

ESSENTIAL TEA TREE OIL AS A TOOL TO COMBAT BLACK SIGATOKA IN BANANA

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Black Sigatoka disease, caused by *Mycosphaerella fijiensis*, is considered the most damaging and costly disease of commercial banana and plantain and is mainly controlled by intensive sprays of synthetic fungicides. Essential tea tree oil derived from *Melaleuca alternifolia* plant was found to be effective against a wide range of plant-pathogenic fungi including black Sigatoka in conventional production systems and was as effective as synthetic fungicides such as tridemorph, difenoconazole, trifloxystrobin and azoxystrobin. This paper provides evidence that tea tree oil offers an attractive alternative for controlling black Sigatoka in banana plantations.

Keywords: bio-fungicide, disease management, disease resistance, *Melaleuca alternifolia*, *Mycosphaerella fijiensis*, banana, plantain, black sigatoka

Black leaf streak, also known as black Sigatoka disease, caused by *Mycosphaerella fijiensis*, is considered the most damaging and costly disease of commercial banana and plantain (Cronshaw 1982; Fullerton 1994; Marin *et al.* 2003; Stover 1980). Infection by conidia or ascospores affects the youngest leaves of the plant (Fullerton 1994; Marin *et al.* 2003), and the first symptom, chlorotic flecks, appears about 15 to 20 days after infection. Subsequently, streaks and necrotic spots appear, often accompanied by extensive leaf death (Marin *et al.* 2003). Damage from this disease causes a significant reduction in the photosynthetic area of the leaf, and fruit yield losses can reach 50%, through premature maturation – a serious problem in fruit grown for export (Chillet *et al.* 2009; Fullerton 1994; Marin *et al.* 2003).

The resulting intensive use of fungicides – up to 60-70 sprays/year in some countries – is a major concern for the environment and human health. This intensive use of fungicides is mainly because of development of resistance to synthetic fungicides by the pathogen populations (Marin *et al.* 2003; Stover 1986).

The global search for plant-protection solutions, that are both environmentally safe and efficacious, is an important aspect of sustainable agriculture. This is driven by the need to supply food to the ever-growing world population, and the call for chemical load reduction.

Tea tree oil (TTO), an essential oil extracted from the plant *Melaleuca alternifolia*, contains many components, mostly terpenes and their alcohols, and has been shown to be an effective antiseptic, fungicide and bactericide (Carson *et al.* 2002; 2006; Cox *et al.* 2001; Hammer *et al.* 2003 and 2004; Markham 1999) and more recently against fungal plant pathogens (Shao *et al.* 2013a; 2013b). Until the last decade, this essential oil had not been tested against plant pathogens

in field-grown agricultural crops. Based on TTO, as an active ingredient, a natural fungicide Timorex Gold (22.3 EC W/V), was prepared for use on plant tissue (Reuveni *et al.* 2009b). This product was found to be effective against a broad range of plant-pathogenic fungi in numerous crops, including vegetables, herbs, grapevines, bananas and fruit trees (Reuveni *et al.* 2009a; Reuveni *et al.* 2009b; Vardi & Reuveni 2009).

Recently, the activity of tea tree oil against *Mycosphaerella fijiensis* and its efficacy against black Sigatoka in field-grown banana plants in South and Central America was tested. The study provided evidence that it represents an additional alternative to existing treatments for controlling black Sigatoka in banana plantations. Some preliminary results have been published (Martillo & Reuveni 2009).

Fungicides

Tea tree oil was used in all trials as an emulsifiable concentrated formulation (Timorex Gold, 22.3 EC W/V; STK group, Israel). For field trials in bananas, TTO was applied similarly to conventional synthetic fungicides, with mineral oil, surfactants, and/or just water. The following fungicides, registered for use against black Sigatoka in banana, were tested for comparison: azoxystrobin (Bankit 25 SC), trifloxystrobin (Flint, Tega 50 WG), difenoconazole (Score, Sico 25 EC), mancozeb (43 SC Duwest 80 WP), flutriafol (Impact, 125 SC) and tridemorph (Calixin 86 OL).

Spore Germination

The sensitivity of *Mycosphaerella fijiensis* ascospores to Timorex Gold was examined. Dry leaf tissue from a banana leaf on stage 6 black Sigatoka lesions containing mature pseudothecia was collected at Monreri Experimental Farm, located on the Atlantic Coast of Costa Rica. The selected tissue was incubated for 48 h at 26°C in plastic bags with a moist paper towel. The infected samples were then removed from the

plastic bag and cut into small pieces of 1-2 cm. The paper with the attached leaf pieces were submerged for 5 minutes in distilled water and then immediately placed inside the top of a petri dish for thirty minutes to allow ascospores discharge over the dish containing 2% water agar amended with various concentrations of TTO. The paper and leaf tissue were then removed and the dishes were incubated at 26°C for 48 hours to allow the ascospores to germinate. The percentage of germinated ascospores was recorded and the inhibition was calculated for each concentration, as a percentage of the germination rate in the controls.

In Uraba, Colombia, leaf tissue samples on stage 6, were collected from two different commercial farms and from wild untreated banana plants. Using the same methodology, the sensitivity of *Mycosphaerella fijiensis* ascospores to TTO formulation at different concentrations was examined.

Single-Leaf Test

A trial was performed in La Blanca, San Marcos, Guatemala. A modification of the “Single leaf test” format, was used where the leaf blade on both sides was treated with a hand aerograph calibrated to spray an equivalent volume to 25 L per hectare. Treatments were arranged in a complete randomized block design replicated 4 times. Evaluated treatments were trifloxystrobin, tridemorph, difenoconazole and TTO. Each treatment was sprayed in emulsion or suspension. Assessments were done five weeks after application by quantifying the disease severity and calculating the area under disease progress curve (AUDPC).

Field trials using whole young plants

Cultivars of Cavendish banana were used, which were susceptible to the pathogen – disease had been evident in these plantations in previous years. Methods of fertilization and other cultural practices for this crop were as recommended to commercial growers in each country.

Experimental Design

Treatments, applied to young banana plants in each trial, were arranged in a randomized complete block design. Plots of various banana plants were replicated four times in all experiments, if not specified differently. Sprays of comparison fungicides and TTO were applied to the banana plants at the time intervals specified for each experiment, and as recommended in each region.

Trial 1. Ecuador

This trial was conducted in 2008 on 8-week-old banana plants, cv. Musa AAA, Williams Hybrid, grown under conventional management in the Vines region, Los Ríos province of Ecuador. Plants were arranged in a randomized complete block design, with four replicates per treatment and five plants per replicate. Four consecutive foliar sprays of either TTO or one of the systemic fungicides, difenoconazole or azoxystrobin were applied at 14-day intervals, each at 0.4 L /ha, by means

of a 15-L-capacity “Cooper Pegleer” Snap Pack sprayer, with a TJ-8001 nozzle. The fungicides were prepared in a water-oil emulsion containing 10% of mineral oil, to provide a final volume application rate of 80 L/ha.

Trial 2. Costa Rica

Another trial was conducted in 2007 in Guápiles, Limón in Costa Rica, on cv. Gran Naine banana plants. It was arranged in a randomized complete block design, with five replicates per treatment, and 16 plants per replicate. From April 13, 2007 (week 14) to June 18, 2007 (week 25) 13 consecutive foliar sprays of TTO formulation at 0.25, 0.5 or 1.0 L/ha, or tridemorph at 0.5 L/ha were applied. Each was mixed with 7 L/ha of mineral oil Spraytex (Texaco) and 1% of NP-7 (Bayer CropScience) and 0.5 L/ha of Nu-Film (Miller Chemical). Foliar applications in all treatments used a Motorblock sprayer (Stihl SR-420), calibrated to apply at 23.6 L /ha. Plants were evaluated and disease was rated weekly by examining each plant of each replicate plot and each treatment.

Trial 3. Guatemala

A similar trial, that used banana plants cv. Gran Naine, was conducted in Rio Bravo, Escuintla, Guatemala. The trial included four replicates per treatment with one plant per replicate. Six consecutive foliar applications of either TTO formulation at 0.5 L/ha, or the systemic fungicides difenoconazole 25 EC and trifloxystrobin 25 SC, each at 0.4 L/ha, were applied at intervals that varied, depending on the infection level on the plants, between 2 and 4 weeks, i.e., at weeks 33 (the first application), 37, 39, 42, 45 and 49 of year 2009. In addition, tank mixes of formulated TTO at the low rate of 0.2 L/ha with each of the fungicides at the full rate of 0.4 L/ha or the half-rate of 0.2 L/ha were applied. TTO, systemic fungicides, and each of their mixtures were each mixed with a mineral oil at a rate of 4 L/ha and Adsee (Duwest, Guatemala) at a rate of 0.04 L/ha. Foliar applications of all treatments were done by means of an air compressor with a low-pressure nozzle at 30 psi, calibrated to apply an average of 15 ml of the mixture per plant – equivalent to an application rate of 20 L /ha. Plants were evaluated weekly by examining each plant in each replicate plot of each treatment.

Trial 4. Brazil

This trial was conducted in 2014 in the Dracena Site, located in the County of Jiquiá, SP, Farm Poço Grande on banana plants cv. Prata. The experimental design was in randomized blocks and included six treatments with four replicates per treatment with 6 plants per replicate. Four different rates of formulated TTO were tested: 0.3 L /ha; 0.4 L /ha; (with addition of mineral oil, in the dosage of 5 L / ha), using the Standard fungicide flutriafol)125 SC) at the dose of 1 L / ha (with mineral oil in the dosage of 15 L / ha). Untreated plants served as controls. Two foliar sprays were applied at 21-d intervals starting at February 2, 2014 at spray volume of 20 L / ha in each application.

Large-scale demonstration trial

A semi-commercial trial was conducted at Banana Growers Association farms, South Stann Creek Big Creek, Belize, from June 30, 2008 to February 22, 2009. A total of 32 applications of formulated TTO were made during this period on an area of 134 ha. Fifteen consecutive applications of TTO were made at an average interval of 7 days, starting on June 30, 2008. These were followed by a single application of (difenoconazole plus mancozeb), and then another five consecutive applications of TTO at an average interval of 6 days. Then a single application of chlorothalonil was used followed by 12 consecutive applications of TTO. A control commercial treatment included conventional chemical compounds such as mancozeb, chlorothalonil, and systemic fungicides, applied on dates in parallel with those of the experimental treatments. TTO and fungicides were each mixed with a mineral oil at a rate of 4 to 6 L/ha, and 1% NP-6 emulsifier and were sprayed at a total volume rate of 18.0 L/ha. The disease variables per plant were recorded weekly.

Disease Assessment

In other trials, the disease was assessed according to the researcher criteria; the number of lesions per each leaf in each stage were used, youngest leaf infected and youngest leaf spotted (YLI and YLS), and Stover modified by Gauhl methodology (Gauhl *et al.*, 2000) were evaluated as disease incidence and severity and all data were harmonized by calculating the area under disease progress curve (AUDPC). Disease damage was converted into the AUDPC using the formula suggested by Shaner and Finney (1977).

In Ecuador disease was assessed by counting the number of lesions on each leaf of each plant, at each black Sigatoka development stage from stage two to stage six.

In Costa Rica the disease severity was recorded. This methodology has six values ranging from 0 to 6 as a function rating the percentage of injured leaf area. The rates are visual observations of each leaf in the plant. In Guatemala the YLI and YLS methodology was used.

Statistical analysis

Analysis of variance (ANOVA) using the SAS GLM (SAS Institute, Inc., Cary, NC) procedure was applied to the data. The Tukey-Kramer HSD Test was applied to determine whether differences between treatments were significant.

Effect of formulated TTO on spore germination

In Costa Rica, the *in vitro* studies revealed that TTO effectively inhibited the germination of ascospores of *M. fijiensis*: at concentrations of 10, 100 and 1000 ppm it inhibited germination of *M. fijiensis* ascospores on 2% water agar by 21, 39 and 100%, respectively, relative to the control.

In experiment conducted in Colombia all evaluated tissue samples either coming from the commercial farms or from the wild plants, showed a similar percentage inhibition of the elongation of the germ tube. In most cases, the inhibition was dependent on the TTO concentration. As product concentration

Table 1. Effect of formulated TTO and fungicides on development of black Sigatoka and lesion expansion, as determined by a Single leaf study performed in La Blanca, San Marcos, Guatemala (November 2 to December 20, 2006)¹

Treatment (L/ha) ²	Disease severity ³	AUDPC
Tridemorph 86 OL 0.5	2.3 B ⁴	57.3 B
Difenoconazole 25 EC 0.4	2.4 B	56.3 B
Trifloxystrobin 25 SC 0.4	1.9 B	24.9 B
TTO 23.8 EC 0.4	2.3 B	70.1 B
Untreated control	3.9 A	251.1 A

¹A "Single leaf test" format, was used where both sides of the leaf blade were sprayed with an equivalent volume to 25 L per hectare.

²Each treatment was sprayed in emulsion or suspension accordingly.

³Assessment was done five weeks after applications by quantifying the disease severity.

⁴Mean numbers within the columns followed by different letters are significantly different ($p < 0.05$) according to Tukey-Kramer HSD Test.

increased, the inhibition percentage of the elongation of the germinating tube also increased. At 10 ppm concentration inhibition ranged between 43 and 78%. As concentration increased results became more homogeneous, and at 100 ppm concentration inhibition range was between 73 and 85%. At 1000 ppm inhibition was 100% in all samples.

Inhibition of Lesion Development and Expansion

The results of a Single Leaf Test (SLT) conducted in La Blanca, San Marcos, Guatemala showed that TTO or each of the three chemical fungicides tested similarly inhibited disease development as expressed by disease severity and the AUDPC, being significantly different from the untreated control (Table 1).

Field Trials Using Whole Plants

Results of the first trial in Ecuador showed that TTO was as effective as difenoconazole and azoxystrobin in controlling black Sigatoka, and that disease development on the treated plants was significantly different from that on the untreated controls (Figure 1; panels a–e). Formulated TTO and the synthetic fungicides all provided excellent disease control, and inhibited the development of lesions at each of the stages 2–6, on all tested leaves. Further analysis to harmonize the data, the calculated disease parameters, revealed that there were no significant differences for the youngest leaf infected, youngest leaf spotted, disease severity and the AUDPC between treatments which received fungicide treatments, but all treatments were different from the untreated control (Table 2).

Similar results were obtained in the second trial in Costa Rica. TTO was as effective as tridemorph in controlling black Sigatoka (Figure 2). Further analysis to harmonize the data, the calculated disease parameters, revealed that there were no significant differences for the youngest leaf infected, youngest leaf spotted, disease severity and the AUDPC between treatments

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Table 2. Efficacy of formulated TTO and systemic fungicides on control of black Sigatoka on young banana plants, Los Rios, Ecuador 2009.

Treatment ¹	YLI	YLS	Total Lesions ²	Severity	AUDPC
TTO 23.8 EC 0.4 L/Ha	7.0 A ³	8.0 A	218 B	26.9 B	15.0 B
Difenoconazole 25 EC 0.4 L/Ha	7.4 A	8.0 A	144 B	26.7 B	9.8 B
Azoxystrobin 25 SC 0.4 L/Ha	7.2 A	9.0 A	203 B	22.2 B	12.0 B
Untreated control	5.4 B	6.0 B	3584 A	36.7 A	272.1 A

¹Four consecutive foliar sprays of either TTO or one of the systemic fungicides, difenoconazole and azoxystrobin were applied at 14-day intervals, each at 0.4 liter/ha, to 8-week-old banana plants. Plants were arranged in a randomized complete block design, with three replicates per treatment and five plants per replicate.

²Plants were evaluated by counting the number of lesions of each stage on each numbered leaf. As shown in Fig. 1 The given data representing the last evaluation as described in M & M section.

³Mean numbers within the columns followed by different letters are significantly different ($p < 0.05$) according to Tukey-Kramer HSD Test.

Table 3. Efficacy of formulated TTO and tridemorph on the control of black sigatoka on young banana plants. (Guapilus, Costa Rica, 2007).

Treatment, Rate (L/ha) ¹	Disease Rating ²			AUDPC
	YLI	YLS	Severity	
TTO 23.8 EC 0.25	4.4 A ³	6.2 B	1.2 B	41.4 B
TTO 23.8 EC 0.5	4.4 A	6.2 B	1.4 B	44.8 B
TTO 23.8 EC 1.0	4.5 A	6.3 B	1.3 B	44.3 B
Tridemorph 0.5	4.6 A	6.8 B	1.0 B	32.3 B
Untreated control	3.8 B	5.1 C	2.3 A	79.9 A

¹Thirteen consecutive foliar sprays of formulated TTO at 0.25, 0.5 or 1.0 L/ha, or tridemorph at 0.5 L/ha were applied to banana plants from April 13, 2007 (week 14) to June 18, 2007 (week 25). Treatments were arranged in a randomized complete block design, with five replicates per treatment, and 16 plants per replicate.

²Plants were evaluated and disease was rated weekly as shown in Figure 2 by examining each plant of each replicate plot and each treatment. The given data representing the last evaluation.

³Mean numbers within the columns followed by different letters are significantly different ($p < 0.05$) according to Tukey-Kramer HSD Test.

Table 4. Efficacy of formulated TTO on the control of black sigatoka on young banana plants. (Brazil, 2014) I.

Treatment (L/ha) ²	Disease severity ³
TTO 0.2	6.0 B ⁴
TTO 0.4	4.0 BC
TTO 0.6	3.7 C
TTO 1.0	3.5 C
Flutriafol 1.0	3.0 C
Untreated control	14.0 A

¹The experiment was conducted in 2014 and included six treatments with four replicates per treatment with 6 plants per replicate and designed in randomized blocks.

²Two foliar sprays were applied at 21-d intervals at spray volume of 20 L / ha in each application.

³Disease assessment was done 35 days after last applications by quantifying the disease severity.

⁴Mean numbers within the columns followed by different letters are significantly different ($p < 0.05$) according to Tukey-Kramer HSD Test.

which received fungicide treatments, but were different from the untreated control (Table 3).

Similar results were obtained in the third trial, in Guatemala, in which TTO at 0.5 L/ha was as effective as difenoconazole or trifloxystrobin (Figure 3). Tank mixes of TTO at the half rate of 0.2 L/ha with either difenoconazole or trifloxystrobin were as effective as any of the components applied alone at the recommended rate (Figure 3). The AUDPC values for TTO, each fungicide at full rate or a tank mix of TTO product and each fungicide at half rates ranged between 83-114 with no significant differences, but were significantly lower than control untreated with a value of 163.

The trial in Brazil provided similar results in which disease severity evaluated at 28 days after the second application showed high efficacy of TTO in controlling black Sigatoka. At a rate of 0.4 L/ha and higher it was as effective as the standard flutriafol fungicide and better than untreated control trees (Table 4).

Large-Scale Demonstration Trial

Thirty-two foliar sprays of TTO were applied to a commercial area of 134 ha, and compared with a control commercial program using protectant and systemic fungicides applied in rotation on the same dates as TTO sprays. The TTO treated area demonstrated efficacy equivalent to the control commercial treatments, as indicated by analyzing the youngest leaf spotted (Figure 4) and youngest leaf infected and AUDPC (Data not shown).

In all trials no symptoms of phytotoxicity in any of the treatments were observed.

Any product that significantly inhibits spore germination, fungal growth, and streak formation should reduce the ability of *M. fijiensis* to cause black leaf streak disease. At appropriate concentrations, the natural formulated TTO significantly inhibited germination of conidia *in vitro*, or lesion development on treated leaves, and limited the expansion of lesions (Tables 1 and 2), and thereby restricted the potential of *M. fijiensis* to infect plant tissue and cause disease.

Black Sigatoka is considered the most damaging and costly disease of banana and plantain (Fullerton 1994; Marin *et al.* 2003; Stover 1986) and its control accounts for 27% of total production costs (Marin *et al.* 2003). It has been esti-

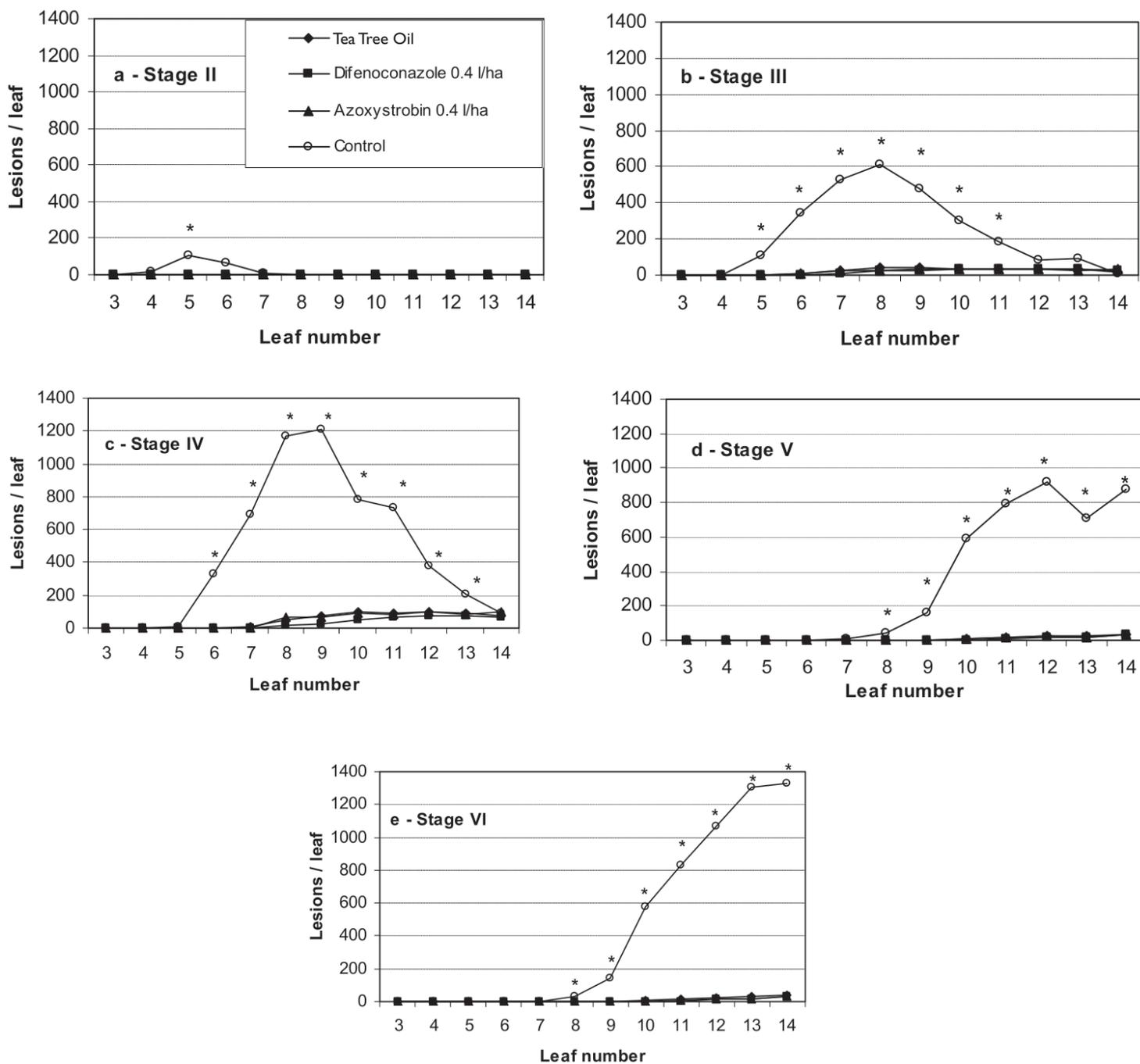


Figure 1 (a–e). Efficacy of formulated TTO and systemic fungicides on control of black Sigatoka on young banana plants, Ecuador 2009. Number of lesions of each stage was counted on each numbered leaf. Stars indicate significant difference ($p < 0.05$) between control untreated and other treatments.

ated that the disease causes more than 38% loss of plantain yield (Marin *et al.* 2003), and even greater losses may occur on export bananas when control measures fail, because the disease destroys the foliage very rapidly if appropriate control measures are not applied. Both growth and yield are affected because of the reduction in the photosynthetic area and premature ripening of fruit, which can occur in the field and during transport and storage (Fullerton, 1994; Marin *et al.* 2003).

The present paper shows that TTO effectively controlled black Sigatoka in whole-plant trials conducted in several countries (Figures 1–3 and Tables 2–4) and in a large-scale demonstration trial conducted in Belize (Figure 4). Due to different

climatic conditions, pressure of the pathogen populations and cultural practices in each country, the number of foliar applications and their intervals was determined according to that recommended for each country and region. As climatic conditions in these regions are characterized by many rainy days and relatively high levels of rainfall, it is a routine approach to add mineral oil to fungicidal spray mixture. Early tests demonstrated that either TTO plus mineral oil or systemic fungicide plus mineral oil provided better results than mineral oil alone at the same rate (E. Martillo, personal communication). Although mineral oil, mainly at higher rates can inhibit black Sigatoka development, it reduced plant growth rate, delayed flowering by four days, and reduced bunch weight of

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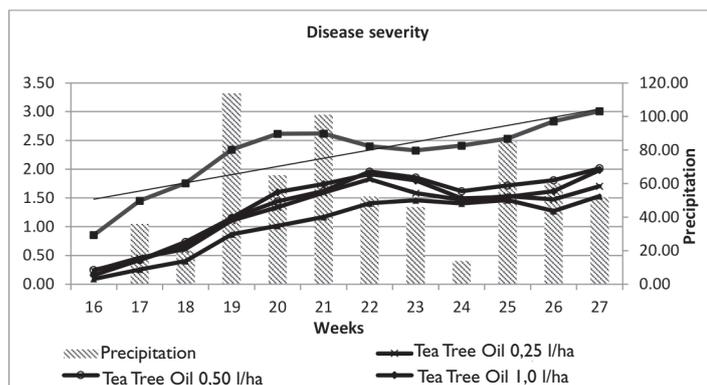


Figure 2. Efficacy of formulated TTO and tridemorph on the control of black sigatoka on young banana plants. (Monreri, Costa Rica, 2007). Disease severity of each treatment was evaluated in each week as described in materials and methods. A significant difference ($p < 0.05$) between control untreated and other treatments was observed.

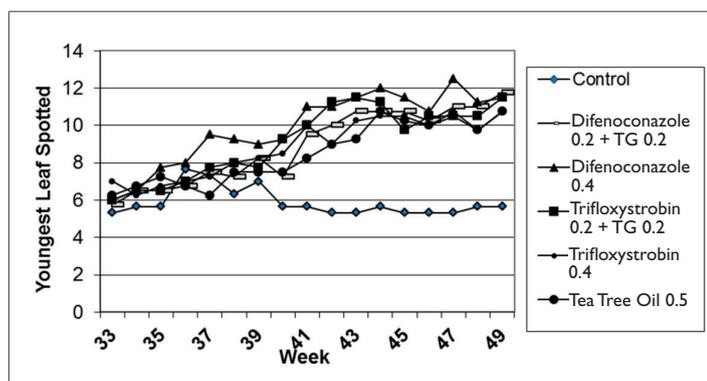


Figure 3. Control of black Sigatoka by formulated TTO (TG), systemic fungicides, and their mixtures; Guatemala, 2009. The youngest leaf spotted of each treatment was evaluated in each week. A significant difference ($p < 0.05$) between control untreated and other treatments was observed.

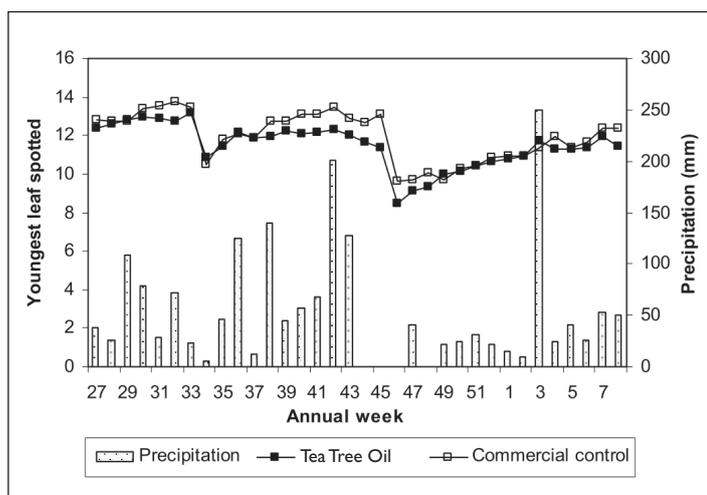


Figure 4. Effects of formulated TTO and commercial treatments with conventional fungicides on control of black Sigatoka in large-scale trial, Belize, 2008–9. The youngest leaf spotted of each treatment was evaluated in each week as described in materials and methods. No significant difference ($p < 0.05$) between the two treatments was observed.

banana plants by 5.6% (Israeli *et al.* 1993). Additionally, at the time when these studies were conducted, synthetic fungicides such as strobilurins or azoles used as standards in this work, were considered to be highly effective against black Sigatoka.

Each of the fungicides tested in the present study has a unique mode of action at the biochemical level. Strobilurin analogs, such as trifloxystrobin or azoxystrobin, inhibit mitochondrial respiration by blocking electron transfer at the cytochrome bc1 complex, also known as complex III (Anke 1995; Brandt *et al.* 1988). The sterol biosynthesis inhibitors, including difenoconazole, are inhibitors of the C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol, a biosynthesis step that occurs during the conversion of lanosterol to ergosterol, the final product of fungal membrane sterol synthesis (Koller & Scheinpflug 1987).

The fungicidal and antimicrobial activities of TTO against fungal pathogens arise from its ability to disrupt the permeability barrier of living organisms' membrane structures (Carson *et al.* 2002; Cox *et al.* 2000; 2001). In yeast cells and isolated mitochondria extract of tea tree components destroy cellular integrity, inhibit respiration and ion transport processes, and increases membrane permeability (Carson *et al.* 2002; Cox *et al.* 2000; 2001). Results obtained with transmission electron microscopy showed that TTO disrupted the fungal cell wall and cell membrane of *M. fijiensis* at stages 4 or 5 of fungal development in the intracellular space of banana leaf mesophyll (Reuveni *et al.* 2012). This may account for the strong curative activity of TTO against black Sigatoka. A similar effect was observed by Shao *et al.* (2013a) against *Botrytis cinerea*.

As each compound has a different mode of action (Anke 1995; Brandt *et al.* 1988; Cox *et al.* 2000; Koller & Scheinpflug 1987; Shao *et al.* 2013a), these modifications could be incorporated into a disease-management program that would minimize the risk of resistance development by *M. fijiensis* (Brent & Hollomon 2007; Marin *et al.* 2003) and, at the same time, maximize disease control. The present study showed that either trifloxystrobin or difenoconazole effectively controlled Black Sigatoka when applied as tank mixes with TTO (Figure 3). Fungicides are combined in mixtures mainly: to widen the spectrum of antifungal activity and to extend its duration; to exploit the synergistic interaction between the compounds, whereby the overall activity can be increased or the amounts used can be reduced without loss of activity; and to delay or reduce the emergence of resistant strains (Gisi 1996). The similarity of the efficacies of the tank mixes of systemic fungicides and TTO (Figure 3) suggests that the tank-mix treatment can be considered as a strategy for control of Black Sigatoka. Although, in principle, combinations of synthetic antifungal compounds could be used to reduce the chemical load of any particular compound applied to crops, concerns about resistance development at lower doses has militated against their use as advised by the Fungicide Resistance Action Committee (FRAC) (Brent & Hollomon, 2007). Further studies are needed in order to demonstrate this resistance-management strategy. Essential tea tree oil, a multicomponent compound, exhibits multisite functional activity and very low probability of promoting resistance or cross-resistance in plant pathogens. Therefore, TTO is an important tool for inclusion in

spray programs, to avoid cross-resistance development during the season. It can be rotated in applications with products to which *M. fijiensis* populations have shown a loss of sensitivity.

The TTO product (Timorex Gold) constitutes an attractive alternative for controlling black Sigatoka and various other diseases (Reuveni et al. 2009a and 2009b; Vardi and Reuveni 2009) in banana plantations, and can be used in both organic and conventional systems.

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References

- Anke T. (1995) The antifungal strobilurins and their possible ecological role. *Can. J. Bot.*, 73(Suppl. 1):S940–S945.
- Brandt U, Schagger H, Jagow G. (1988) Characterization of binding of the methoxyacrylate inhibitors mitochondrial cytochrome c reductase. *Eur. J. Biochem.* 173:499–505.
- Brent KJ, Hollomon DW. (2007) Fungicide resistance in crop pathogens: how can it be managed? *Fungicide Resistance Action Committee*, 2nd rev. edit. 56 pages. Crop Life International, Brussels, Belgium.
- Carson CF, Hammer KA, Riley TV. (2006) Maleleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin. Microb. Rev.* 19:50–62.
- Carson CF, Mee BJ, Riley TV. (2002) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob Agents Chemother.* 46:1914–20.
- Chillet M, Abadie C, Hubert O, Chilin-Charles Y, de Lapeyre de Bellaire L. (2009) Sigatoka disease reduces the greenlife of bananas. *Crop Prot.* 28:41–45.
- Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG. (2000) The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microb.* 88:170–175.
- Cox SD, Mann CM, Markham JL, Gustafson JE, Warmington JR, Wyllie SG. (2001) Determining the antimicrobial actions of tea tree oil. *Molecules* 6:87–91.
- Cronshaw DK. (1982) Management of banana leaf spot (sigatoka) disease in the Windward Islands. *Trop. Pest Manag.* 28:136–146.
- Fullerton RA. (1994) Compendium of tropical fruit diseases. Pages 12–14 in: *Sigatoka Leaf Diseases*. RC. Ploetz, GA. Zentmyer, WT. Nishijima, KG. Rohrbach. & HD. Ohr, eds. APS Press, St. Paul, MN, USA.
- Gisi U. (1996) Synergistic interaction of fungicides in mixtures. *Phytopath.* 86:1273–1279.
- Hammer KA, Carson CF, Riley TV. (2003) Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *J. Appl. Microb.* 95:853–860.
- Hammer KA, Carson CF, Riley TV. (2004) Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*. *J. Antimicro. Chemo.* 53:1081–1085.
- Israeli Y, Shabi E, Slabaughc WR. (1993) Effect of banana spray oil on banana yield in the absence of Sigatoka (*Mycosphaerella* sp.). *Sci. Hort.* 56: 107–117
- Jacome LH, Schuh W. (1992) Effects of leaf wetness duration and temperature on development of black Sigatoka disease on banana infected by *Mycosphaerella fijiensis* var. *difformis*. *Phytopath.* 82:515–520.
- Koller W, Scheinpflug H. (1987) Fungal resistance to sterol biosynthesis inhibitors: A new challenge. *Plant Dis.* 71:1066–1074.
- Marin D, Romero R, Guzman M, Sutton T. 2003. Black sigatoka: an increasing threat to banana cultivation. *Plant. Dis.* 87:208–222.
- Markham J. (1999) Tea tree – the genus *Melaleuca*. Pages 169–190 in: *Biological Activity of Tea Tree Oil*. J. Southwell, & R. Lowe, eds. Har. Ac. Pub. The Netherlands.
- Martillo EE, Reuveni M. (2009) A new potent bio-fungicide for the control of Banana Black Sigatoka. *Phytopath.* 99:S80 (Abst.).
- Reuveni M, Arroyo CJ, Henriquez JL. (2009a) A new tea tree oil-based organic fungicide for the control of grape powdery and downy mildews. *Phytopath.* 99:S108 (Abst.).
- Reuveni M, Neifeld D, Dayan D, Kotzer Y. (2009b) BM-608 – A novel organic product based on essential tea tree oil for the control of fungal diseases in tomato. *Acta Hort.* 808:129–132.
- Reuveni M, Sanchez E, Barbier M, Ramirez F. (2012) Mode of activity of Timorex Gold against black Sigatoka (*Mycosphaerella fijiensis*) on banana leaves. 4th Int. Banana cong. 20–24 Feb. 2010. San Jose, Costa Rica.
- Shao X, Cheng S, Wang H, Yu D, Mungai C. (2013a) The possible mechanism of antifungal action of tea tree oil on *Botrytis cinerea*. *J. Appl. Microb.* 114:1642–1649.
- Shao X, Wang H, Xu F, Cheng S. (2013b). Effects and possible mechanisms of tea tree oil vapor treatment on the main disease in postharvest strawberry fruit. *Post. Biol. Tech.* 77: 94–101.
- Shanner G, Finney RE. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopath.* 67:1051–1056.
- Stover RH. (1980) Sigatoka leaf spots of bananas and plantains. *Plant Dis.* 64:750–755.
- Stover RH. (1986) Disease management strategies and the survival of the banana industry. *Ann. Rev. Phytopath.* 24:83–91.
- Vardi Y, Reuveni M. (2009) Antifungal activity of a new broad spectrum bio-fungicide in the controlling of plant diseases. *Phytopath.* 99:S134 (Abst.).

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