 0.45-µm filters, micropipettes, and a stereomicroscope.

2.2. Protocols

Banana sampling and anthracnose lesion development

• Step 1. Fruit sample preparation

– Collect 50 fruits. *Note:* fruits should be collected in a packing station before the postharvest fungicide treatment. In order to obtain more anthracnose lesions, it is advised to collect fruits on bunches that were not covered with a plastic sleeve during their growth [2].

– Bruise fruits in order to induce a faster development of large anthracnose lesions. For this purpose, use a rounded rod and bruise the fruit in several parts by exerting a pressure on the fruit skin with the rounded extremity of the rod. *Note:* most contaminations occur at the apex of the fruit where it is advised to make more injuries.

• Step 2. Anthracnose lesion development

– Store the fruits in a box, inside a plastic perforated bag, to avoid their desiccation. *Note:* plastic bags must be perforated to prevent the modification of the atmosphere, which would slow down fruit ripening.

– Transfer fruits into a controlled environment cabinet regulated at 25 °C until they are ripe and anthracnose lesions are well formed (10–20 d). *Caution:* the relative humidity should not go below 85%. Do not use ethylene in order to hasten fruit ripening, since this treatment could induce the development of lesions caused by other non-pathogenic *Colletotrichum* species [3].

Agar plate preparation

• Step 3. Media and fungicide solution preparation

– Prepare PDA media (potato extract 4 g, glucose 20 g, agar 15 g).

– Prepare a stock solution of chloramphenicol in ethanol (100 $mg \cdot mL^{-1}$) and add 1 mL of this solution to 1 L of the medium after autoclaving.

– Prepare agar + chloramphenicol plates (agar 30 $g \cdot L^{-1}$, chloramphenicol 100 $mg \cdot L^{-1}$) for single-spore isolations.

– Prepare agar plates (30 g $agar \cdot L^{-1}$).

– Prepare fungicide-amended agar plates (30 $g \cdot L^{-1}$ + the necessary amount of the fungicide to reach the correct concentration).

Note: fungicides should be added after autoclaving. Stock solutions are prepared in order to add a volume of 500–1000 µL of fungicide solution for 1 L of final amended agar media. If the fungicide is miscible in alcohol, add directly the necessary amount of stock solution before pouring onto plates. If the fungicide is miscible in water, filter the stock solution through a 0.45-µm sieve before adding the fungicide to the agar medium. Examples of fungicide concentrations commonly used are: thiabendazole (1 $mg \cdot L^{-1}$), bitertanol or other SBI fungicides [(0.001, 0.01, 0.1 or 1) $mg \cdot L^{-1}$].

Caution: fungicide-amended agar plates should not be conserved for more than one week.

Single-spore isolation

• Step 4

Select 30 fruits bearing a sporulating anthracnose lesion (conidia are formed in pink-salmon-coloured acervuli).

• Step 5

For each fruit, transfer and spread a small part of the pink-salmon spore mass over an agar + chloramphenicol plate.

• Step 6

Incubate at 25–30 °C for 3–4 h to initiate conidial germination.

• Step 7

Under a stereomicroscope pick up single germinated spores and transfer them to PDA plates. *Note:* only one strain per fruit is conserved, so that the final sample constitutes 30 single-spore strains from 30 different fruits.

Colony radial growth test

• Step 8

Three to four days after the single-spore isolation, remove a 4-mm^2 plug from the edge of the colony and transfer it to non-amended agar media (control). Repeat the operation for each fungicide concentration tested.

• Step 9

Store the plates at 25 °C for 3 d.

• Step 10

For each strain, measure the diameter of the colony (mm) with a ruler on pure agar (L_{colony}) and on fungicide-amended agar ($L_{fungicide}$).

Data analysis

• Step 11

For antimitotic products (thiabendazole), calculate the percentage of resistant strains on the whole sample (30 strains). *Caution:* some isolates grow only slightly on thiabendazole (2–3 mm) and should not be considered as resistant. Only isolates with a growth superior to 50% of the control should be considered as resistant.

• Step 12

Calculation for SBI products (bitertanol):

(a) Calculations for the concentration 0.1 $mg \cdot L^{-1}$.

– Calculate the growth inhibition (GI) for each fungicide concentration: $GI = [1 - (L_{fungicide} / L_{colony})] \times 100$.

– Determine, for each fungicide concentration, the distribution of sensitivity for each strain using the following growth inhibition classes: [0–10[, [10–20[, [20–30[, [30–40[,

[40–50[, [50–60[, [60–70[, [70–80[, [80–90[and [90–100].
– Calculate the frequency of strains in each growth inhibition class and make a graphic representation of this distribution.
– Compare this distribution with that obtained for wild strains from the unsprayed reference area. Particularly, calculate the percentage of classes not represented (NRC) in the untreated area population (low inhibition classes), as well as the mean growth inhibition (MGI). A statistical analysis of the distribution of the two populations will show whether the distribution in the fungal population in contact with the fungicide is different from that of the reference population.

(b) Calculations for a wide range of concentrations [(0.001 to 1) $mg \cdot L^{-1}$].
– For each concentration, determine the GI of each strain. Calculate the average of GI for the population.
– Determine the relation: [GI/fungicide concentration] (graphic representation, model).
– Determine the concentration inducing a 50% growth inhibition (EC50)

2.3. Troubleshooting

Two main problems can occur:
(a) Very few lesions are observed on ripe fruits and it is not possible to obtain 30 isolates from the sample of 50 fruits: initial levels of inoculum are too low.

Solution: harvest fruits from bunches that were not covered with a plastic sleeve and from badly-kept banana plots (poor sanitation practices). Sampling is better in the rainy season.

(b) Fruits do not ripen properly and anthracnose lesions do not develop: humidity inside the room is too low or CO_2 is too high.

Solution: take care that the plastic bag is perforated and that the humidity inside the room is not low.

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Paper 7:

Genetic structure of *Mycosphaerella fijiensis* populations in Costa Rica

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Abstract
Mycosphaerella fijiensis is the causal agent of black leaf streak of banana, the most important threat to banana production in many countries and particularly in Costa Rica where the climate is very conducive for the disease. Currently, the main control measure is the frequent application of fungicides. However, apart from environmental concerns, this approach is not sustainable due to the abrupt or gradual development of fungicide resistance. To analyze the population dynamics of fungicide resistance, we developed molecular diagnostics for strobilurin resistance, using the cytochrome b gene (cytb), in *M. fijiensis*. We also developed molecular markers for the mating type idiomorphs (mat1-1 and mat1-2) and primers for five VNTR loci to estimate population genetic parameters. Monospore isolates were collected at three plantations that are 20-30 km apart (Cartagena, San Pablo and Zent) in the Limón province that represents the heart of the Costa Rican banana production area. Ninety-five isolates were obtained from a distant wild-type population that was never sprayed with fungicides in the Herédia province. In total, 665 isolates were assayed for mat1-1, mat1-2, VNTR and cytb. The mating type genes segregated in a 1:1 ratio indicating that the sampled populations most likely are randomly mating. The strobilurin diagnostic indicated that the wild-type population is entirely sensitive and that two of the three commercial populations are entirely resistant. The Zent population contained 8% of sensitive strains even though strobilurins were still used in that plantation. The VNTR primers identified 33 alleles and high levels of gene diversity within each population (h = 0.402-0.487). Analysis of molecular variance revealed that 92% of the total variation was within populations with only 8% due to differentiation among them. Levels of gene flow were high (Nm = 4-14 individuals per population pair per year) but populations were still slightly but significantly differentiated. These analyses provide an excellent basis for future research into fungicide resistance in Costa Rican populations of *M. fijiensis* as well as comparative analyses with other banana-producing areas.

Conclusion

In the present deliverable we stressed various innovative possibilities contributing to a reduction of chemical nematicides, insecticides, and also fungicides in banana agrosystems. Gaps of knowledge and technical deficiencies to control, in particular, soilborne pests -including plant-parasitic nematodes and black weevil- were substantial. This opens the promising track of integrated crop protection in bananas.

The solutions proposed here in the different (and next coming) papers to bridge these gaps are relevant with the objective of reducing pesticides in bananas, while promoting sustainability.

It is therefore on major interest to further go on in the capitalization of these innovating solutions. The outcomes from this knowledge are threefold:

- A focus is done in DR1.16 on the technical ways to implement many of the solutions. The necessity to advise farmers and to train technical advisors is also underlined.

- Two relevant and significative initiatives allowing to further disseminate the knowledge from the presented solutions and its appropriation, are running in the two bigger European regions producing bananas: In the MAC region i.e. Madeira + Azores + Canaries (Portugal and Spain) with a project called “BIOMUSA”, and, in the French West Indies, with the project “Plan Banane Durable”. Defined with the key contribution of two members of the Banana CS, respectively, ICIA (Spain), and CIRAD (France), these projects similarly mobilize banana growers, researchers and different other stakeholders dealing with pesticide reduction and more sustainable banana cropping systems, to ensure dissemination of innovative techniques.

- Part of the scientific knowledge presented here will be further refined and completed within the ENDURE NoE through collaborations in the framework of RA2.1 “Prevention of pest damage at the cropping system level” and RA2.3 “Exploitation of landscape and community ecology” in 2010 in the 3rd JPA.

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