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Assessment of Fungicide Resistance of a Population of *Mycosphaerella* Spp. on Señorita Banana Variety (Sucrier Group)

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ABSTRACT

Mycosphaerella leaf spot diseases have threatened world banana production. Yield losses from 40-50% have been reported in some cases. During the second cropping cycle, plantain yield loss due to black Sigatoka could reach 76%, and 100% loss of exportable fruits. In commercial banana plantations, fungicide has still become the main tool in mitigating development and effect of the disease; however, the dependence of fungicide for the control of banana diseases has led to a new problem of fungicide resistance. This research was to study the fitness and to assess fungicide resistance of *Mycosphaerella* pathogens causing banana leaf spot diseases from exposure of different fungicide pressures. The treatments were established based on a management scheme of fungicide resistance as follows: a) consecutive application of protectant (Mancozeb) fungicide; b) consecutive application of systemic (azoxystrobin) fungicide; c) alternate application of protectant and systemic fungicides; d) mixture application of protectant and systemic fungicides; e) control (i.e., no fungicide application). Fungicide treatments significantly reduced conidial density and its germination, and affected youngest leaf spotted (YLS) and the severity of *Mycosphaerella* leaf spot diseases. Consecutive azoxystrobin applications consistently resulted in the lowest conidial densities that were significantly different from consecutive applications of mancozeb, and the unsprayed control. The lowest conidial germination resulted by application of azoxystrobin but it was not significantly different from the other fungicide treatments. The fungicide application, however, significantly differed from the untreated control. Ten consecutive applications of azoxystrobin resulted in a 5.1-fold pathogen resistance as compared with control. Development of azoxystrobin resistance was also observed in a mixed application of azoxystrobin and mancozeb, consecutive application of mancozeb, and alternate application of azoxystrobin and mancozeb.

Keywords: azoxystrobin, conidial density, conidial germination, disease severity, systemic fungicide, youngest leaf spotted
Abbreviations: BLSD, black leaf streak disease; **DDT**, disease development time; **EC_{50}**, 50% of effective concentration; **FRAC**, Fungicide Resistance Action Committee; **RCBD**, randomized complete block design; **SD**, sigatoka disease; **UPLB**, University of the Philippines Los Baños; **YLS**, youngest leaf spotted

INTRODUCTION

Mycosphaerella leaf spot diseases have threatened world banana production. It does not only affect banana leaves, but also bunch weight and fruit quality. Yield losses from 40-100% have been reported in some cases (Meredith 1970; Martinez 1997; Mourichoun *et al*. 1997; Porras and Perez 1997; Cordeiro *et al.* 1998; Martinez *et al.* 1998; Vicente 1998; Ploetz 2000a; Burt *et al*. 1997 *in* Sanchez and Zapata 2002). During the second cropping cycle, plantain yield loss due to black Sigatoka could reach 76% (Ngongo 2002), and 100% loss of exportable fruits (Porras and Perez 1997) due to premature bunch (Meredith 1970; Balbín and Zapata 2001). In Mexico, disease incidence of Cavendish plantation reached 100% (Orozco-Santos *et al.* 1995, 2001).

In commercial banana plantations, fungicide has still become the main tool in mitigating development and reducing effect of the diseases. With around 20–25 sprays a year (Peterson 1998), it consumed 15-45% of the final retail price (Jones 2002; Martinez *et al*. 2002) or increased the production cost by 40-45% (Ngongo 2002). The cost of control was predicted at around $1,000/ha/year. Jones (1995) reported that plantain growers in Venezuela applied 12-15 fungicide treatments a year to control black Sigatoka consuming about USD 350/ha.

The dependence of fungicides for the control of banana diseases has led to a new problem of fungicide resistance. It was reported for the first time in the 1960s when systemic fungicides started to be used, paralleling the introduction of new compounds that attack specific biochemical targets in the pathogen (Eckert 1988). Adaptation to fungicide was clearly demonstrated by the loss in efficacy of certain products used for chemical control such as benzimidazoles and triazoles (Guzman *et al.* 2000; Ploetz 2000b; Romero 2000 *in* Martínez *et al*. 2002). Resistance of *M. fijiensis*, the causal agent of black leaf streak disease (BLSD), to benzimidazoles was reported in the 1970s from Central America (Smith 1988). However, Smith (1988) further stated that reentry of benzimidazole after resistance occurs is possible. This suggests that resistance strains of *M. fijiensis* are not as competitive as the wild-sensitive strains due to their loss of fitness.

A shift in sensitivity of *M. musicola*, the causal agent of Sigatoka disease (SD), was first detected with propiconazole in 1995 as it was reported in Australia by Peterson *et al*. (2002); a shift in sensitivity to tebuconazole was detected in *M. musicola* populations that had not been sprayed with tebuconazole, but with propiconazole. It represented cross resistance from propiconazole to tebuconazole. A shift in sensitivity to triazoles was linked to excessive use (8-12 applications), the number of consecutive applications (3-5), and applications to heavily diseased plants (no deleafing), which was reversed when the product or similar products were withheld for 6-12 months.

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Original Research Paper

In 1997 the first strobilurin (azoxystrobin) fungicide was introduced. Due to its excellent efficacy and favorable environment and toxicological profile, the introduction represented a significant step forward in the integrated control of BLSD. The fungicide has a site-specific mode of action by the inhibition of electron transport at the Qo site of cytochrome bc_1. However, in 2000, the first strobilurin-resistant individuals were reported in Costa Rica. Resistance to strobilurin has reached high levels on some farms in the main banana production zones of Costa Rica (Knight *et al.* 2002).

Regarding the implementation of chemical control measure, Mourichoun (2002) stated that in the future, the aim will be to gain alternative chemical control but without excluding it completely. Beresford (1994) stated that fungicide resistance is manageable where there is a loss of fitness with resistance. In many cases, there is lack of data. All too frequent, theoretical strategies are debated and implemented but not tested (Delp 1988). A rational chemical control should be generated as part of the integrated pest management.

This research's objectives were to study the fitness and to assess the fungicide resistance of *Mycosphaerella* pathogens causing banana leaf spot diseases from exposure to different fungicide pressures. Specifically, the study aimed to:

1. Determine the frequency of occurrence of *P. musae* and *P. fijiensis* from Señorita banana variety (Sucrier group) as the test material of fungicide assessment,
2. Count the density (nos. 1.237 cm^{-2}), measure the length, calculate the germination, and assess the azoxystrobin sensitivity of *Mycosphaerella* spp. conidia taken from Señorita banana variety (Sucrier group) previously subjected to different fungicide pressures.
3. Evaluate the virulence of Mycosphaerella leaf spot pathogens on Señorita banana variety (Sucrier group) previously subjected to different fungicide pressures.

MATERIALS AND METHODS

Field experiment

The study was conducted at lowland Santa Rosa, Laguna, the Philippines (14° 14′ 39.1″ N and 121° 03′ 34.0″ E, 23 masl) from November 2004 to May 2005. Using an orchard of six-month old Senorita banana cultivar, the experiment was arranged in randomized complete block design with three replications, and nine plants per plot. The treatments were established based on the management scheme of fungicide resistance proposed by Wade (1988) as follows:

A. Consecutive application of protectant (mancozeb = Dithane M-45 (Rohm and Haas)) fungicide (P);
B. Consecutive application of systemic (azoxystrobin = Amistar® (Syngenta)) fungicide (S);
C. Alternate application of protectant and systemic fungicides (P → S → P → S → P → S);
D. Mixture application of protectant and systemic fungicides (S+P → P → P → S+P → P → P); and
E. Control (no fungicide application).

Fungicide sprays were applied 10 times at weekly intervals with semi automatic sprayer, using recommended dosage of the fungicides (3 g/l for mancozeb and 0.625 ml/l of azoxystrobin). Observation was done weekly toward youngest leaf spotted (YLS): the first fully unfurled leaf with 10 or more discrete, mature, necrotic lesions or one large necrotic area with 10 light-colored dry centers (Meredith 1970; Mobambo *et al.* 1993), and disease severity of Mycosphaerella leaf spot diseases (Carlier *et al.* 2002).

Laboratory observations

The effect of field treatments was also assessed in the laboratory of Plant Pathology, University of the Philippines Los Baños (UPLB) from November 2004 to May 2005. Mature-necrotic leaf spot were collected every fortnight from the field experimental site. Observations were addressed to the frequency occurrence of *P. musae* and *P. fijiensis* (Orjeda 1998), density and germination of *Mycosphaerella* spp. conidia (Gaviria *et al.* 1999), and 50% effective concentration (EC_{50}) of azoxystrobin fungicide of *Mycosphaerella* spp. (Gaviria *et al.* 1999).

Greenhouse virulence test

The effect of fungicide applications against the virulence of *Mycophaerella* spp. was assessed in greenhouse of the Department of Plant Pathology, UPLB from May to July 2005. Mature-necrotic leaf spots were collected from each treatment of fungicide application from the field experiment. Modifying the method used by Mora *et al.* (2002), the spots then were extracted in sterile distilled water to collect *Mycosphaerella* spp. propagules. After filtered with muslin cloth, the inoculum was adjusted in 10^4 dilution, sprayed on two-month old seedling of Senorita banana variety. Observation was done in six-hour interval for the first three days after inoculation to see conidial development, and daily interval to asses incubation period (time from inoculation to first symptom appeared), and disease development time (DDT; time from inoculation to necrotic) (Mobambo *et al*. 1994; Orjeda 1998).

Statistical analysis

Frequency of occurrence of *P. musae* and *P. fijiensis* is presented as percent of each fungus from the total observed conidia. Density and germination of *Mycosphaerella* spp. conidia; and YLS and disease severity of Mycosphaerella leaf spot were analyzed with variance analysis in randomized complete block design (RCBD). To see the effect of number of fungicide application on conidial germination, the data was analyzed in regression. Azoxystrobin sensitivity of *Mycosphaerella* spp. is represented by EC_{50} obtained from Probit regression using PriProbit software version 1.63. The time needed for disease development is presented in a schematic figure.

RESULTS AND DISCUSSION

The pathogens

Both *P. musae* (49.53%) and *P. fijiensis* (50.47%) were found from the pathogen population in the research area. Settlement of the Señorita plantation provided a good chance for both *Mycosphaerlla* pathogens to develop. Instead of cultural practices such as mixed cropping and deleafing, no fungicide was applied by the banana farmer. Sanchez and Zapata (2002) reported that frequency of *P. musae* and *P. fijiensis* were significantly affected by the interactions between banana clones and dates (resulting from varied situation of rainfall, humidity and temperature), indicating that high or low inoculum production depends on the planting material and the effects of environmental conditions on the development of each material. In terms of genetic variation of the fungus, Carlier (2004) reported that the level of genetic differentiation between population was highest at the global scale (*Fst* = 0.52 between some continents) and almost nil at the local scale (*Fst* = 0 between nearby plantations).

Conidial density

After one application, the fungicide treatment did not significantly affect conidial density of *Mycosphaerella* spp., while succeeding applications yielded significant effects. Consecutive azoxystrobin application (treatment B of the field experiment) consistently resulted in the lowest conidial density, which was significantly different from consecutive application of mancozeb (treatment A) and the control (E) (**Table 1**).

In general, all the treatments where systemic azoxystrobin fungicide was applied, either consecutively, alternate or mixed with protectant mancozeb effectively reduced the production of conidia. Conidial density 1.237 cm^{-2} mature necrotic spot ranged from 6.56 to 247 conidia.

Table 1 Conidial density of *Mycosphaerella* spp. (nos. 1.237 cm^{-2}) taken from mature necrotic leaf spots of Señorita banana variety (Sucrier group) previously subjected to different fungicide pressures.[1)]

Treatment[2)]	Conidial density at … week(s) after weekly fungicide application						
	1	3	5	7	9	10	Mean
A	159.00 a	77.61 b	165.90 b	139.90 b	146.40 b	182.90 ab	145.29
B	24.78 a	6.72 c	87.56 c	60.72 c	41.06 d	6.56 c	37.90
C	54.72 a	74.33 b	49.67 c	71.06 c	93.11 c	144.50 b	81.23
D	22.33 a	37.28 c	80.94 c	28.45 c	98.78 c	198.70 a	77.75
E	121.50 a	201.80 a	247.30 a	201.10 a	216.10 a	205.30 a	198.85

1) Means in the same column followed by the same letter are not significantly different according to Tukey's test (α = 0.05).
2) Treatment:
A. Consecutive protectant (mancozeb) fungicide application
B. Consecutive systemic (azoxystrobin) fungicide application
C. Alternate protectant and systemic fungicide application
D. Mixture of protectant and systemic fungicide application
E. Control or no fungicide application

Table 2 Conidial germination (%) of *Mycosphaerella* spp. taken from mature necrotic leaf spots of Señorita banana variety (AA Sucrier group) previously subjected to different fungicide pressures.[1)]

Treatment[2)]	Conidial germination at … week(s) after weekly fungicide application						
	1	3	5	7	9	10	Average
A	43.85 ab	23.58 a	4.56 a	0.43 a	3.83 a	4.89 a	13.52
B	0.30 b	22.83 a	21.29 a	8.73 a	14.40 a	17.68 a	14.21
C	6.31 ab	3.45 a	7.22 a	13.65 a	16.87 a	15.57 a	10.51
D	0.52 b	8.88 a	6.36 a	9.02 a	28.01 a	7.77 a	10.09
E	68.89 a	21.15 a	19.29 a	17.73 a	48.83 a	41.24 a	36.19

1) Means in the same column followed by the same letter are not significantly different according to Tukey's test (α = 0.05).
2) Treatment:
A. Consecutive protectant (mancozeb) fungicide application
B. Consecutive systemic (azoxystrobin) fungicide application
C. Alternate protectant and systemic fungicide application
D. Mixture of protectant and systemic fungicide application
E. Control or no fungicide application

Table 3 Proportion of the final conidial germination from the normal conidial density of *Mycosphaerella* spp. taken from mature necrotic leaf spot of Señorita banana variety (Sucrier group) previously subjected to different fungicide pressures.[1)]

Treatment[1)]	Average conidial density (nos. 1.237cm^{-2})	Percent reduction from "E"	Average conidial germination (%)	Percent reduction from "E"	Final conidial germination (%)
A	145.29 a	73.07	13.52 b	37.36	9.88 b
B	37.90 b	19.06	14.21 b	39.26	2.71 b
C	81.23 b	40.85	10.51 b	29.04	4.29 b
D	77.75 b	39.10	10.09 b	27.88	3.94 b
E	198.85 a	-	36.19 a	-	36.19 a

1) Calculated based on the average of 10 fungicide applications
2) Treatment:
A. Consecutive protectant (mancozeb) fungicide application
B. Consecutive systemic (azoxystrobin) fungicide application
C. Alternate protectant and systemic fungicides application
D. Mixture of protectant and systemic fungicides application
E. Control or no fungicide application

Conidial germination

In contrast to conidial production, the fungicide treatments affected conidial germination only after one application. The lowest conidial germination was observed after application of azoxystrobin but was not significantly different from the other fungicide treatments. They were, however, significantly different from the control (**Table 2**). Even though the remaining fungicide application did not affect conidial germination, in general, the fungicide treatments were found to reduce conidial germination.

Application of the systemic azoxystrobin fungicide, either singly, alternate, or mixed with protectant fungicide, reduced the ability of the fungicide to control conidial germination. Regression analysis addressed to conidial germination of the *Mycosphaerella* spp. population subjected to azoxystrobin spray showed that the three mode of applications resulted in the positive regression coefficient as follows: $Y = 4.035 + 0.127X$ ($R = 0.095$), $Y = -0.16 + 0.57X$ ($R = 0.824$), and $Y = 3.543 + 0.227X$ ($R = 0.368$), consecutively for single, alternate, and mixture application of azoxystrobin and mancozeb, where Y = conidial germination, X = application frequency. This phenomenon also showed the trend of resistance of the pathogen toward azoxystrobin, where progressive application of the fungicide increased conidial germination. On the other hand, the effectiveness of consecutive application of mancozeb has increased with the addition of application frequency, as shown by the formula of $Y = 8.175 – 0.173X$ ($R = 0.71$). The negative trend of conidial germination on control with equation $Y = 6.895 – 0.029X$ ($R = 0.030$) happened because of dry condition at the end of the experiment.

The effectiveness of fungicide treatments on the reproductive capacity of the pathogens can be attributed, firstly, by reduced conidial production, and secondly, by reduced germination capacity. Although the percent conidial germination of the fungicide treatments ranged from 10.09 to 14.21, the real conidial germination on those treatments was lower. The most effective treatment that reduced conidial density of *Mycosphaerella* spp. was by consecutive application of azoxystrobin as much as 2.71% followed by treatments D, C, A, and E, respectively, as much as 3.94, 4.29, 9.88, and 36.19% (**Table 3**).

Azoxystrobin sensitivity

Fungicide treatments reduced sensitivity of *Mycosphaerella* spp. conidia to azoxystrobin as shown by the increase of EC_{50}. **Table 4** shows that 10 consecutive applications of azoxystrobin (B) resulted in a 5.1-fold resistance of the fungus from the control. This was followed by mixed application of azoxystrobin and mancozeb (D), then, the consecu-

Table 4 Azoxystrobin sensitivity of *Mycosphaerella* spp. taken from mature necrotic leaf spot of Señorita banana variety (Sucrier group) previously subjected to ten applications of different fungicide pressures.

Treatment[1)]	50% effective concentration (EC_{50})	Increase of resistance from the control[2)]
A	0.03729	3.05-fold
B	0.06232	5.10-fold
C	0.04822	3.95-fold
D	0.03621	2.96-fold
E	0.01222	-

[1)] Treatment:
A. Consecutive protectant (mancozeb) fungicide application
B. Consecutive systemic (azoxystrobin) fungicide application
C. Alternate protectant and systemic fungicide application
D. Mixture of protectant and systemic fungicide application
E. Control or no fungicide application
[2)] Proportion of treatment A, B, C, and D toward treatment E

tive application of mancozeb (A), and alternate application of azoxystrobin and mancozeb (C). A similar result was reported by Heaney *et al*. (1998, in Ploetz 2000a) where intensive used of azoxystrobin (> 10 continuous applications) on banana in Costa Rica revealed in a small recovered population that was sufficiently insensitive to affect the control. Jones (1995) recommended alternate application of propiconazole and benomyl during the rainy season to control black Sigatoka in Venezuela. Surprisingly that consecutive application of mancozeb also caused a 3.05-fold resistance to azoxystrobin. The mechanism by which this case occurs may be due to cross-resistance between mancozeb and azoxystrobin. Resistance mechanism of *M. fijiensis* to fungicide was due to the presence of mutation G143A in the cytochrome b protein, the site where is targeted by the fungicide (Knight *et al.* 2002; Marin *et al.* 2003), or F1291 and additional mechanisms (Fungicide Resistance Action Committee = FRAC, n.d.). Mutation of G143A was present in field isolates from banana farms that had received 4-6 applications of strobilurins per year for the previous two years (Marin *et al.* 2003), while FRAC (1998) recommended a maximum of eight sprays per season for strobilurin/azoxystrobin.

Youngest leaf spotted

Fungicide treatments significantly affected YLS of Mycosphaerella leaf spot diseases after 2, 3, 4 and 7 fungicide applications. Different fungicide pressures reduced the aggressiveness of the pathogens as shown by the higher YLS value. In all observations, the lowest YLS was obtained by plants with no fungicide application (control), while the highest varied among the other treatments. However, the average YLS from all observations showed that alternate and mixed application of azoxystrobin and mancozeb yielded better control as shown by higher YLS than the others (**Table 5**). In terms of range of YLS, screening conducted by Danniels and Bryde (1999) showed the variation of YLS from leaf number 4.6-16, where the YLS on Seniorita was occurred on leaf number 6.3 and judged as susceptible variety. Rodriquez *et al*. (2002) reported that treatment of F20 and Tilt 250EC resulted in YLS of 9-10 on Paracido al Rey (AAA) banana and 7-9 on CEMSA3/4 (ABB) plantain affected by black Sigatoka, while Mobambo *et al*. (1994) reported 5.8 on susceptible Obino l'Ewai and 9-10.7 on its hybrids.

Disease severity

Fungicide treatments significantly reduced the severity of Mycosphaerella leaf spot disease even after only two applications. Although the severity varied among the observation, it showed that there was no significant difference among the fungicide treatments (treatments A, B, C, and D) (**Table 6**).

Both of YLS and disease severity at the end of the experiment were not significantly affected by fungicide treatments, perhaps because of very dry conditions. Vawdrey and Grice (2005) reported a different finding from their evaluation in Australia. The report mentioned that strobilurin fungicides trifloxystrobin, pyraclostrobin and azoxystrobin proved more effective than the industry standards propiconazole and mancozeb at controlling Sigatoka disease. Balbin and Zapata (2001) reported that alternate application of contact and systemic fungicide combined with optimum fertilization and deleafing resulted in lower disease severity

Table 5 Youngest leaf spotted (YLS) of Mycosphaerella leaf spot diseases on Señorita banana variety (Sucrier group) previously subjected to different fungicide pressures.[1)]

Treatment[2)]	YLS at … week(s) after weekly fungicide application										Average
	1	2	3	4	5	6	7	8	9	10	
A	7.33 a	6.22 a	7.33 ab	6.78 ab	7.78 a	6.44 a	5.89 ab	6.22 a	5.77 a	5.56 a	6.53
B	8.00 a	6.67 a	7.67 ab	6.78 ab	7.22 a	7.00 a	6.11 ab	6.55 a	6.11 a	5.56 a	6.77
C	7.78 a	7.22 a	8.22 a	7.67 a	8.22 a	6.78 a	8.00 a	6.21 a	6.44 a	6.11 a	7.27
D	7.67 a	7.22 a	7.89 a	7.89 a	8.89 a	7.33 a	6.67 ab	6.78 a	6.22 a	6.33 a	7.29
E	6.11 a	5.89 b	6.22 b	6.11 b	6.22 a	5.33 a	5.00 b	5.33 a	4.55 a	4.11 a	5.49

[1)] Means in the same column followed by the same letter are not significantly different according to Tukey's test ($\alpha = 0.05$).
[2)] Treatment:
A. Consecutive protectant (mancozeb) fungicide application
B. Consecutive systemic (azoxystrobin) fungicide application
C. Alternate protectant and systemic fungicide application
D. Mixture of protectant and systemic fungicide application
E. Control or no fungicide application

Table 6 Severity (%) of Mycosphaerella leaf spot diseases on Señorita banana variety (Sucrier group) previously subjected to different fungicide pressures.[1)]

Treatments[2)]	Severity (%) at … week(s) after weekly fungicide application										Average
	1	2	3	4	5	6	7	8	9	10	
A	17.63 a	19.77 b	17.62 ab	19.40 a	20.85 ab	24.88 b	24.70 ab	22.60 b	23.83 b	27.41 a	21.87
B	16.64 a	21.33 ab	19.70 ab	19.65 a	18.40 ab	21.19 b	23.92 ab	25.27 ab	22.71 b	27.03 a	21.58
C	16.69 a	20.08 b	17.16 b	18.88 a	18.54 ab	23.09 b	20.21 b	25.87 ab	25.01 b	24.68 a	21.02
D	18.30 a	19.22 b	18.26 ab	17.00 a	16.01 b	20.95 b	22.13 b	23.87 ab	24.33 b	24.47 a	20.45
E	22.37 a	26.97 a	22.97 a	23.69 a	24.96 a	33.81 a	30.00 a	31.59 a	35.18 a	34.68 a	28.62

[1)] Means in the same column followed by the same letter are not significantly different according to Tukey's test ($\alpha = 0.05$).
[2)] Treatment:
A. Consecutive protectant (mancozeb) fungicide application
B. Consecutive systemic (azoxystrobin) fungicide application
C. Alternate protectant and systemic fungicide application
D. Mixture of protectant and systemic fungicide application
E. Control or no fungicide application

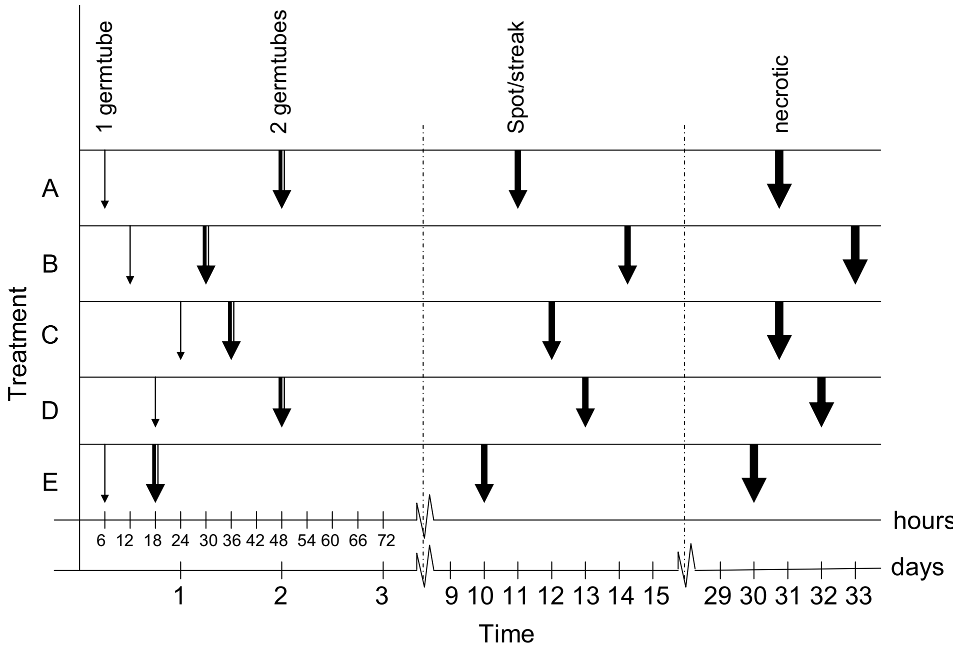

Fig. 1 Time needed for the infection process of *Mycosphaerella* spp. and the symptom development under greenhouse artificial inoculation test. Treatments were different inoculum sources from Señorita banana variety (Sucrier group) previously subjected to consecutive application of protectant (mancozeb) fungicide (A); consecutive application of systemic (azoxystrobin) fungicide (B); alternate application of protectant and systemic fungicides (C); mixture application of protectant and systemic fungicides (D); and control or no fungicide application (E). Spot/streak = spot is only just visible to the naked eye as a minute yellowish-green speck (about 1.0 × 5.0 mm); necrotic = stage when the center of the spot dries out, turns light grey, and is surrounded by a well-defined black ring, which is, in turn, surrounded by a bright yellow halo. These spots remain visible after the leaf has dried out because the ring persists.

of yellow Sigatoka on Plantain. However, from an economic point of view, this control measure combination did not sustain benefit/cost ratio compare to the control.

Greenhouse test

Artificial inoculation of Señorita banana leaves with 6.13×10^4 *Mycosphaerella* spp. conidia/ml collected from different treatment at field set up deposited 0–14 conidia/cm^2 of the banana leaf (average of 2.203 ± 1.783 conidia/cm^2). These conidia germinated within 6 to 48 hrs, produced initial spot or streak (incubation time) within 10 to 14 days, and developed necrotic spot (DDT) within 30 to 33 days (**Fig. 1**). Wild type isolate (indicated by treatment E) collected from control (no fungicide application) grew faster and produced earlier symptom than those collected from fungicide-treated plots.

Among the treatments, the wild isolate collected from control field experiment germinated, produced initial spot/streak and developed necrotic symptoms earlier than the remaining isolates. The conidia were observed to produce one or two germtubes within 48 hrs. Although the germtubes elongated, no penetration was observed until 72 hrs after inoculation. The most resistant isolate, collected from consecutive field application of azoxystrobin (treatment B), germinated earlier but produced symptom later than those collected from alternate and/or mixture application of azoxystrobin and mancozeb (treatment C and D). Isolate collected from consecutive field application of mancozeb resulted in similar time of germination and symptom production most compared to the wild isolate.

Previous research reported longer incubation periods of 17-20 and 22-24 days for *M. musicola* and *M. fijiensis*, respectively (Moulim-Pefoura *et al.* 1996), and 20.1 to 29.4 days for *M. fijiensis* (Romero and Sutton 1997). Variation of *Mycosphaerela* spp. developments were caused by source of isolate (Romero and Sutton 1997; Torres *et al.* 2000), susceptibility of banana varieties/accessions (Mobambo *et al.* 1994; Vicente 1998; Danniels and Bryde 1999; Mora *et al.* 2002), progeny (Moens *et al.* 2002), clone (Torres *et al.* 2000), and weather conditions (Vicente 1998).

CONCLUSIONS

1. Both *P. musae* and *P. fijiensis* were found in the research area, respectively comprising 49.53 and 50.47% of the pathogen population;
2. All the fungicide treatments effectively reduced YLS and Mycosphaerella leaf spot severity;
3. Consecutive application of azoxystrobin excellently controlled Mycosphaerella leaf spot diseases through reduction of conidial production and conidial germination. However, it caused 5.1-fold resistance of *Mycosphaerella* spp. conidial population compare to those of the control (no fungicide application). This resistance problem was reduced by mixing or alternating the application of azoxystrobin with mancozeb.

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