Centre Africain de Recherches sur Bananiers et Plantains

ETUDE SUR LES CONDITIONS DE REINTRODUCTION DES FONGICIDES SYSTEMIQUES DANS LES PROGRAMMES DE LUTTE CONTRE LA MALADIE DES RAIES NOIRES AU CAMEROUN DANS LA ZONE DE PRODUCTION DE LA BANANE DESSERT D'EXPORTATION

RAPPORT NARRATIF INTERMEDIAIRE N°5
Première campagne de monitoring à la CDC/Del Monte juin 2009

Contrat de service N°146 – 762/786/798/801(Cris)

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1. Description

1.1. Nom du bénéficiaire du contrat de subvention:
Centre Africain de Recherches sur bananiers et Plantains (CARBAP)

1.2. Nom et fonction de la personne de contact :
Kodjo Tomekpe, Directeur du centre

1.3. Nom des partenaires de l'Action:
CIRAD (Centre International de Recherches Agronomiques pour le Développement) et Bayer CropScience

1.4. Intitulé de l'Action:
Etude sur les conditions de réintroduction des fongicides systémiques dans les programmes de lutte contre la Maladie des Raies Noires au Cameroun

1.5. Numéro du contrat:
N° 146 – 762/786/798/801 (Cris)

1.6. Date de début et date de fin de la période de reporting:
1er avril 2009 au 15 septembre 2009

1.7. Pays ou région(s) cible(s):
Cameroun

1.8. Bénéficiaires finaux et/ou groupes cibles[1] (si différents) (y inclus le nombre de femmes et d'hommes):
Plantations agro Industrielles de bananes destinées à l'export

1.9. Pays dans lequel/lesquels les activités sont réalisées (si différent du point 1.7):

2. Evaluation de la mise en œuvre des activités de l'Action

2.1. Rappel du contexte de l'étude

La maladie des raies noires (MRN) est la principale contrainte parasitaire des plantations agro-industrielles de bananes dessert. Cette maladie foliaire, présente dans la majeure partie des zones de production de bananes dans le monde, est provoquée par le champignon ascomycète et aérien *Mycosphaerella fijiensis*. Les attaques de ce champignon peuvent entraîner une réduction de l'activité photosynthétique et des pertes de rendement variant de

10 à 100%. Toutefois, l'effet le plus important de la maladie est indirect car les régimes récoltés sur les plants fortement affectés ont une durée de conservation fortement réduite et ne peuvent donc pas être exportés. En l'absence de variétés résistantes (non disponibles à ce jour), la culture intensive de la banane dessert pour l'export n'est donc réalisable qu'au moyen d'un contrôle chimique rigoureux de cette maladie. Au Cameroun, *M. fijiensis* a été signalé pour la première fois en 1981. A la fin des années 80, une méthode d'avertissement utilisant des descripteurs biologiques a été mise au point et appliquée avec succès, limitant ainsi le nombre d'applications à 12-14 par an. Cette lutte raisonnée par avertissement reposait fortement sur l'emploi de fongicides systémiques ayant un fort effet curatif. Malheureusement, depuis 1996 l'apparition de souches résistantes aux fongicides systémiques a entraîné l'abandon de cette stratégie au détriment d'une méthode de lutte plus systématique reposant majoritairement sur l'emploi de fongicides de contact. Les fongicides de contact ne provoquent pas l'apparition de souches résistantes, mais ils n'ont pas d'effet curatif sur la maladie, et sont donc utilisés préventivement. Ainsi, en 2006, malgré un souci constant de continuer à piloter la lutte chimique par l'observation de descripteurs biologiques, environ 40 traitements ont été effectués sur la majorité des plantations. Cette augmentation du nombre de traitements a entrainé une augmentation du coût de la lutte, mais également des risques environnementaux. En effet, en plus de l'augmentation des quantités de matière active liées à l'accroissement du nombre de traitements, les fongicides de contact sont épandus à des doses plus importantes que les fongicides systémiques. De nouvelles stratégies de traitement doivent être aujourd'hui redéfinies pour retrouver une situation plus durable sur les plans économiques et environnementaux

2.2. Rappel des objectifs de l'étude et de la méthodologie

2.2.1. Objectifs

Les observations récentes des derniers monitoring montrent qu'il y a une baisse des niveaux de résistance dans certaines plantations commerciales du Cameroun, plus particulièrement depuis que les fongicides systémiques ne sont plus ou peu employés. Cette évolution permet de penser que les phénomènes de résistance aux fongicides sont peut être réversibles.
Plusieurs mécanismes peuvent être à l'origine de cette évolution récente des niveaux de résistance :

- des flux de gènes provenant des zones non traitées (effectifs élevés de populations sensibles aux fongicides), vers les plantations commerciales (effectifs faibles de populations résistantes) qui pourraient entrainer une « dilution » progressive du phénomène de résistance
- une perte de compétitivité des souches résistantes qui seraient alors progressivement éliminées lorsque la pression de sélection fongicide est arrêtée (plus de traitements avec des fongicides systémiques)

L'objectif de cette étude est ainsi de :

- Mesurer l'évolution dans le temps du niveau de résistance aux fongicides systémiques dans les populations pathogènes de *M. fijiensis* des plantations industrielles du Cameroun.
- Définir les conditions d'un réemploi éventuel des fongicides systémiques dans le cadre des différentes stratégies de traitement utilisées au Cameroun. Plus particulièrement est visée la possibilité de réutiliser des stratégies de traitement basées sur un système d'avertissement.

2.2.2. Méthodologie

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Le programme de travail de cette étude a été regroupé en 5 activités spécifiques qui permettront de répondre aux objectifs de l'étude :

Activité spécifique 1. Améliorer les méthodes d'évaluation de la résistance aux fongicides.

Activité spécifique 2. Evaluer les niveaux de résistance dans les différentes plantations commerciales du Cameroun.

Activité spécifique 3. Mesurer les flux de gènes entre les plantations non traitées et les plantations commerciales.

Activité spécifique 4. Mesurer l'impact de stratégies de traitement sur la résistance aux fongicides

Activité spécifique 5. Evaluer la compétitivité des souches résistantes par rapport aux souches sensibles

2.3. Résumé de l'Action

Au cours de cette période, les activités ont concerné l'évaluation des niveaux de résistance à la CDC/Delmonte. Il s'agit de la première campagne d'évaluation qui a été réalisée en juin 2009, pour la méthode ascospores sur les 13 secteurs choisis pour cette plantation. Trois fongicides de la famille des triazoles ont été proposés par la CDC/Delmonte et analysés au cours de cette campagne : BAYCOR 500 SC, OPAL 75 EC et SICO 250 EC.

2.4. Activities and results

2.4.1. Introduction

Black Leaf Streak Disease (BLSD) is one of the major constrains of industrial production of banana. The infection of this fungal pathogen can lead to a significant reduction of the photosynthetic activity and serious loss of yield varying between 10 to 100%. However, the most important effect of this disease is indirect because of the strong reduction of green life of bunches harvested on highly infested plants, which bunches cannot be exported. The intensive production of banana for exportation depends on an intensive chemical control of the disease. By the end of the 80's, a forecasting system using biological descriptors was put in place in Cameroon and successfully limited the number of annual applications to 12-14 cycles. This forcasting system was strongly based on the use of systemic fungicides having a strong curative effect. Unfortunately, since 1996, the appearance of resistant strains to the systemic fungicides led to the failure of this strategy. The control strategy evolved then to a systematic strategy based on the employment of contact fungicides. This type of fungicides never promotes the appearance of resistant strains, but do not have either a curative effect and should be used like protectants. So in 206, inspite of continuous management of disease control using biological descriptors, about 40 treatments were realised on the main

plantations. This evolution in the number of treatment led to increasing cost of disease control and also to more important environmental impact.

In order to evaluate the possible reintroduction of the forecasting strategies based on the use of systemic fungicide, it is important to continuously monitor the evolution of the sensitivity of fungal populations inside industrial plantations of Cameroon.

In this project, this monitoring will be realised by CARBAP on many sectors of CDC/Delmonte plantation. So, 90 analyses are expected by year in this plantation. One part of these analyses is realised with the common ascosporic method on a certain number of sectors and fungicides defined together with the technical plantation manager. The second part of analyses will be expected for the comparison of two different methods: ascosporic and conidial methods on 3 sectors and for all the group of systemic fungicides (benzimidazoles, triazoles and strobilurines).

a. For the comparison of the ascosporic and conidial method - 24 analyses

Sectors	**Number of analyses (2 methods)**	**Fungicides**
Tiko	4+4	bankit, callis, tilt et tega
Ekona	4+4	
mussaka	4+4	

b. for analyses with ascosporic method : 68 analyses

Sectors	**Number of analyses (2 periods)**	**Fungicides**
Tiko	6 +6	baycor
Ekona	3 + 3	
mussaka	3 + 3	
Tiko	6 + 3	sico
Ekona	3 + 3	
mussaka	2 + 2	
Tiko	6 + 6	opal
Ekona	3 + 3	
mussaka	2 +2	

In this report, we present the results of the first campaign realized in june 2009 for the ascosporic method on 9 selected sectors in Tiko, 2 sectors in Mussaka and 3 in Ekona. Three fungicides were proposed by CDC/Delmonte: Baycor 500 SC, Sico 250 EC and Opal 75 EC. In this report we show results for 39 analyses.

2.4.2. Protocol of the ascosporic method

2.4.2.1 Leaf sampling

Leaf samples were collected from industrial plantations which apply fungicides on a regular basis (farm samples) and from smallholders' plots where fungicides have never been applied (baseline sample).
Leaf samples consist of necrotic tissue where the disease symptoms had reached grade 6 (necrotic leaf area with spots showing a clear grey centre).
For each field necrotic tissues of at least 20 banana trees were collected.

Farm samples:
Leaf samples were collected from 12/06/09 to 08/07/09 in 9 different sectors of the plantation.

- Tiko
-

Sample collected	Fungicide tested
Mondoni 1 F5C3 Mondoni 2 F7C21 Moquo F3C26 Mafanja 1 Cable 0 Mafanja 2 F1C8 Pungo F2C13 Benoe F1 C2 Bwinga F1 C25/28 Esuke F1 C14	Sico, Baycor and Opal

- Ekona
-

Sample collected	Fungicide tested
Ekona G8/9 Ekona J6	Sico, Baycor and Opal

- Mussaka

Sample collected	Fungicide tested
Mussaka F1C13 Mussaka F5C19	Sico, Baycor and Opal

Baseline samples :
For Tiko plantation, samples from smallholders' fields were collected on plantains along the road between Tiko and Mungo bridge, the 08/07/09 for Sico, Baycor and Opal.

For Ekona and Ekona plantation, samples from smallholders' fields were collected on plantains along the road between Ekona and Buea, the 08/07/09 for Sico, Baycor and Opal.

2.4.2.2. Isolation and cultivation of ascospores

- Necrotic leaf samples (containing perithecia) are collected in the plantations.
- After an incubation period of 48 hours at room temperature, leaf segments were stapled to a disc of about 90 mm diameter of filter paper with the abaxial surface down (lower leaf side down).
- The leaf segments are submerged for 10 minutes in bi-distilled water and then immediately placed inside the lid of a Petri dish and suspended over the water agar amended with different fungicide concentrations.
- A minimum of 3 hours is needed for discharge of the ascospores.
- Afterwards the filter paper with the attached leaf segment was removed and the plates were stored at 25 °C for 48 hours.

- The sensitivity of the fungal population was evaluated for the samples collected in different sectors of Del Monte plantations. The fungicides tested were: Sico 250 EC (a.i: difenoconazole), Baycor 300 EC (a.i: bitertanol) and Opal 75 EC (a.i: epoxyconazole).

Different concentrations used for these analyses:

- For Sico: 0 and 0.1 ppm
- For Baycor: 0 and 0.1 ppm
- For Opal: 0 and 0.1 ppm

2.4.2.3 Evaluation of fungal growth

- The length of the germ tubes of the ascospores were visually assessed with a microscope.
- Fifty spores were observed per sample for Sico 250 EC, Baycor 300 EC, and Opal 75 EC.
- Response to Sico 250 EC, Baycor 300 EC, and Opal 75 EC was estimated by examining the germ tube lengths. Germ tube lengths were measured with the aid of a microscope outfitted with an indexed objective lens. The relationship between the real length and the observed length is expressed through a conversion factor, specified by the microscope manufacturer.
- The results of spore growth are presented as a percentage of the control (ascospores germinating on agar not amended with the fungicide).

2.4.3 Results

These results will be presented according to the name of the 3 plantations of CDC (Tiko, Ekona and Mussaka), for better understanding by the companies.

2.4.3.1 Tiko plantation

- Sico 250 EC (Triazole – difenoconazole)– table 1; figures 1 and 2

For difenoconazole, the average percentage of inhibition for the 9 different sectors (60 %) is lower than in the untreated smallholder plantation (82%). A significant proportion of the average population (31%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows a significant shift in sensitivity to difenocanazole in this plantation. 5 sectors show a stronger shift in sensitivity to this fungicide : Mondoni 2 F7C21 (IC = 45 ; 59 % strains with less than 50 % inhibition) ; Mondoni 1 F5C3 (IC = 43 ; 70 % strains with less

than 50 % inhibition) ; Moquo F3C26 (IC = 41 ; 53 % strains with less than 50 % inhibition) ; Bwinga F1C25/28 (IC = 45 ; 54 % strains with less than 50 % inhibition) ; Mafanja 1 Cable 0 (IC = 62 ; 26 % strains with less than 50 % inhibition). No previous data are available with this fungicide in this plantation.

- Baycor 300 EC (Triazole – bitertanol) – table 2; figures 3 and 4

For bitertanol, the average percentage of inhibition for the 9 different sectors (59 %) is lower than in the untreated smallholder plantation (76%). A significant proportion of the average population (30%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows a significant shift in sensitivity to bitertanol in this plantation. 3 sectors show a stronger shift in sensitivity to this fungicide : Bwinga F1C25/28 (IC = 34 ; 70 % strains with less than 50 % inhibition) ; Benoe F1C2 (IC = 45 ; 68 % strains with less than 50 % inhibition) ; Mondoni 1 F5C3 (IC = 55 ; 70 % strains with less than 50 % inhibition). No previous data are available with this fungicide in this plantation. Compared with previous data, the evolution in this plantation shows a regular shift in sensitivity to bitertanol.

- Opal 75 EC (epoxyconazole) – Tableau 3; figures 5 and 6

For epoxyconazole, the average percentage of inhibition for the 9 different sectors (71 %) is comparable to the untreated smallholder plantation (79%). A low proportion of the average population (9%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows no significant shift in sensitivity to epoxyconazole in this plantation. However, 2 sectors show a slight shift in sensitivity to this fungicide: Benoe F1C2 (IC = 58 ; 30 % strains with less than 50 % inhibition) ; Bwinga F1C25/28 (IC = 55 ; 20 % strains with less than 50 % inhibition. No previous data are available with this fungicide in this plantation.

Conclusions and recommendations for Tiko farm

A shift in sensitivity to Triazoles is significant in this plantation. This shift is more pronounced for difenoconazole and bitertanol but is very slight for epoxyconazole. By another hand the situation is fluctuable according to the various sectors of the plantation, Bwinga, Benoe, Mondoni being most affected; Esuke and Pungo being less affected.

Frac recommendations is the following : not more than 8 applications should be done with this family of fungicides which include the following products: Tilt, Folicur, Baycor, Sico, Opal, Punch, Vectra. These fungicides should be fully used in alternation with other mode of actions. Because of the shift in sensitivity observed for Sico and Baycor, in some sectors of Tiko farm, we recommend to reduce this maximum number to 6-4 applications of triazole. This is especially true for Mondoni, Moquo, Bwinga and Benoe.

By another hand, we recommend also to reinforce deleafing management programs in this plantation (a poor quality of deleafing was noticed this year in many sectors) because high population levels can accelerate the selection of resistant strains inside the farms.

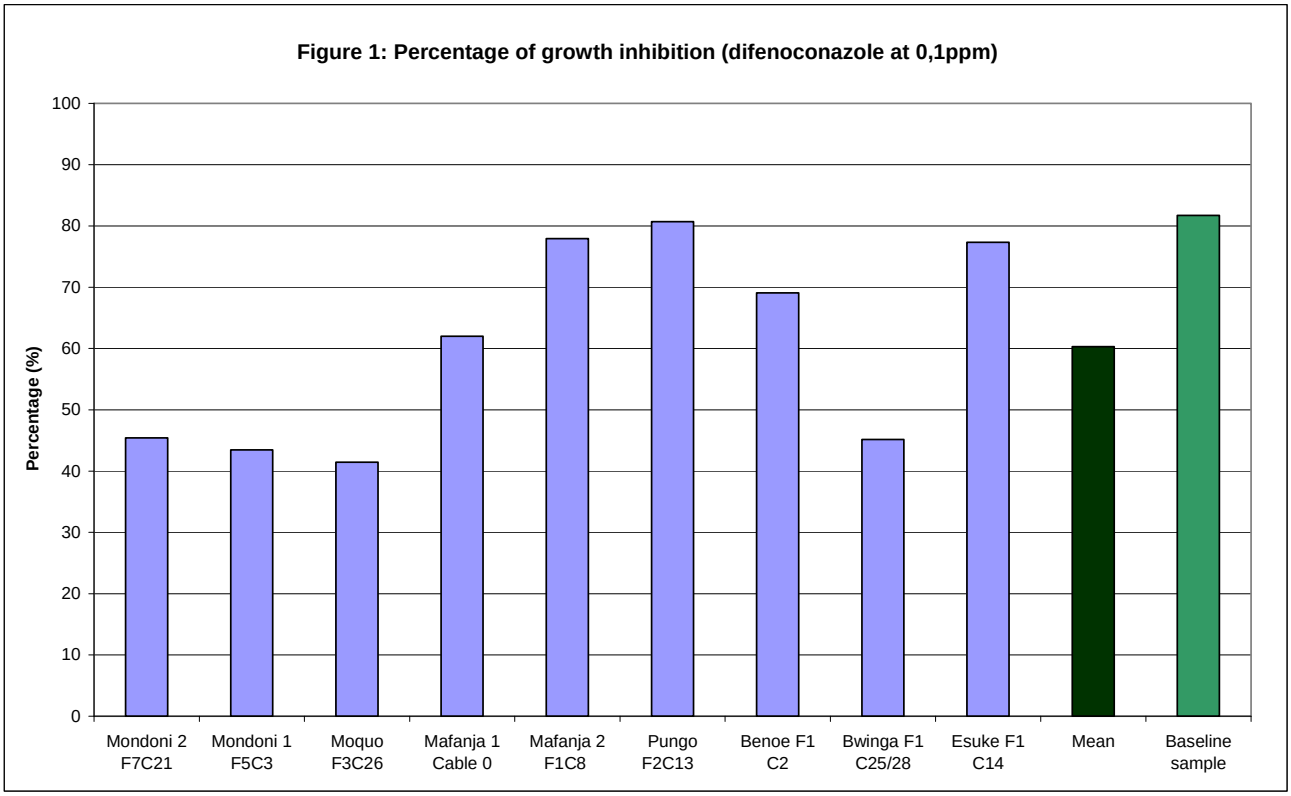
Table 1: **Summary of results concerning the sensitivity of *M. fijiensis* populations to Sico 250 EC (difenoconazole at 0.1 ppm)**

SICO	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			**0-10**	**11-30**	**31-50**	**51-70**	**71-90**	**91-100**	
Mondoni 2 F7C21	**45**	148	2	10	47	35	6	0	**59**
Mondoni 1 F5C3	**43**	140	4	16	50	18	10	0	**71**
Moquo F3C26	**41**	162	2	12	34	38	4	0	**53**
Mafanja 1 Cable 0	**62**	100	0	4	22	32	42	0	**26**
Mafanja 2 F1C8	**78**	78	0	0	2	10	88	0	**2**
Pungo F2C13	**81**	60	0	0	0	0	100	0	**0**
Benoe F1 C2	**69**	92	0	0	16	14	70	0	**16**
Bwinga F1 C25/28	**45**	166	0	18	36	38	8	0	**54**
Esuke F1 C14	**77**	65	0	0	0	16	84	0	**0**
Mean	**60**	**112**	**1**	**7**	**23**	**23**	**46**	**0**	**31**
Baseline sample	**82**	**56**	**0**	**0**	**0**	**0**	**98**	**0**	**0**

Summary of the results of previous monitoring

June-09	**60**	**112**	**1**	**7**	**23**	**22**	**46**	**0**	**31**

Figure 1: Percentage of growth inhibition (difenoconazole at 0,1ppm)



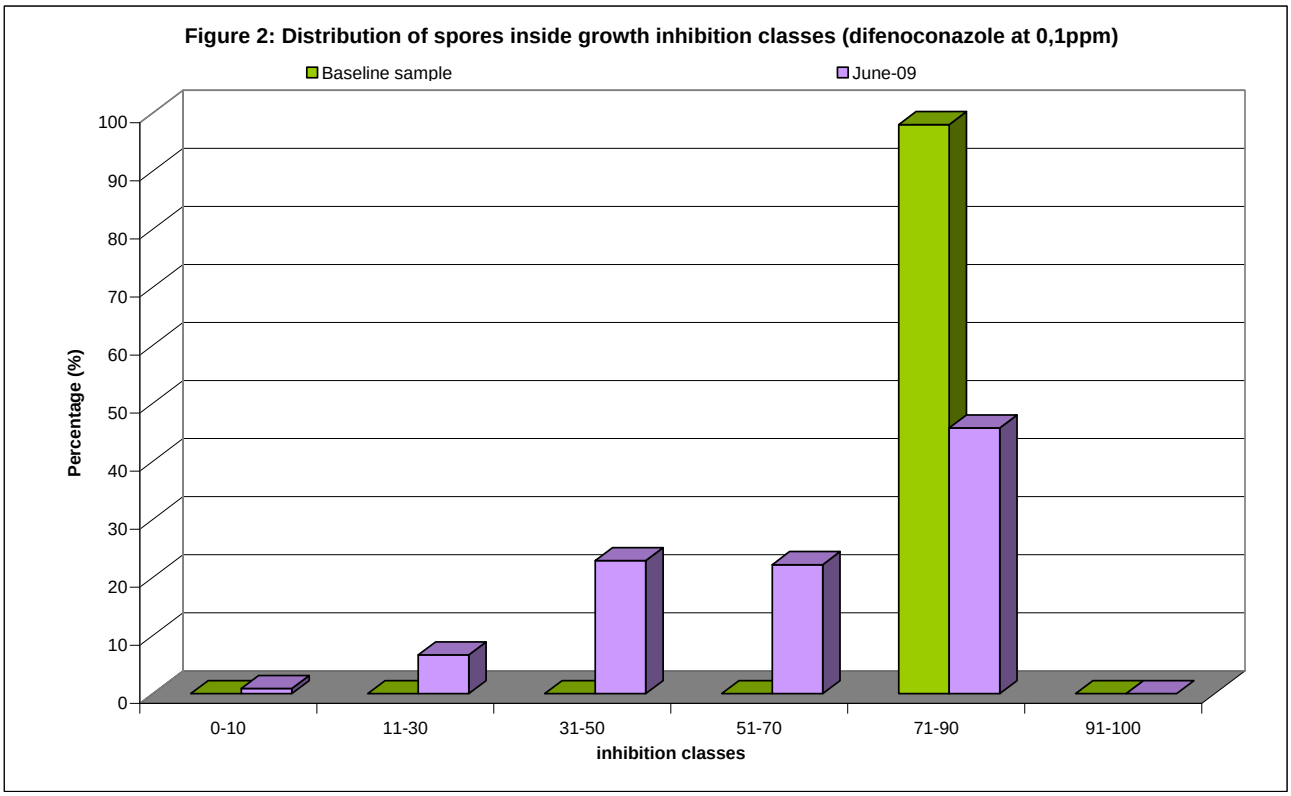


Table 2: **Summary of results concerning the sensitivity of *M. fijiensis* populations to BAYCOR (bitertanol at 0.1 ppm)**

BAYCOR	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			**0-10**	**11-30**	**31-50**	**51-70**	**71-90**	**91-100**	
Mondoni 2 F7C21	**62**	103	0	0	16	66	18	0	**16**
Mondoni 1 F5C3	**55**	105	0	2	48	38	12	0	**50**
Moquo F3C26	**62**	105	0	4	10	64	22	0	**14**
Mafanja 1 Cable 0	**70**	78	0	0	6	26	68	0	**6**
Mafanja 2 F1C8	**66**	122	0	0	12	46	42	0	**12**
Pungo F2C13	**75**	78	0	0	2	24	74	0	**2**
Benoe F1 C2	**45**	162	0	16	52	16	16	0	**68**
Bwinga F1 C25/28	**34**	198	10	32	28	30	0	0	**70**
Esuke F1 C14	**58**	119	0	0	29	53	18	0	**29**
Mean	**59**	**119**	**1**	**6**	**23**	**40**	**30**	**0**	**30**
Baseline sample	**76**	**73**	**0**	**0**	**0**	**8**	**92**	**0**	**0**

Summary of the results of previous monitoring

déc-05	68	64	0	0	4	59	38	0	4
déc-06	65	84	0	0	11	51	38	0	11
nov-07	63	108	0	1	11	58	30	0	12
june-09	**59**	**119**	**1**	**6**	**22**	**40**	**30**	**0**	**30**

Figure 3: Percentage of growth inhibition (bitertanol at 0,1ppm)

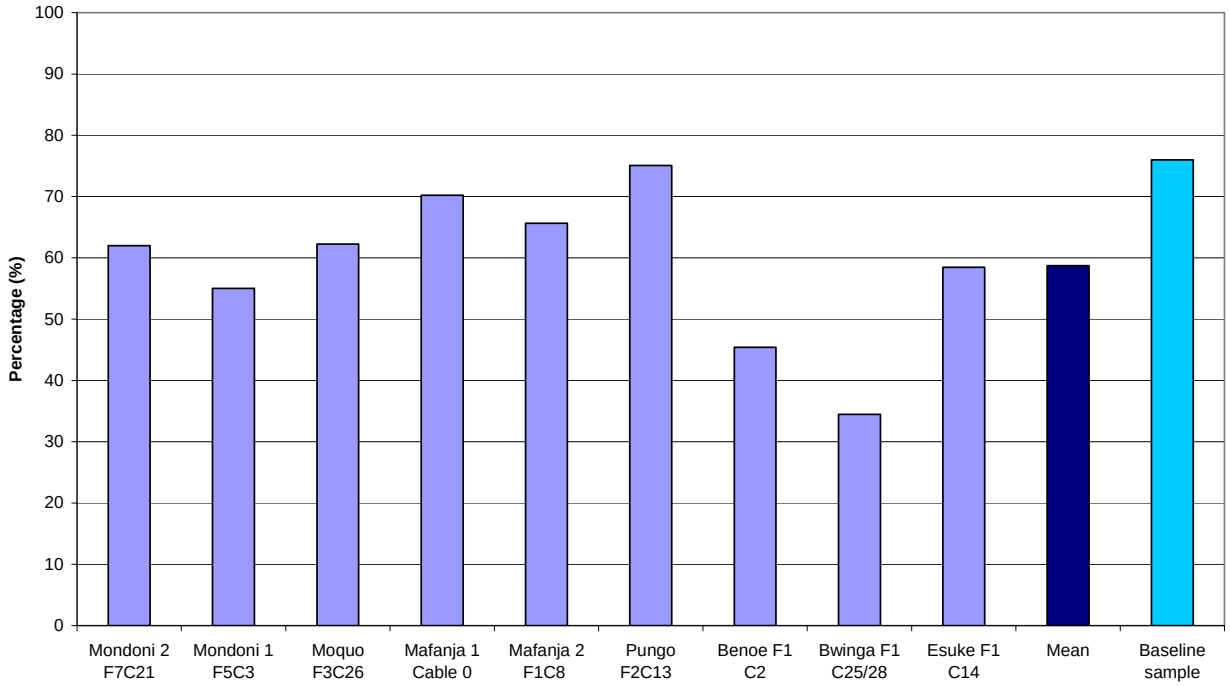


Figure 4: Distribution of spores inside growth inhibition classes (bitertanol at 0,1ppm)

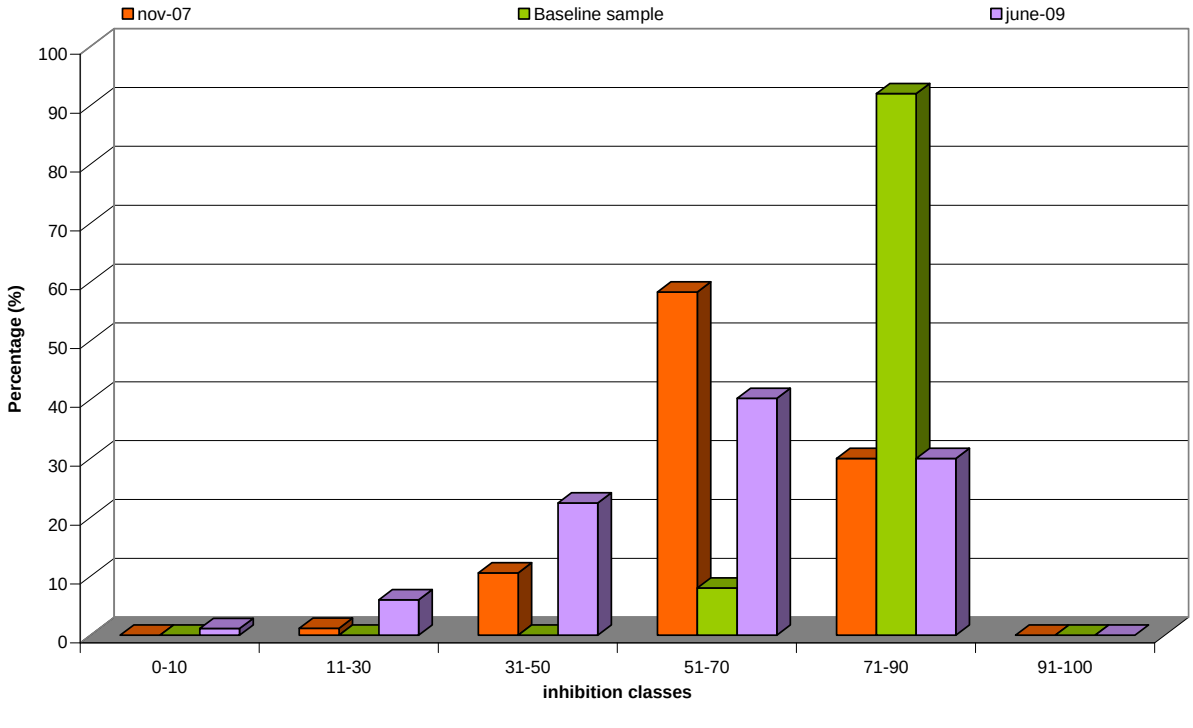
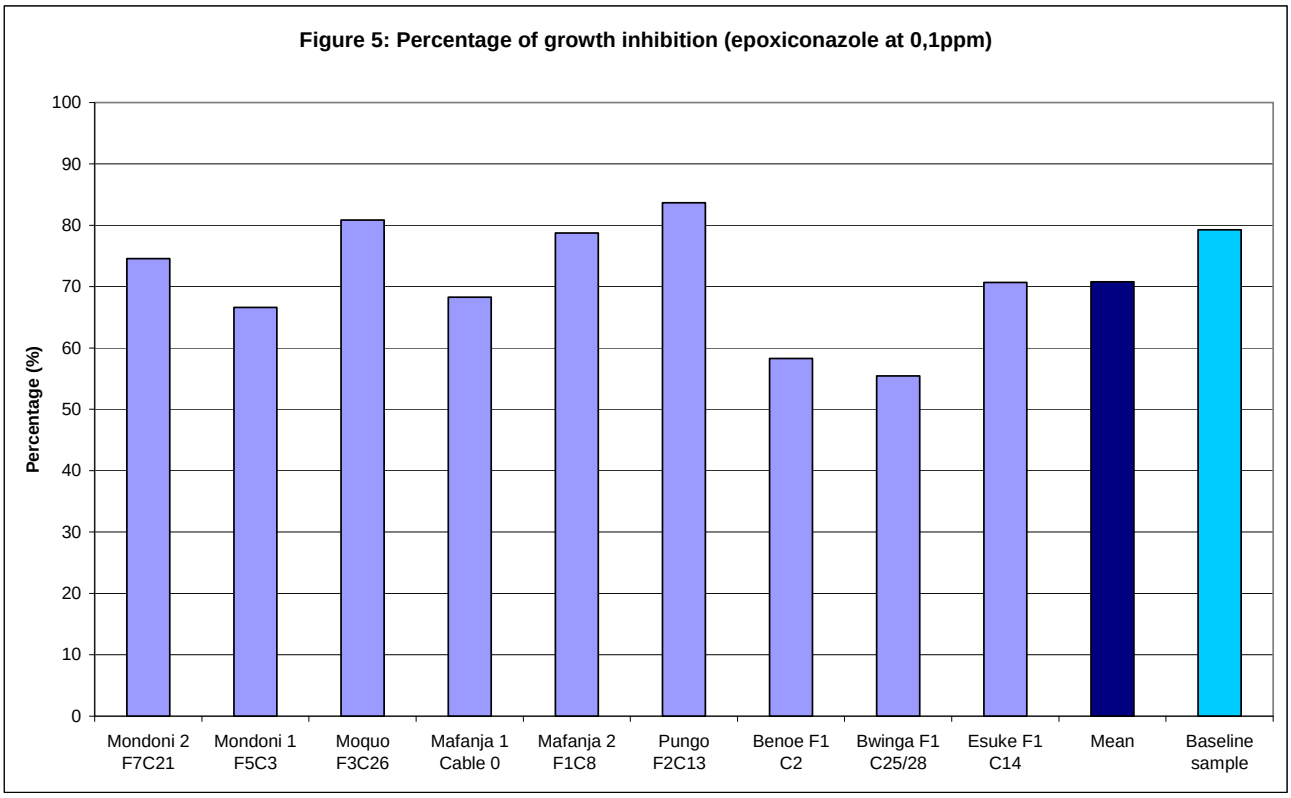


Table 3: **Summary of results concerning the sensitivity of *M. fijiensis* populations to Opal 75 EC (epoxyconazole at 0.1 ppm).**

OPAL Sectors	% inhibition	Lenghts of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
			0-10	11-30	31-50	51-70	71-90	91-100	
Mondoni 2 F7C21	**75**	69	0	0	8	24	68	0	**8**
Mondoni 1 F5C3	**67**	83	0	0	8	56	36	0	**8**
Moquo F3C26	**81**	53	0	0	0	10	90	0	**0**
Mafanja 1 Cable 0	**68**	83	0	0	8	36	56	0	**8**
Mafanja 2 F1C8	**79**	75	0	0	6	20	72	2	**6**
Pungo F2C13	**84**	51	0	0	0	6	74	20	**0**
Benoe F1 C2	**58**	124	0	0	30	34	36	0	**30**
Bwinga F1 C25/28	**55**	135	0	2	20	62	16	0	**22**
Esuke F1 C14	**71**	84	0	0	2	56	42	0	**2**
Mean	**71**	**84**	**0**	**0**	**9**	**34**	**54**	**2**	**9**
Baseline sample	**79**	**63**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

Summary of the results of previous monitoring

june-09	71	84	0	0	9	34	54	2	9



2.4.3.2. Ekona plantation

-Sico 250 EC (Triazole- difenoconazole) - table 1; figures 1 and 2

For difenoconazole, the average percentage of inhibition for the 2 different sectors (76 %) is comparable to the untreated smallholder plantation (81%). No strains had an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows no shift in sensitivity to difenoconazole in this plantation. No previous data are available with this fungicide in this farm.

- Baycor 300 EC (Triazole-bitertanol) – table 2; figures 3 and 4

For bitertanol, the average percentage of inhibition for the 2 different sectors (76 %) is comparable to the untreated smallholder plantation (79%). A very low proportion of the average population (1%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows no shift in sensitivity to bitertanol in this plantation. This situation is comparable with previous data on this farm.

- Opal 75 EC (triazole - epoxyconazole) table 3; figures 5 and 6

For epoxyconazole, the average percentage of inhibition for the 2 different sectors (76 %) is comparable to the untreated smallholder plantation (77%). A very low proportion of the average population (4%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows no shift in sensitivity to epoxyconazole in this plantation. No previous data are available with this fungicide in this farm.

Conclusions and recommendations for Ekona farm

No shift in sensitivity to any of the Triazoles tested has been noticed in this farm. This is confluent with previous data on this farm with bitertanol.

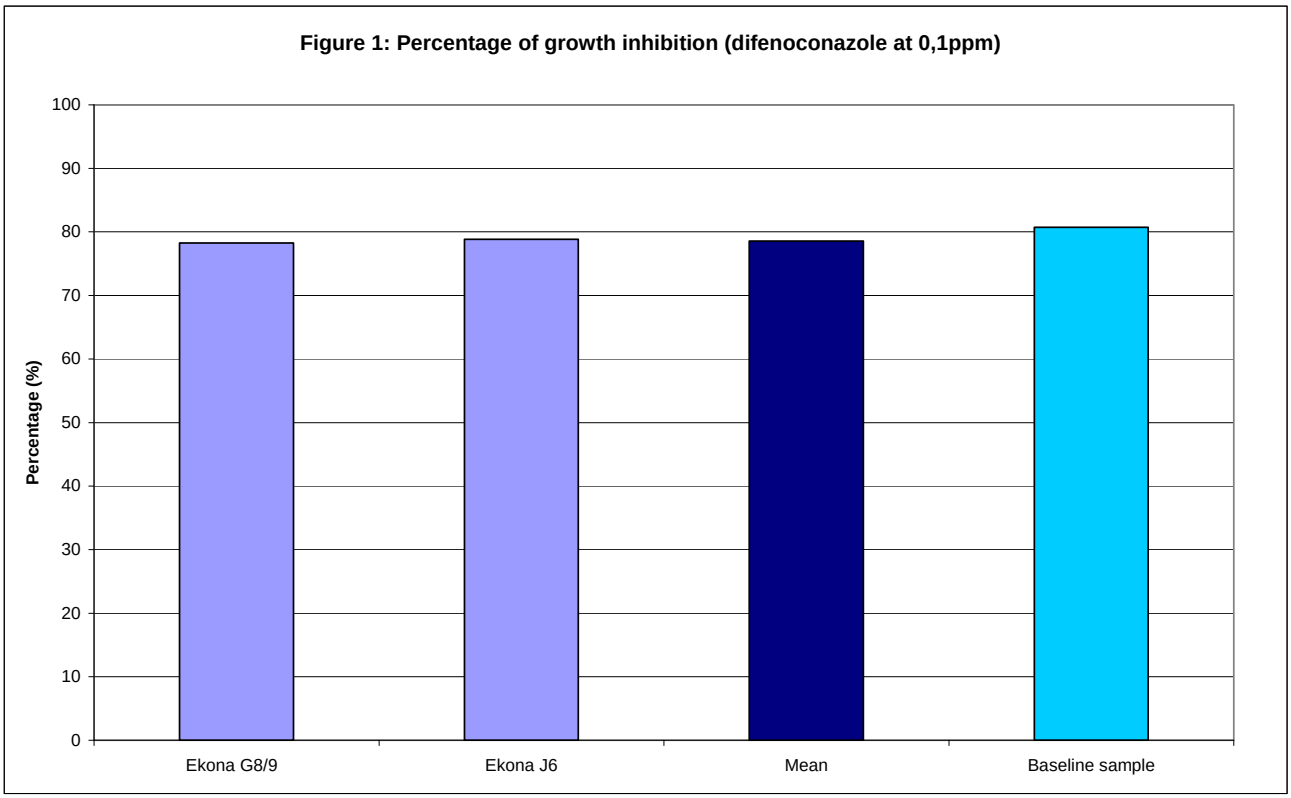
We recommend to fulfil with Frac's recommendations in this farm : not more than 8 applications should be done with this family of fungicides which include the following products: Tilt, Folicur, Baycor, Sico, Opal, Punch, Vectra. These fungicides should be fully used in alternation with other mode of actions.

<u>Table 1</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to SICO (0.1 ppm of difenoconazole).

Sico Fields	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
			0-10	11-30	31-50	51-70	71-90	91-100	
Ekona G8/9	**78**	**73**	0	0	0	2	98	0	**0**
Ekona J6	**79**	**79**	0	0	0	8	92	0	**0**
Mean	**79**	**76**	**0**	**0**	**0**	**5**	**95**	**0**	**0**
Baseline sample	**81**	**60**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

Summary of the results of previous monitoring

june-09	79	76	0	0	0	5	95	0	0



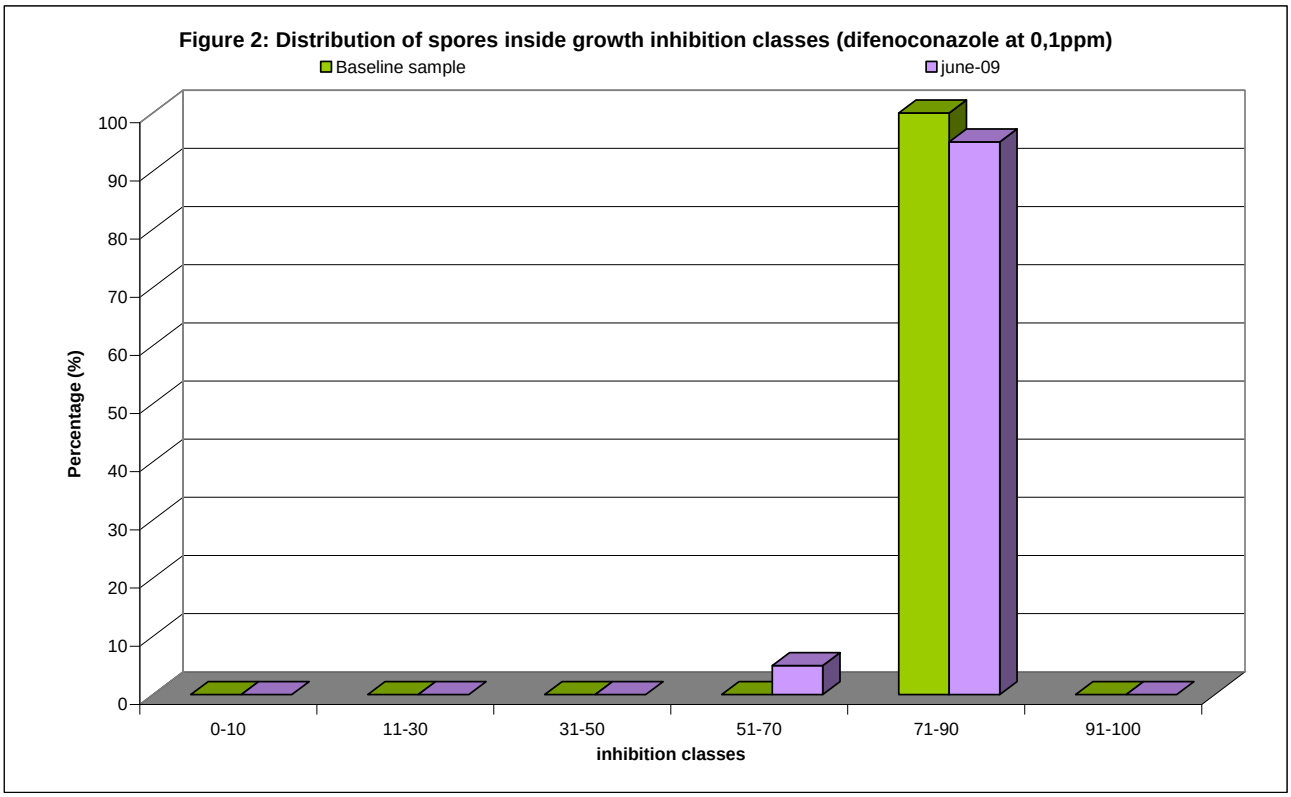


Table 2 :. **Summary of results concerning the sensitivity of *M. fijiensis* populations to BAYCOR (0.1 ppm of bitertanol).**

BAYCOR	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition <50 %
Fields			0-10	11-30	31-50	51-70	71-90	91-100	
Ekona G8/9	**76**	**81**	0	0	0	6	94	0	**0**
Ekona J6	**77**	**88**	0	0	2	28	70	0	**2**
Mean	**76**	**84**	**0**	**0**	**1**	**17**	**82**	**0**	**1**
Baseline sample	**79**	**66**	**0**	**0**	**0**	**0**	**100**	**0**	**0**

Summary of the results of previous monitoring

déc-05	83	39	0	0	0	2	98	0	0
dec-06	74	63	0	1	17	28	14	40	18
nov-07	70	72	0	0	0	41	59	0	0
june-09	76	84	0	0	1	17	82	0	1

Figure 3: Percentage of growth inhibition (bitertanol at 0,1ppm)

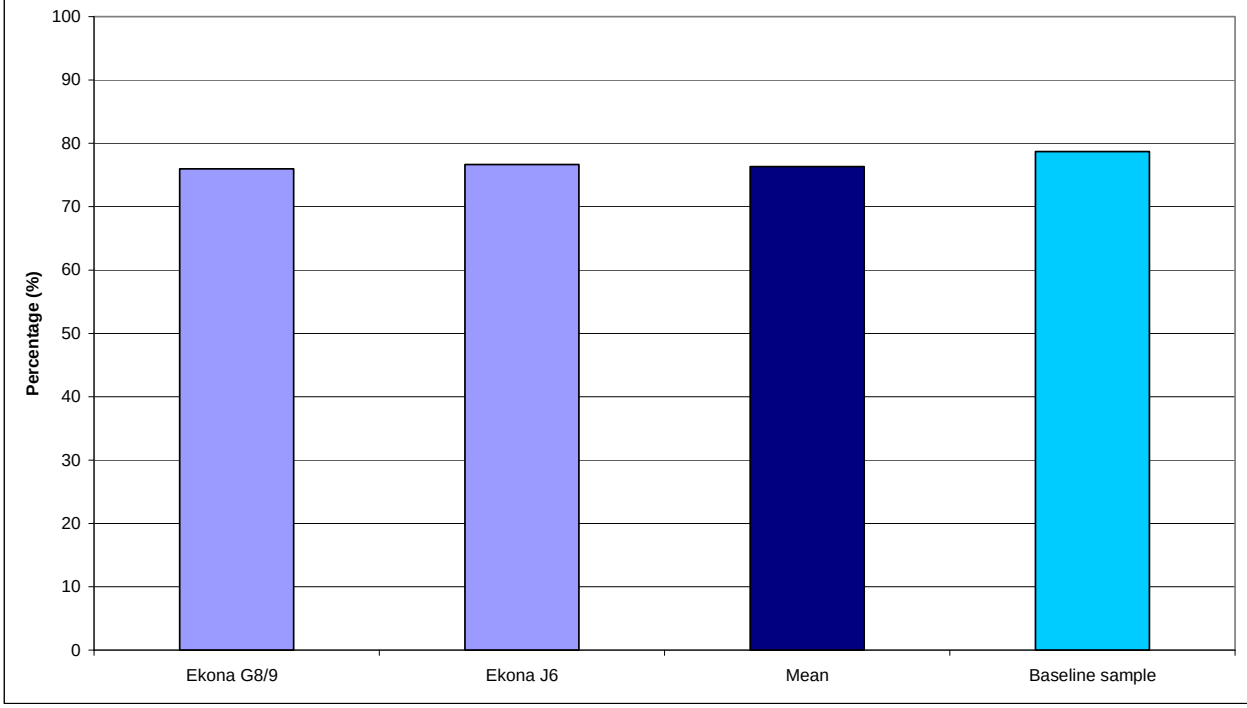
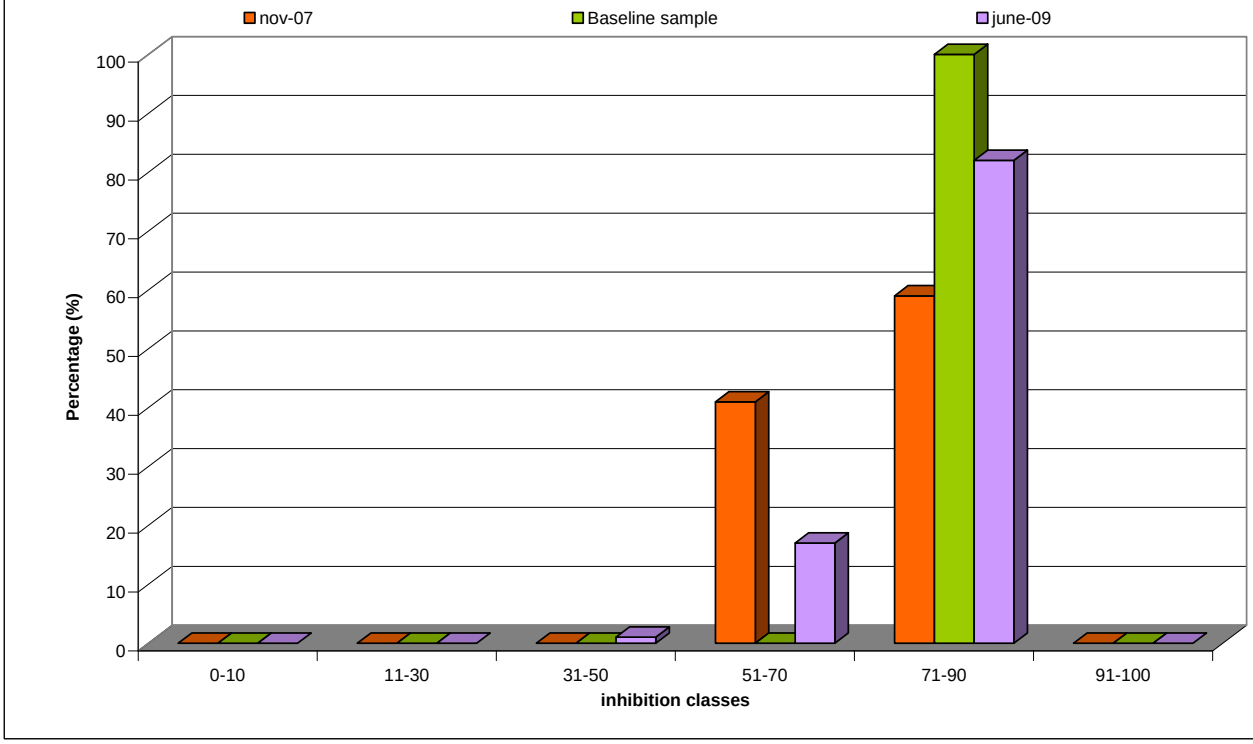
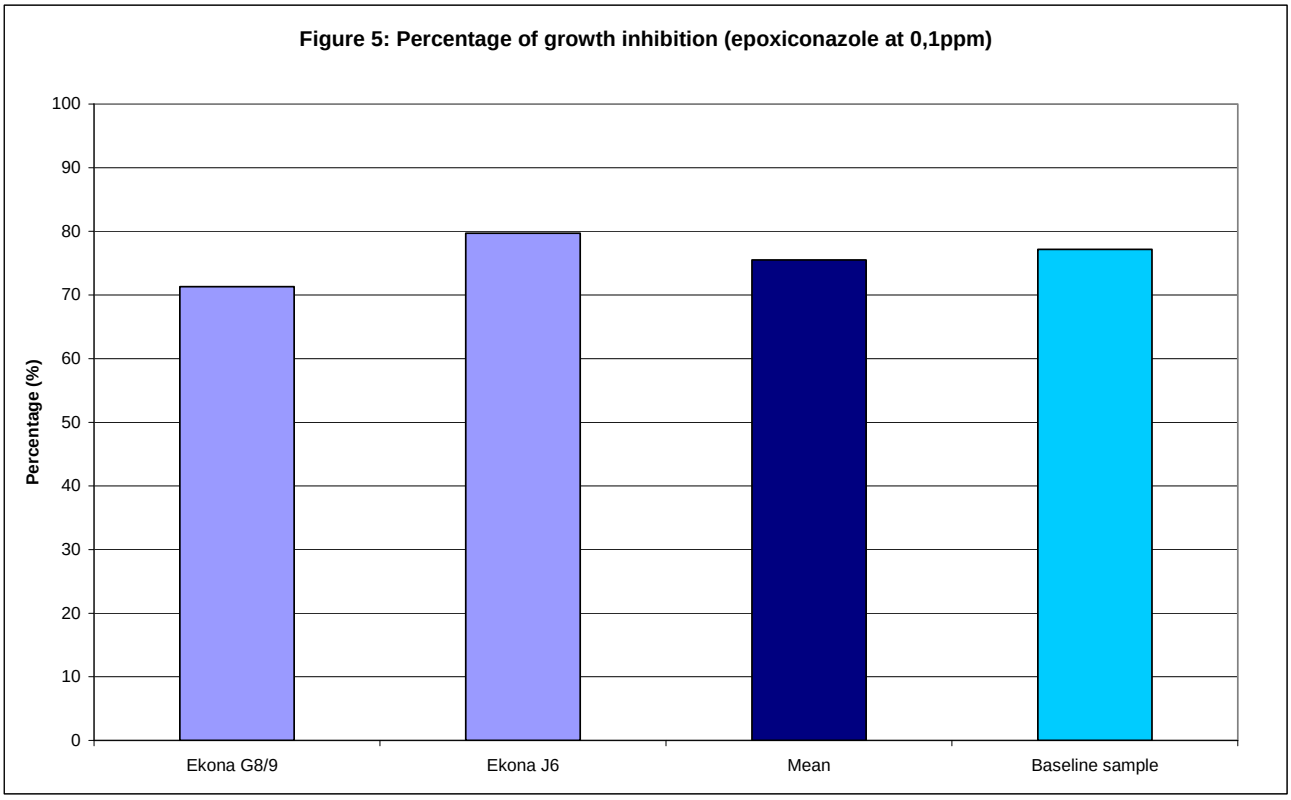


Figure 4: Distribution of spores inside growth inhibition classes (bitertanol at 0,1ppm)



<u>Table 3</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to OPAL (0.1 ppm of bitertanol).

Opal Fields	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
			0-10	11-30	31-50	51-70	71-90	91-100	
Ekona G8/9	**71**	**97**	0	0	8	26	66	0	**8**
Ekona J6	**80**	**76**	0	0	0	14	86	0	**0**
Mean	**76**	**86**	**0**	**0**	**4**	**20**	**76**	**0**	**4**
Baseline sample	**77**	**71**	**0**	**0**	**0**	**8**	**92**	**0**	**0**
Summary of the results of previous monitoring									
june-09	**76**	**86**	**0**	**0**	**4**	**20**	**76**	**0**	**4**



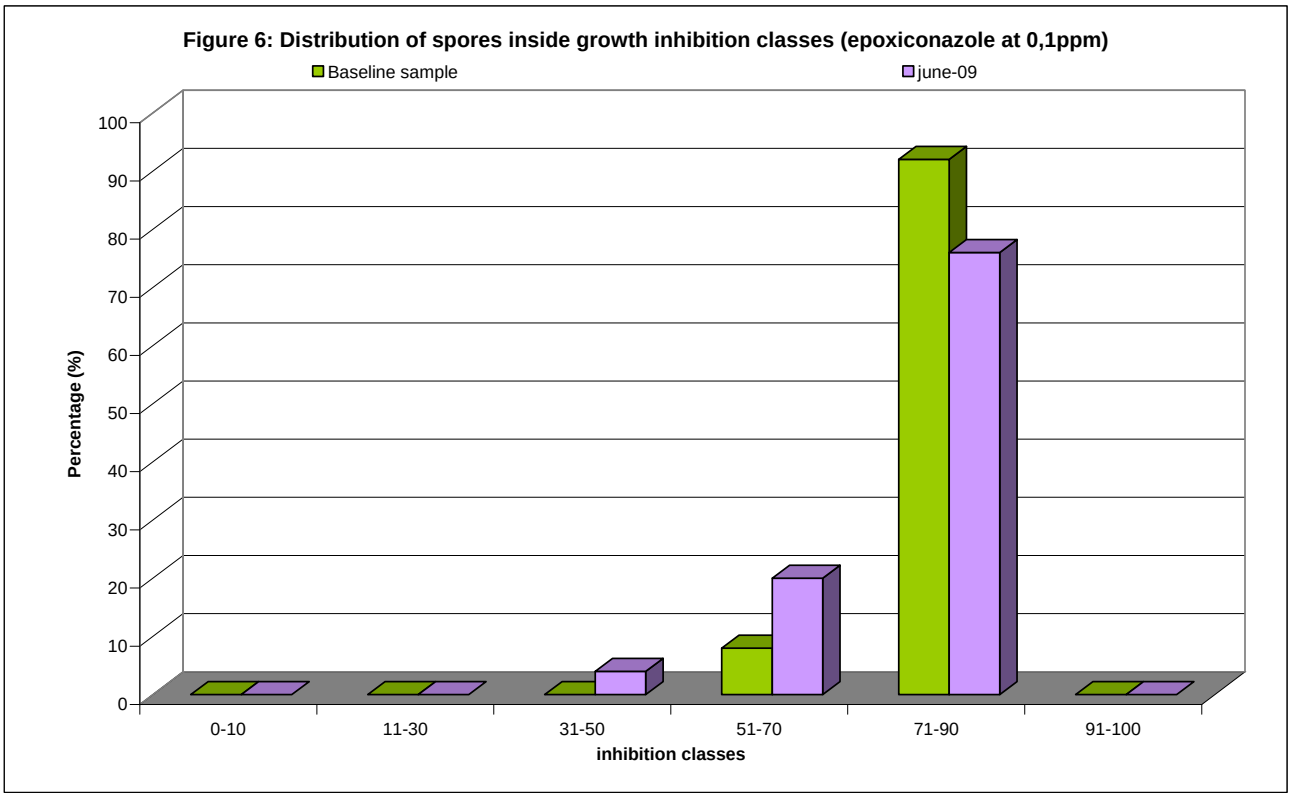


Figure 6: Distribution of spores inside growth inhibition classes (epoxiconazole at 0,1ppm)
Baseline sample
june-09
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
0-10
11-30
31-50
51-70
71-90
91-100
inhibition classes

2.4.3.3. Mussaka plantation

- Sico 250 EC (Triazole – difenoconazole) – table 1; figures 1 and 2

For difenoconazole, the average percentage of inhibition for the 2 different sectors (69 %) is lower than in the untreated smallholder plantation (81%). A low proportion of the average population (4%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows an unsignificant shift in sensitivity to difenoconazole in this plantation. No previous data are available with this fungicide in this farm.

- Baycor 300 EC (Triazole-bitertanol) - table 2; figures 3 and 4

For bitertanol, the average percentage of inhibition for the 2 different sectors (67 %) is lower than in the untreated smallholder plantation (79%). A low proportion of the average population (14%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). This shows a low shift in sensitivity to bitertanol in this plantation. These data are similar to previous monitorings (dec 2006 and November 2007) whiched showed similar low shift for bitertanol in this farm. Nevertheless this shift is not more pronounced today.

- Opal 75 EC (epoxyconazole) – table 3; figures 5 and 6

For epoxyconazole, the average percentage of inhibition for the 2 different sectors (61 %) is lower than in the untreated smallholder plantation (77%). A significant proportion of the average population (29%) has an inhibition inferior to 50%, phenotype which is not encountered in untreated smallholder populations (100% strains have an inhibition > 50%). Nevertheless, the lower classes of the distribution (0-30 % IC) are not represented in the fungal population. This shows a low shift in sensitivity to epoxyconazole in this plantation. These data are similar to previous monitorings (dec 2006 and November 2007) whiched showed similar low shift for bitertanol in this farm. No previous data are available with this fungicide in this farm.

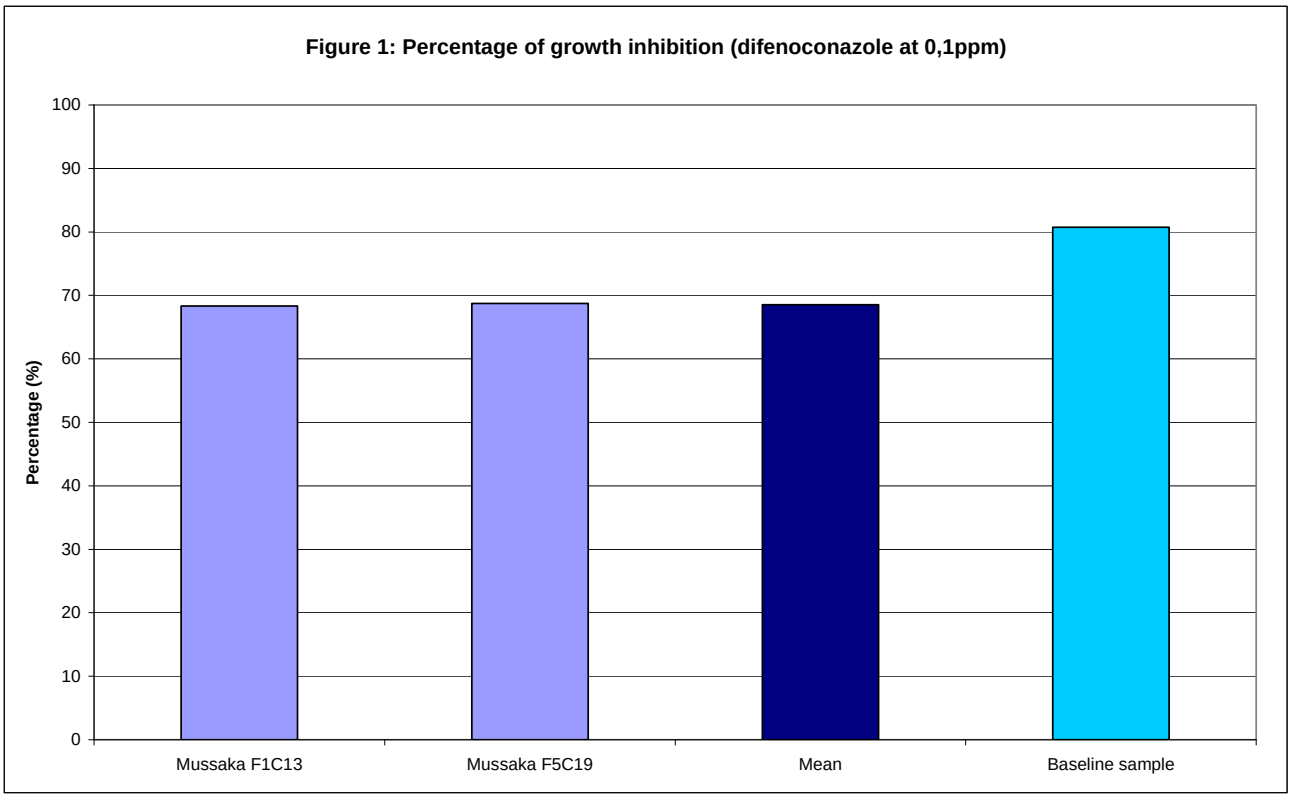
Conclusions and recommendations for Mussaka farm

A very low shift in sensitivity to Triazoles has been noticed in this farm. This shift is more pronounced for epoxyconazole and bitertanol.

We recommend to fulfill with Frac's recommendations in this farm: not more than 8 applications should be done with this family of fungicides which include the following products: Tilt, Folicur, Baycor, Sico, Opal, Punch, Vectra. These fungicides should be fully used in alternation with other mode of actions. Nevertheless, particular attention should be paid in the future to this low shift. By another hand, we recommend also to reinforce deleafing management programs in this plantation (a poor quality of deleafing was noticed this year in many sectors) because high population levels can accelerate the selection of resistant strains inside the farms.

Table 1: Summary of results concerning the sensitivity of *M. fijiensis* populations to Sico (difenoconazole at 0.1 ppm)

Sico	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka F1C13	**68**	99	0	0	6	36	58	0	**6**
Mussaka F5C19	**69**	100	0	0	2	46	52	0	**2**
Mean	**69**	**99**	**0**	**0**	**4**	**41**	**55**	**0**	**4**
Baseline sample	**81**	**60**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
History									
june-09	**69**	**99**	**0**	**0**	**4**	**41**	**55**	**0**	**4**



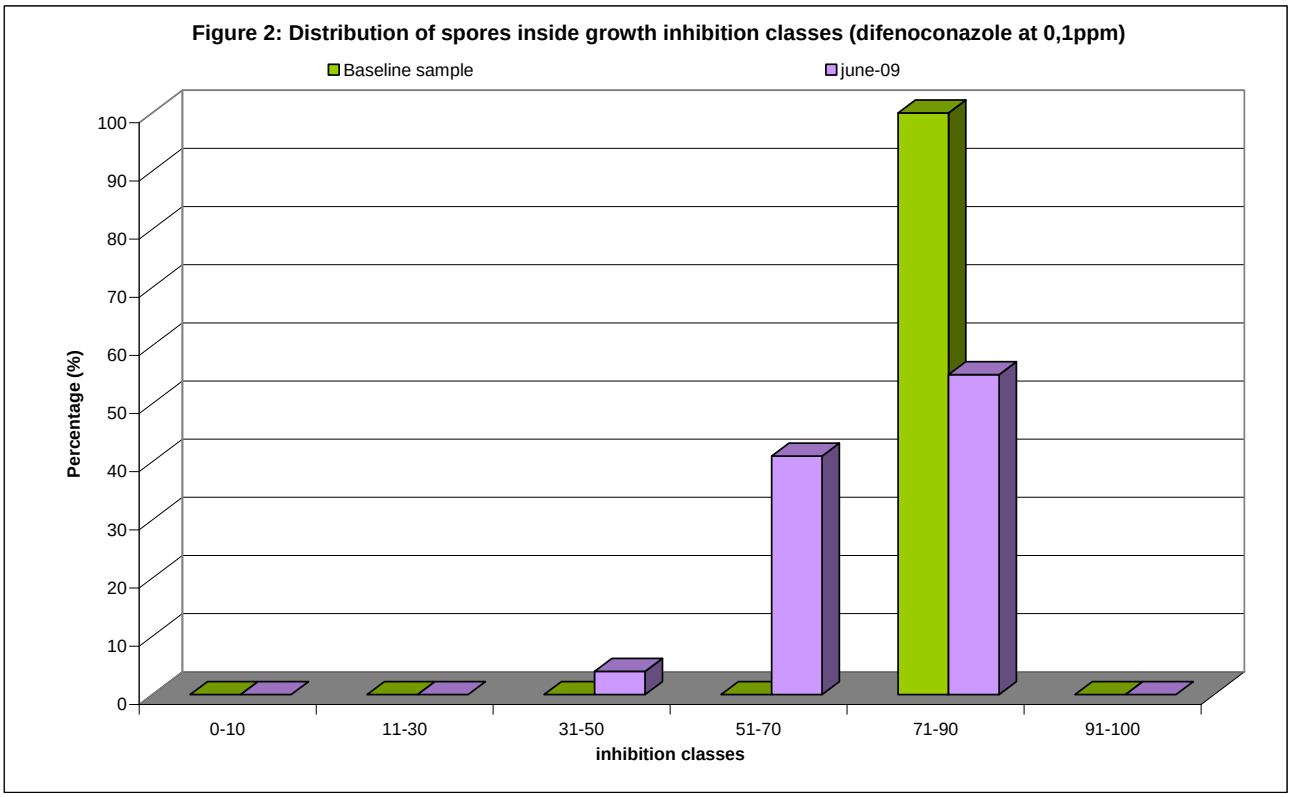


Table 2: **Summary of results concerning the sensitivity of *M. fijiensis* populations to BAYCOR (bitertanol at 0.1 ppm)**

BAYCOR	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
Sectors			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka F1C13	**67**	104	0	0	12	30	58	0	**12**
Mussaka F5C19	**66**	108	0	0	16	32	52	0	**16**
Mean	**67**	**106**	**0**	**0**	**14**	**31**	**55**	**0**	**14**
Baseline sample	**79**	**66**	**0**	**0**	**0**	**0**	**100**	**0**	**0**
History									
déc-05	**78**	**48**	**0**	**0**	**0**	**9**	**86**	**5**	**0**
dec-06	**56**	**110**	**0**	**7**	**20**	**59**	**14**	**0**	**27**
nov-07	**66**	**85**	**0**	**0**	**10**	**44**	**45**	**1**	**10**
june-09	**67**	**106**	**0**	**0**	**14**	**31**	**55**	**0**	**14**

Figure 3: Percentage of growth inhibition (bitertanol at 0,1ppm)

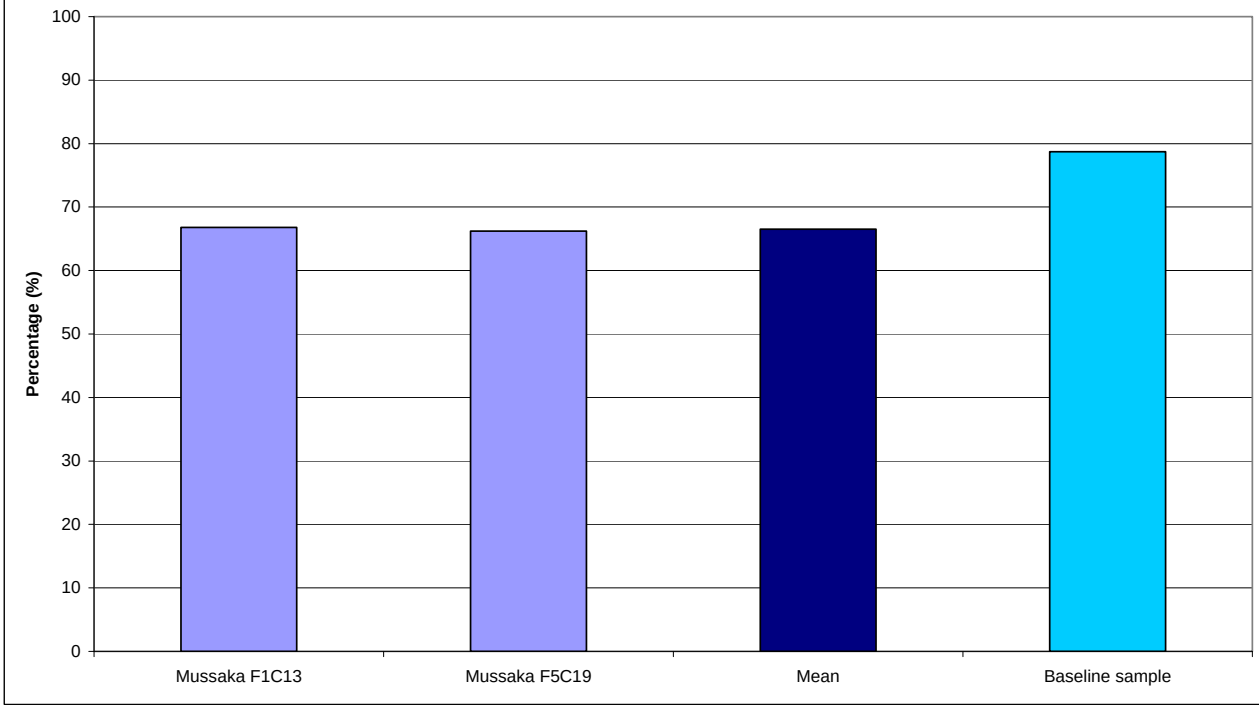
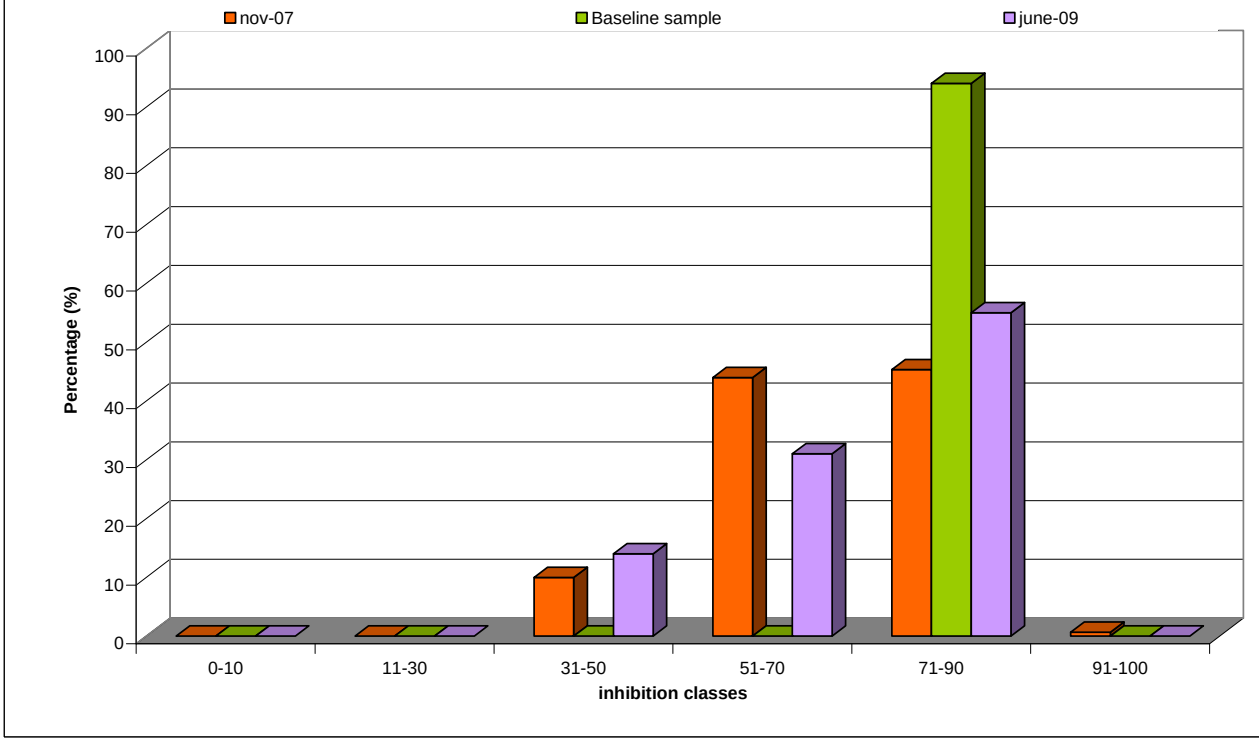
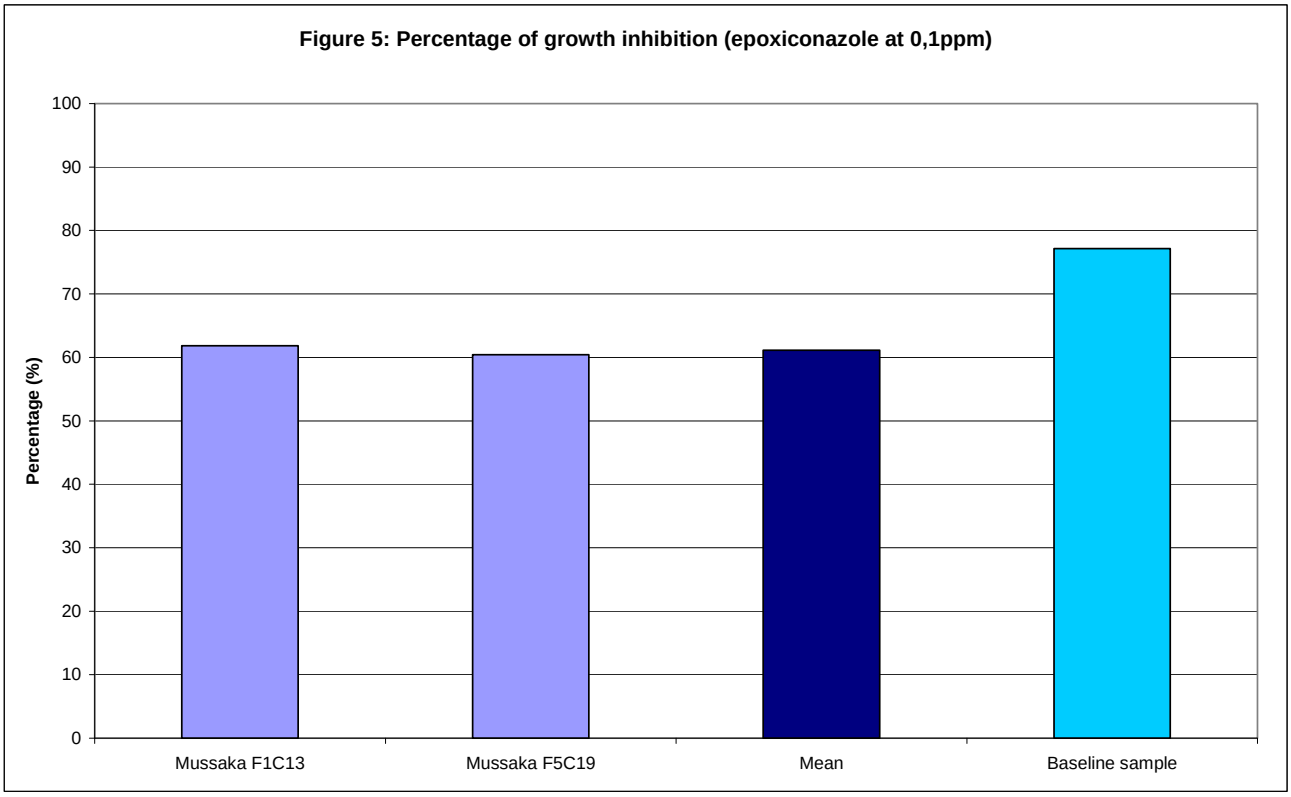


Figure 4: Distribution of spores inside growth inhibition classes (bitertanol at 0,1ppm)



<u>Table 3</u>: Summary of results concerning the sensitivity of *M. fijiensis* populations to Opal (epoxyconazole at 0.1 ppm).

Opal / Sectors	% inhibition	Lenght of the germ tubes (µm)	Growth inhibition classes						% spores inhibition < 50 %
			0-10	11-30	31-50	51-70	71-90	91-100	
Mussaka F1C13	**62**	119	**0**	**4**	**20**	**30**	**46**	**0**	**24**
Mussaka F5C19	**60**	126	0	0	34	28	38	0	**34**
Mean	**61**	**123**	**0**	**2**	**27**	**29**	**42**	**0**	**29**
Baseline sample	**77**	**71**	**0**	**0**	**0**	**8**	**92**	**0**	**0**
history									
june-09	**61**	**123**	**0**	**2**	**27**	**29**	**42**	**0**	**29**



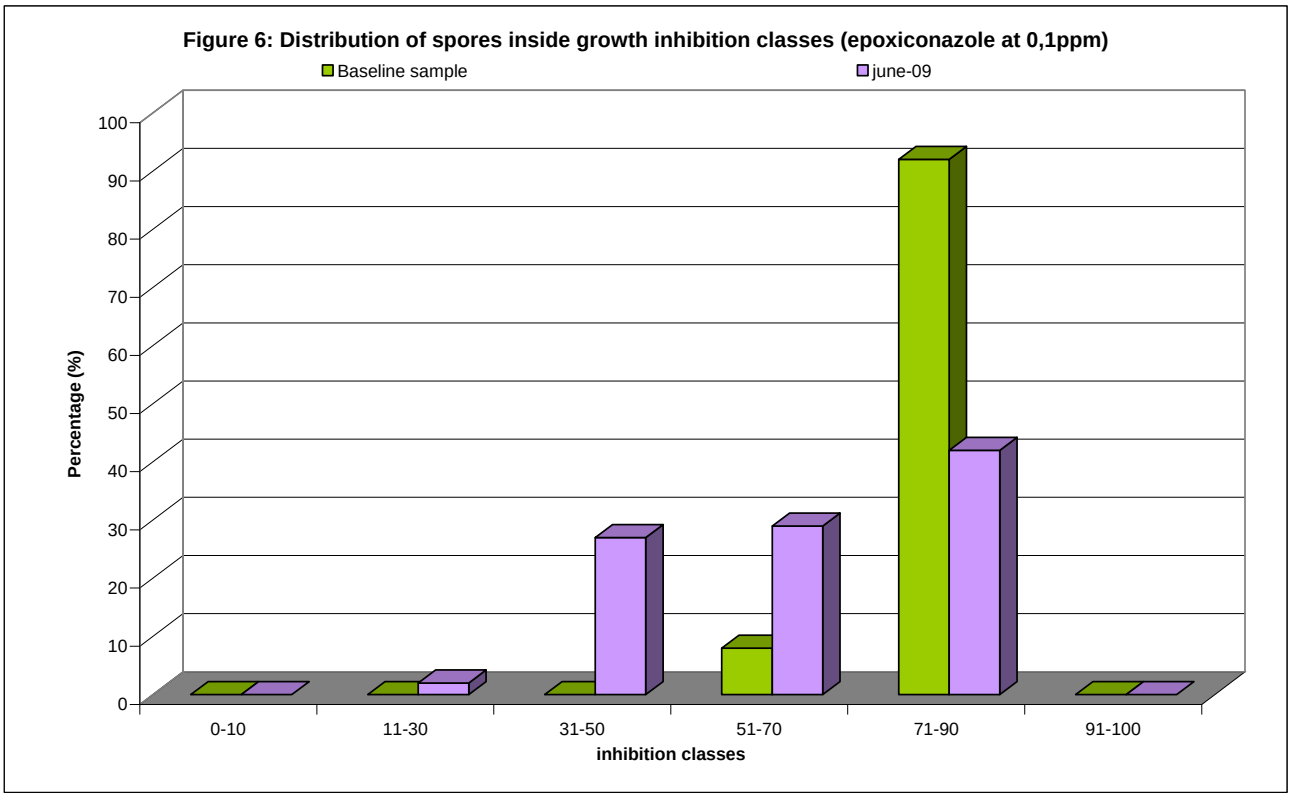


Figure 6: Distribution of spores inside growth inhibition classes (epoxiconazole at 0,1ppm)
Baseline sample
june-09
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
0-10
11-30
31-50
51-70
71-90
91-100
inhibition classes