

# Response of the black Sigatoka pathogen *Mycosphaerella fijiensis* to Calixin (tridemorph) in vitro.

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RESPONSE OF THE BLACK SIGATOKA PATHOGEN  
*MYCOSPHAERELLA FIJIENSIS* TO CALIXIN (TRIDEMORPH)  
IN VITRO.

R.H. STOVER.

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**ABSTRACT** - After 28 days storage of leaf samples at room temperatures of 22°C - 27°C germination fell sharply and there was no germination at 42 days. There was no significant change in germination and germ tube length when leaf samples were stored 42 days in a refrigerator at 11°C.

The ED<sub>50</sub> (50% reduction in ascospore germ tube length) was determined for 14 different leaf samples by probit analyses following ascospore discharge onto plain agar amended with the following concentrations of tridemorph : 0, 0.005, 0.01, 0.05, 0.1, 1.0, 10.0, 25.0 and 50.0 ppm. The average ED<sub>50</sub> was 0.079 ± 0.014 ppm. Most ED<sub>50</sub>s fell in the range of 0.05 to 0.1 ppm. At 0.1 ppm the average germ tube reduction for 45 leaf samples was 57% and 94% at 10.0 ppm.

Since the ED<sub>50</sub> was highly variable, the frequency distribution for germ tube inhibition at 0.1 and 10.0 ppm was studied using measurements for 1,800 individual ascospores from 31 leaf samples. Some 66% of the ascospores fell in the range of 31-65% inhibition at 0.1 ppm. At 10.0 ppm more than 90% of the ascospores fell in the range of 91-100 inhibition. A similar distribution was obtained with 45 leaf samples using the average of 50 ascospores per sample. However, when only 14 samples were used averaging 50 ascospores per sample distribution results were not comparable at 0.1 ppm. The level of 45-65% inhibition was higher and there were no spores falling into the category of 66-75% inhibition. At levels of 16-30% and 76-85% inhibition results were similar for all sampling methods.

At tridemorph levels below 1.0 ppm results for the same leaf sample analysed by two different laboratories were highly variable between laboratories.

To detect possible shifts in sensitivity to tridemorph, it is recommended that germ tube inhibition results from a large number of leaf samples over a specific time period be analysed at 0.1 and 10.0 ppm which approximates the ED<sub>50</sub> and ED<sub>90</sub> for *Mycosphaerella fijiensis*.

## INTRODUCTION

Calixin (tridemorph) along with the triazole and benzimidazole fungicides is an important component of integrated spray programs for the control of black Sigatoka (*Mycosphaerella fijiensis*). Methods of use have been recently

ACTION IN VITRO DU TRIDEMORPHE (CALIXINE) SUR  
*MYCOSPHAERELLA FIJIENSIS* AGENT DE LA MALADIE  
DES RAIES NOIRES.

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**RESUME** - La germination des ascospores n'est pas affectée lorsque les échantillons foliaires sont conservés pendant 28 jours à 22-27°C. Par contre plus aucune germination n'est notée au-delà de 42 jours. Aucun changement significatif n'est observé tant pour la germination que pour la croissance des tubes germinatifs lorsque les échantillons sont conservés 42 jours à 11°C.

La CI 50 (réduction de 50 p. 100 de la longueur des tubes germinatifs) a été déterminée sur 40 échantillons après libération d'ascospores sur des milieux contenant différentes concentrations de tridemorph ; 0, 0.005, 0.01, 0.05, 0.1, 1.0, 10, 25, 50 ppm. La CI 50 moyenne est évaluée à 0.079 ± 0.014 ppm. La plupart des valeurs se situent entre 0.05 et 0.1 ppm. A 0.1 ppm la réduction des tubes germinatifs est de 57 p. 100 et de 94 p. 100 à 10 ppm.

Une grande variabilité est observée pour les CI 50 et une analyse plus fine sur la croissance a été réalisée sur 1800 ascospores obtenus à partir de 31 échantillons foliaires. 66 p. 100 des ascospores présentent une réduction de croissance de 31-65 p. 100 à 0.1 ppm. A 10 ppm plus de 90 p. 100 des ascospores présentent une réduction de 91-100 p. 100. Une distribution similaire est observée avec 45 échantillons foliaires (50 ascospores par échantillon). Des résultats différents sont obtenus avec 14 échantillons seulement et toujours 50 ascospores par échantillon.

A des concentrations de tridemorphe inférieures à 1.0 ppm, des différences sont notées entre résultats obtenus dans différents laboratoires.

Pour évaluer des différences de sensibilité au tridemorphe, il est recommandé d'évaluer l'inhibition de croissance des tubes germinatifs d'un nombre suffisant d'échantillons foliaires aux concentrations 0.1 et 10 ppm qui, approximativement, correspondent aux CI 50 et CI 90 de *Mycosphaerella fijiensis*.

reviewed (Anon., 1989 ; Stover, 1990). Tridemorph is the only fungicide involved in the inhibition of ergosterol biosynthesis in which there has never been a shift to reduced fungus sensitivity. Nevertheless, periodic monitoring is carried out using *M. fijiensis* following the guidelines (Anon., 1988) of the Fungicide Resistance Action Committee (FRAC).

\* - LA LIMA - Honduras.

First results using the FRAC guidelines indicated concentrations would have to be modified to determine the ED<sub>50</sub> (the dose of tridemorph reducing germ tube growth 50%). The results of using different concentrations of tridemorph with different geographic populations of the pathogen are described. In addition, the influence of leaf sample storage conditions on pathogen response was studied.

## METHODS

Methods were based on techniques outlined for monitoring pathogen response to benomyl (Anon. 1983) and subsequently modified for triazole fungicide (Anon., 1988). This involves measuring germination and germ tube growth on plain agar amended with various concentrations of fungicide.

Ascospores-bearing leaf tissue is incubated in plastic bags with moist towelling for 48 hr at laboratory temperatures (22°C-27°C). Leaf tissue for ascospore discharge is removed, cut into 1.0-2.0 cm<sup>2</sup>, numbered with ink and stapled to 9.0 cm diameter N° 1 filter paper (Photo 1). The filter paper with attached leaf pieces is submerged in tap water for 5.0 minutes and then placed inside the top of a Petri plate over a 2.0% water agar for 1 hr for ascospore discharge and then removed. Ascospores are located and identified using 45X and 100X magnifications. Care must be taken not to confuse ascospores of *Mycosphaerella fijiensis* with those of other *Mycosphaerella* spp. (Anon., 1983 ; Stover, 1969). Commercial tridemorph was added to cooled 2.0% water agar at concentrations of 0., 0.005, 0.01, 0.05, 0.1, 1.0, 10.0, 25.0 and 50.0 ppm. Following ascospore discharge as described above plates are incubated at 26°C ± 1°C for 48 hr. Pending examination, plates are stored in closed plastic bags in a refrigerator at 11°C ± 1°C. Germination percentage and germ tube length are measured on 50 ascospores per plate (Photos 2 and 3). An ascospore is considered to have germinated if the germ tube is at least 5μ(microns) long.

### Effect of Leaf Storage Conditions on Ascospore Germ Tube Response to Tridemorph.

A large leaf sample was collected from Santa Catalina Farm, Honduras, and divided into two parts. One part was stored in a plastic bag at a fluctuating room temperature of 22°C - 24°C minimum and 25°C - 27°C maximum. One sample was stored in a plastic bag in a refrigerator at 11°C ± 1°C. Samples were removed and ascospores discharged after 7, 14, 28 and 42 days of storage. ED<sub>50</sub> values were determined using the MSTAT-C Michigan State University Program where probit values were regressed against log 10 concentrations of tridemorph.

### Response of Ascospores from Different Geographic Areas and ED<sub>50</sub>s.

During 1988 and 1989 samples were collected from 9 different areas : 1) Honduras : Corozal Cable 2 ; 2) Honduras : Corozal Cable 16 ; 3) Honduras : Santa Rosa Cable 38 ; 4) Honduras : Santa Rosa Cable 170 ; 5) Panama : Farm 76 ; 6) Belize : Farm 4 ; 7) Honduras : Santa Catalina ; 8) Ecuador 5 ; 9) Ecuador 6. All areas were commercial and had received 4 to 6 sprays of Calixin annually except Santa Catalina which received none. Samples were analysed as described above. In addition, four other samples from Honduras and Costa Rica were analysed at 0.001, 0.01, 0.1, 1.0 and 10.0 ppm. The ED<sub>50</sub>s were determined using the MSTAT-C Michigan State University Program.

### Frequency Distribution of Germ Tube Inhibition Caused by Tridemorph of Individual Ascospores and Ascospores Populations.

Approximately 1,800 individual ascospore germ tubes from Santa Catalina growing at 0.1 and 10.0 ppm of tridemorph were plotted on a frequency distribution graph. At 0.1 ppm the frequency distribution ranged from 5-15 to 86-100% inhibition and from 71-80 to 91-100% inhibition at 10.0 ppm. In addition, the average germ tube

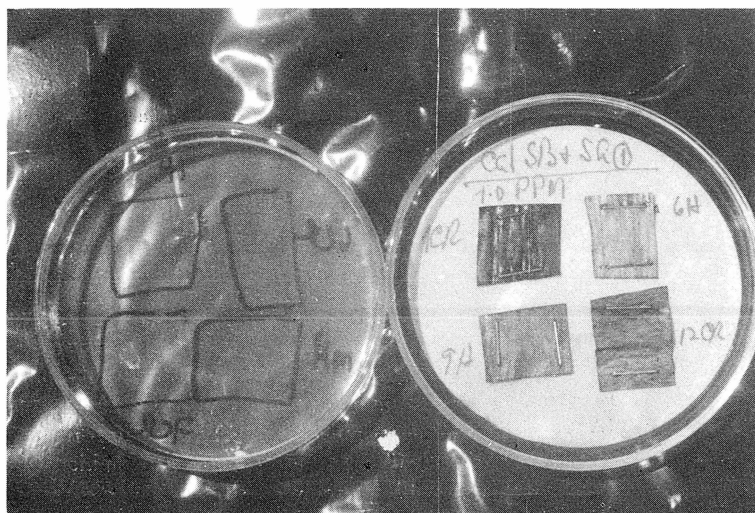
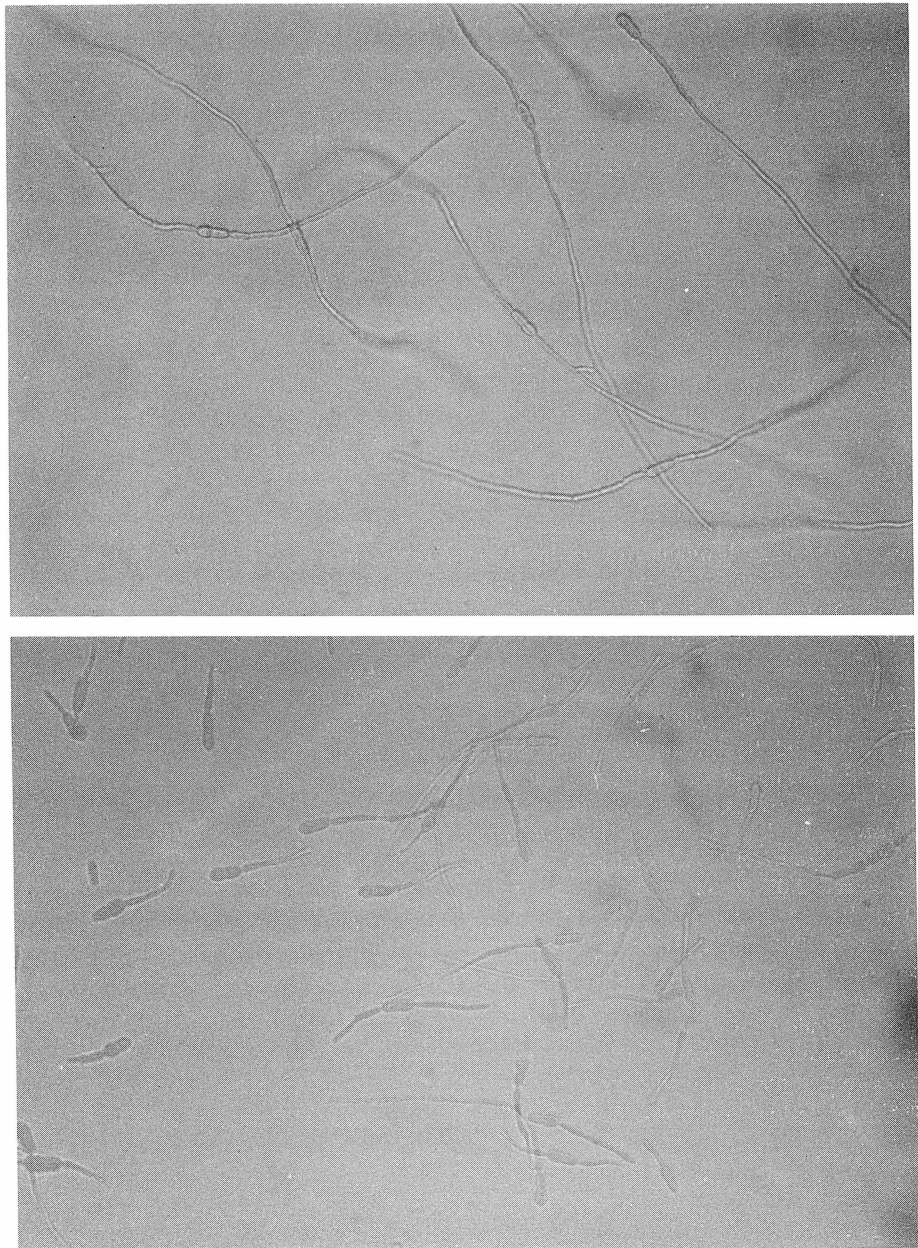


Photo 1 - Banana leaf pieces stapled on filter paper in upper portion of Petri plate ready for moistening and ascospore discharge (right). Pieces are outlined with crayon and labelled on the outside of the lower Petri dish (left).



**Photo 2** - Appearance of germ tubes from ascospores after 48 hours at 26°C. Upper : Control ; lower : 0.1 ppm of tridemorph. 200 X.

length of 50 ascospores from 45 leaf samples was plotted. Finally, average germ tube growth of 14 leaf samples containing 50 ascospores per sample were plotted at 0.1 ppm and 12 samples at 10.0 ppm.

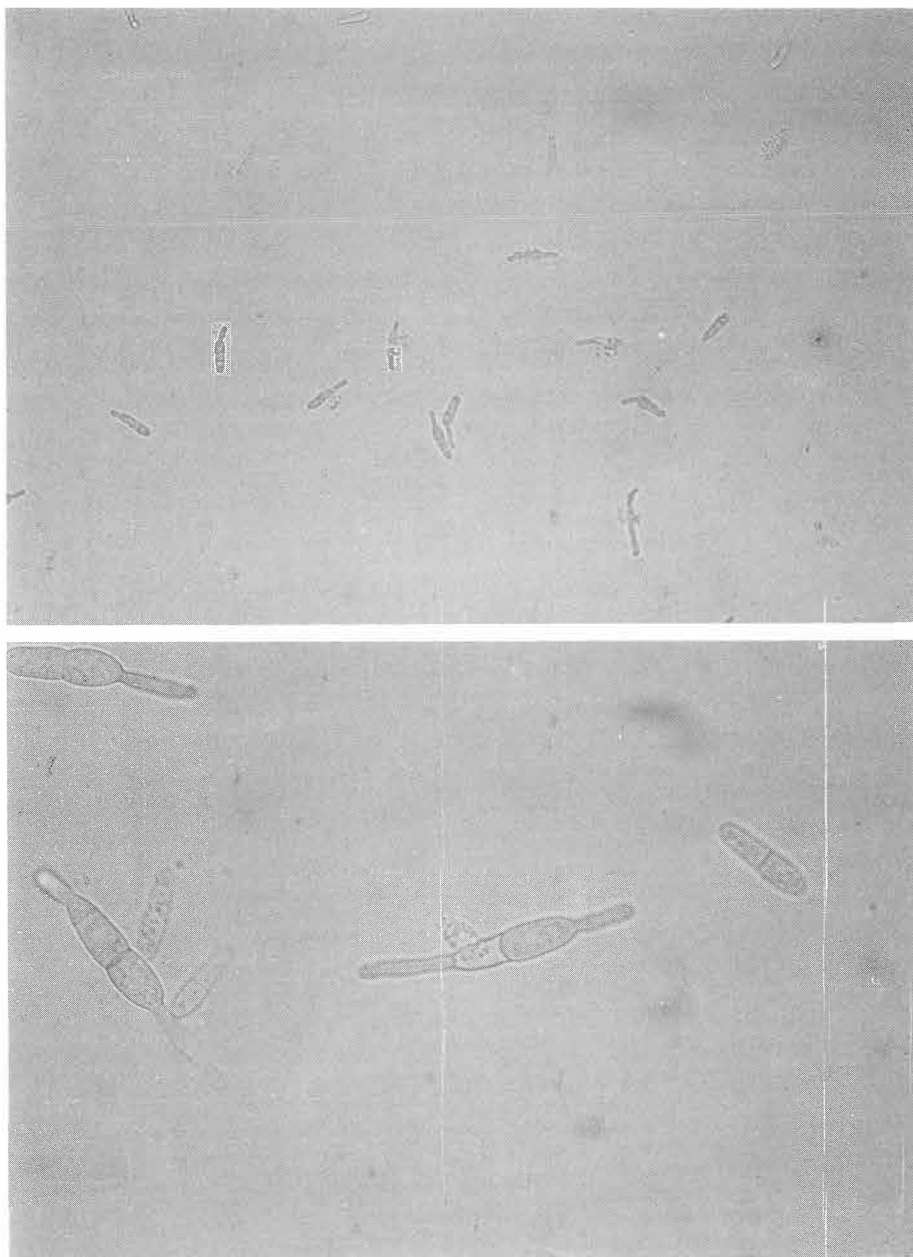
#### **Variation Between Different Laboratories Analysing the Same Leaf Sample.**

Three leaf samples from Honduras and Panama were divided equally between two laboratories and analysed by two different technicians at 0.005, 0.01, 0.05, 0.1, 1.0, 5.0, 10.0, 25.0 and 50.0 ppm of tridemorph.

## **RESULTS**

### **Effect of Storage Conditions for Leaf Samples on Ascospore Response to Tridemorph.**

After 28 days storage of leaf samples at room or laboratory temperatures of 22°C - 27°C germination fell sharply ; there was no germination at 42 days (Table 1). Hence leaf samples should not be held longer than 3 weeks at ambient temperatures. Samples kept well with no significant change in germination when stored in a closed plastic bag at 11°C in the refrigerator for 42 days. Germ tube length was also reduced after 28 days storage at ambient temperatures (Tables 2 and 3). The ED<sub>50</sub> values were highly variable (Table 2) with a range of 0.049 to 0.429. The latter reading is far above ED<sub>50</sub> values from different geographic areas (see next section) and is considered aberrant.



**Photo 3** - Appearance of germ tubes from ascospores growing in 10.0 ppm of tridemorph after 48 hours at 26°C. Upper 200 X ; lower 800 X.

**TABLE 1** - Percentage germination of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples stored at different lengths of time in the laboratory or refrigerator.

Calixin ppm	Laboratory				Refrigerator			
	Days storage				Days storage			
	7	14	28	42	7	14	28	42
Control	100.0	100.0	29.1	0	100.0	100.0	100.0	100.0
0.005	100.0	100.0	19.4	0	100.0	100.0	100.0	100.0
0.01	100.0	100.0	17.7	0	100.0	100.0	100.0	100.0
0.05	100.0	100.0	17.0	0	100.0	100.0	100.0	100.0
0.1	100.0	100.0	38.9	0	100.0	100.0	100.0	100.0
1.0	100.0	100.0	6.0	0	100.0	100.0	100.0	100.0
10.0	0	67.5	0	0	87.3	66.4	43.2	0

**TABLE 2 - Percentage reduction in germ tube length of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples stored at different lengths of time in the laboratory or refrigerator.**

Calixin ppm	Laboratory				Refrigerator			
	Days storage				Days storage			
	7	14	28	42	7	14	28	42
0.005	15.3	35.2	9.6	-	20.8	30.1	16.9	31.0
0.01	17.1	38.0	13.3	-	19.4	38.4	15.8	38.6
0.05	27.8	44.8	16.3	-	34.1	44.9	23.4	34.2
0.1	34.9	49.4	24.8	-	39.2	50.1	27.9	46.5
1.0	54.0	68.4	55.4	-	63.0	75.8	32.8	76.7
10.0	100.0	92.2	100.0	-	91.4	94.4	81.4	100.0
ED <sub>50</sub>	0.189	0.050	-	-	0.146	0.049	0.0429	0.055

**TABLE 3 - Average germ tube length in microns of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples stored at different lengths of time in the laboratory or refrigerator.**

Calixin ppm	Laboratory				Refrigerator			
	Days storage				Days storage			
	7	14	28	42	7	14	28	42
Range control	153.8-369.2	194.9-338.4	102.6-194.9		153.8-307.7	205.4-420.5	184.6-287.2	205.0-359.0
Control average	231.8	265.4	140.1		218.0	291.2	231.8	255.2
0.005	196.3	171.9	126.7	no germination	172.7	203.7	192.6	176.0
0.01	192.2	164.5	121.4		175.8	179.5	195.1	156.7
0.05	167.4	146.5	117.3		143.6	160.4	177.6	167.8
0.1	150.8	134.2	105.4		132.5	145.2	167.2	136.6
1.0	106.7	83.9	62.5		80.7	70.6	155.7	59.5
10.0	0	20.8	0		18.7	16.3	43.0	0

**TABLE 4 - Percentage reduction in germ tube length of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples from nine different areas.**

Calixin ppm	Sample number									Av.
	1	2	3	4	5	6	7	8	9	
0.005	25.2	27.5	28.6	43.4	47.8	20.1	22.1	35.8	N.D.	30.8
0.01	32.2	37.3	33.3	55.2	50.8	32.3	30.6	38.1	38.3	39.6
0.05	29.3	46.8	48.3	67.7	57.0	34.8	18.9	42.0	41.9	43.8
0.1	46.2	38.4	63.0	53.4	56.8	44.9	56.2	55.5	47.0	62.8
1.0	81.8	67.9	89.2	82.2	79.5	64.9	57.0	61.6	62.6	72.3
10.0	87.3	89.1	100.0	94.2	N.D.	100.0	90.0	89.2	100.0	93.5
25.0	86.5	92.9	N.D.	95.6	92.6	100.0	93.3	100.0	100.0	94.8
50.0	94.0	94.0	100.0	95.0	100.0	100.0	94.8	100.0	100.0	97.5
ED <sub>50</sub>	0.110	0.085	0.034	0.017	0.013	0.093	0.156	0.053	0.052	

Average ED<sub>50</sub> 0.0527 ± 0.0342

**Response of Ascospore from Different Geographic Areas to Tridemorph and Variation in the ED<sub>50</sub>.**

Germination, germ tube length, percentage reduction in germ tube length and the ED<sub>50</sub> for various concentrations of tridemorph are shown in Table 4 to 6. For the nine different leaf samples the ED<sub>50</sub> ranged from 0.013 to 0.156 ppm with an average of 0.068 ± 0.016. The least significant difference using the t test and Duncans multiple

range tests were calculated although statisticians do not recommend these tests where dosages of the same material are being compared. They indicate there were no significant differences in percentage reduction in germ tube length among 10.0, 25.0 and 50.0 ppm, between 0.1 and 1.0 ppm and 0.01 and 0.05 ppm. With respect to germ tube length, differences were statistically significant between 0.05, 0.1, 1.0 and 10.0 ppm (Table 5). The preferred method of comparing dosages is the probit of the Log<sub>10</sub> concentration

**TABLE 5 - Average germ tube length in microns of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples from nine different areas.**

Calaxin ppm	Sample number									
	1		2		3		4		5	
Range	153.8 276.9	%	133.3 266.7	%	112.8 256.4	%	153.8 369.2	%	234.9 344.3	%
Control	215.8	100	201.8	100	173.7	100	250.0	100	287.7	100
0.005	161.4	74	146.3	72	124.1	68	141.7	40	150.3	0
0.01	146.0	44	126.6	50	115.9	56	112.0	8	141.5	0
0.05	152.6	58	107.3	26	89.8	16	80.8	4	123.8	0
0.1	116.1	6	124.3	50	64.2	8	116.5	16	124.3	0
1.0	39.2	0	64.8	0	18.7	0	44.5	0	59.0	0
10.0	27.5	0	21.9	0	0	0	19.0	0	N.D.	
25.0	29.2	0	14.3	0	N.D.	-	10.9	0	21.3	0
50.0	13.0	0	12.2	0	0	0	12.6	0	0	0
	6		7		8		9		Average & Std. Error	
Range	153.8 307.7		156.5 286.9		130.4 300.0		125.2 268.7			
Control	213.5	100	215.3	100	199.1	100	176.8	100	214.8 ± 11.8	
0.005	170.5	94	167.7	72	127.9	48	N.D.	-	148.7 ± 6.1	
0.01	144.6	42	149.4	42	123.3	40	108.2	14	129.7 ± 5.3	
0.05	139.3	36	174.4	74	115.5	42	102.7	12	120.7 ± 10.1	
0.1	117.7	8	94.2	12	88.6	6	93.7	20	80.0 ± 15.5	
1.0	74.9	0	92.6	2	76.4	0	66.1	2	59.6 ± 7.4	
10.0	0	0	21.2	0	21.5	0	0	0	13.9 ± 4.2	
25.0	0	0	14.5	0	0	0	0	0	11.3 ± 3.8	
50.0	0	0	11.2	0	0	0	0	0	5.4 ± 2.2	

Notes : % equal percentage germ tubes falling with control range.

**TABLE 6 - Percentage germination of ascospore of *Mycosphaerella fijiensis* discharged from banana leaf samples from nine different areas.**

Calaxin ppm	Sample number								
	1	2	3	4	5	6	7	8	9
Control	90	74	52	84	100	100	100	82	54
0.005	87	69	51	77	58	100	100	30	ND
0.01	93	68	46	37	97	100	100	61	83
0.05	78	76	41	79	81	79	98	33	75
0.1	78	83	50	62	69	100	96	28	53
1.0	63	73	48	52	62	92	91	39	95
10.0	35	14	0	14	ND	0	83	37	0
25.0	44	34	ND	8	21	0	56	0	0
50.0	20	36	0	12	0	0	48	0	0

shown for six leaf samples in figures 1 and 2. These indicate significant differences among different leaf samples.

Since differences in percentage reduction in germ tube growth were small or non significant in some of the above concentrations studied, germ tube reduction was studied at the following concentrations : 0.001, 0.01, 0.1, 1.0 and 10.0 ppm. At concentrations below 0.1 ppm there was little difference between 0.001 and 0.01 ppm in two of the four samples (Tables 7 and 8). The ED<sub>50</sub> values were : 0.158, 0.191, 0.013 and 0.169.

The ED<sub>50</sub> values for 14 leaf samples at one range of concentrations and 4 at the second range are summarized in Table 9. The average for the 14 leaf samples at range 1 was  $0.079 \pm 0.014$ . The average for the 4 leaf samples using the second range was 0.133 ppm.

#### Frequency Distribution of Germ Tube Inhibition Caused by Tridemorph of Individual Ascospores and Ascospores Populations.

Frequency distribution of percentage inhibition at

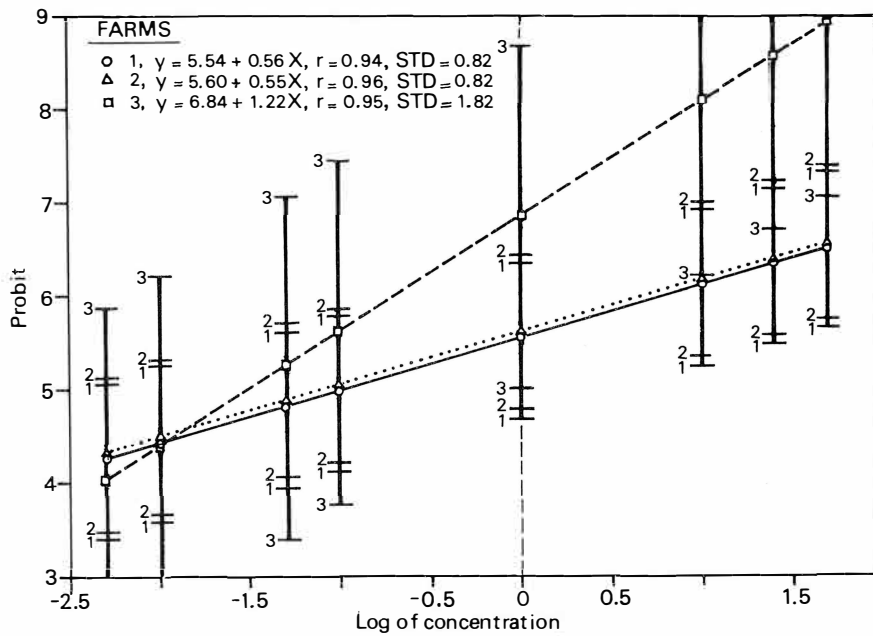


FIGURE 1 - Probit inhibition values regressed against log concentration of tride-morph for Farms 1, 2 and 3.

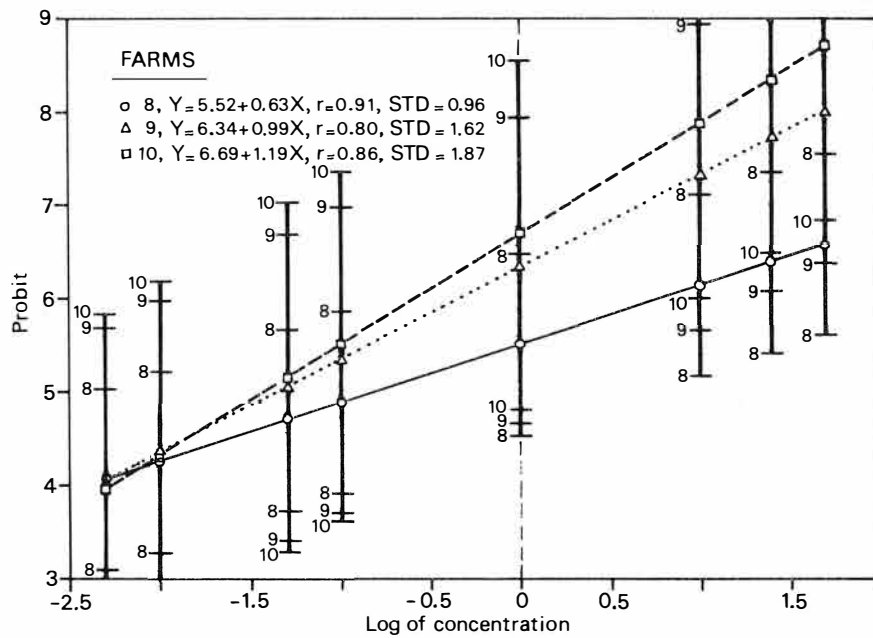


FIGURE 2 - Probit inhibition values regressed against log concentration of tride-morph for Farms 8, 9 and 10.

TABLEAU 7 - Percentage reduction in germ tube length of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples from Honduras and Costa Rica.

Calixin ppm	Honduras		Costa Rica	
	Santa Catalina	Santa Barbara	Rio Barbilla	San Alberto
0.001	14.2	25.9	33.0	3.3
0.01	12.7	24.6	45.4	20.1
0.1	36.9	30.1	65.0	46.0
1.0	73.7	60.0	78.0	68.6
10.0	92.0	87.0	94.7	90.9
ED50	0.158	0.191	0.013	0.169

TABLE 8 - Average germ tube length in microns of ascospores of *Mycosphaerella fijiensis* discharged from banana leaf samples from Honduras and Costa Rica.

Calixin ppm	Honduras				Costa Rica			
	Santa Catalina		Santa Barbara		Rio Barbilla		San Alberto	
	Length	%	Length	%	Length	%	Length	%
Range	153.8 307.7		143.6 276.9		164.1 379.5		133.3 246.1	
Control	214.1	100	195.9	100	260.5	100	176.7	100
0.001	183.8	98	145.2	72	174.5	68	170.9	92
0.01	186.9	90	147.7	70	142.2	32	141.0	66
0.1	135.2	32	136.4	50	91.1	0	95.5	0
1.0	56.4	0	78.4	0	57.4	0	55.4	0
10.0	17.2	0	25.4	0	13.9	0	16.1	0

Notes : % equal percentage germ tubes falling within control range.

TABLE 9 - Summary of ED<sub>50</sub>s for 14 samples and 4 samples from two different concentration ranges of tridemorph.

Sample	Range 1	Sample	Range 2
1	0.110	1	0.158
2	0.085	2	0.191
3	0.034	3	0.013
4	0.017	4	0.169
5	0.013		
6	0.093		
7	0.156		
8	0.053		
9	0.052		
10	0.189		
11	0.146		
12	0.050		
13	0.049		
14	0.055		
Average	0.079		0.133
±	0.014		

Notes : Concentration Range 1 is in ppm of tridemorph : 0.005, 0.01, 0.05, 0.1, 1.0, 10.0, 25.0 and 50.0. Concentration Range 2 is 0.001, 0.01, 0.1, 1.0 and 10.0 ppm.

Data taken from Tables 2 and 4.

The 0.429 reading from Table 2 is excluded as being aberrant.

0.01 ppm of tridemorph of ascospore of germ tube length of 1,800 individual spores from 31 leaf samples all collected at Santa Catalina, Honduras, are shown in Figure 3. Inhibition frequencies are spread over a broad range from 16 to 85% inhibition with more than 50% of the germ tube falling in the range of 56 to 85% inhibition. When inhibition distribution is based on 45 different leaf samples using the average inhibition of 50 ascospores in each sample, results are similar to individual ascospore distribution. Detailed data for the 45 leaf samples are given in Appendixes 1 and 2. Fourteen random samples of 50 ascospores each (Appendix 1) were plotted at 0.1 ppm. Results are more variable (Figure 3). Maximum inhibition is almost double the larger population of ascospores at the 46-55% level of inhibition. Between 16 and 30% inhibition and 76-85% inhibition results are similar for all three sample sizes.

Frequency distribution of germ tube inhibition at

10.0 ppm is less variable than at 0.1 ppm (Figure 4). More than 90% of the ascospores fell in the range of 81 to 100% germ tube inhibition at 10.0 ppm.

#### Variation in Germ Tube Inhibition Between Laboratories Analysing the Same Leaf Sample.

Three leaf samples from different areas were analysed by two laboratories called T and R (Table 10). Tridemorph concentrations were 0.005, 0.01, 0.05, 0.1, 1.0, 5.0, 10.0, 25.0 and 50.0 ppm. Since previous studies indicated differences between certain of these concentrations were not statistically significant data were combined as follows : (0.005), (0.01 and 0.05), (0.1 and 1.0) and (10.0, 25.0 and 50.0 ppm). The data show high levels of variation between laboratories at levels of 1.0 ppm and lower with a tendency to very low levels of inhibition in Laboratory T compared with the overall average of a larger number of samples (columns A and B, Table 10). Data from de Lapeyre de Bellaire (1990) using conidia of *Pseudocercospora musae* (common or yellow Sigatoka) are included in Table 10. This close relative of *Paracercospora fijiensis* is less sensitive to tridemorph.

#### DISCUSSION

Previous studies of the response of *M. fijiensis* to tridemorph were carried out at the relatively high concentrations of 1.0, 5.0, 10.0, 50.0 and 100.0 ppm where there is little or no difference among 10.0, 50.0 and 100.0 ppm (Cronshaw and Lorenz, 1988 ; Cronshaw and Akers, 1990). Studies reported here show the ED<sub>50</sub> is usually between 0.05 and 0.1 ppm with a large variability among leaf samples and even between laboratories analysing the same sample. Variability in germ tube growth is much less at concentrations of 1.0 to 10.0 ppm. The ED<sub>90</sub> appears to be around 10.0 ppm. According to de Lapeyre de Bellaire (1990) the ED<sub>50</sub> and ED<sub>90</sub> for *Pseudocercospora musae* was 3.0 ppm and 24.0 - 29.0 ppm, respectively.

Romero and Marin (1990) studied the response of *M. fijiensis* to propiconazole. They used a range of concentrations of 0.01 to 0.08 at 0.01 ppm intervals. The ED<sub>50</sub>



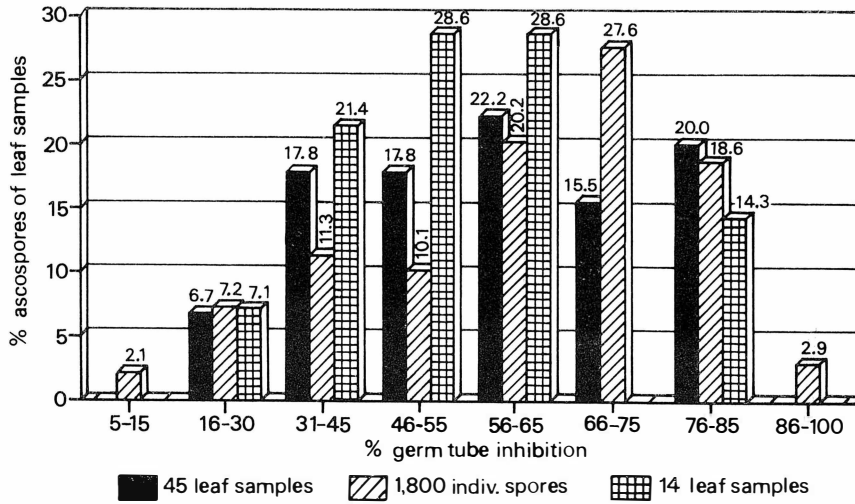


FIGURE 3 - Frequency distribution of percentage germ tube inhibition at 0.1 ppm tridemorph.

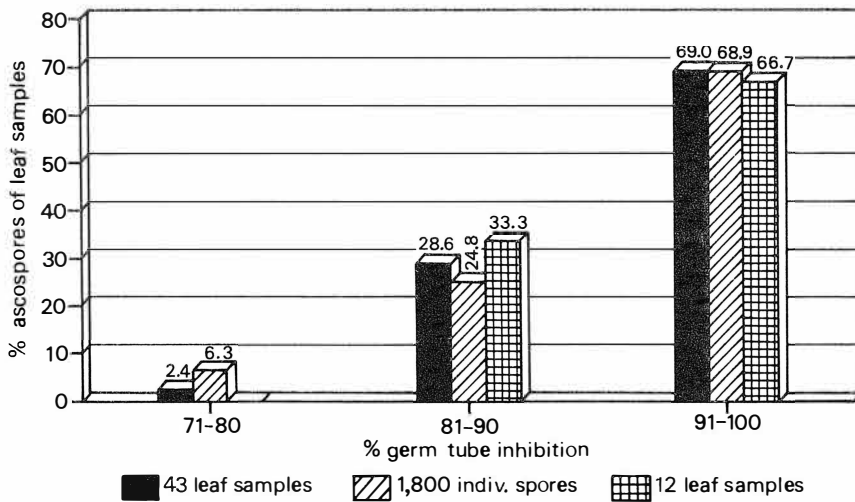


FIGURE 4 - Frequency distribution of percentage germ tube inhibition at 10.0 ppm of tridemorph.

TABLE 10 - A comparison of percentage reduction in germ tube length of sample from different areas processed by two different laboratories.

ppm	A	B	C	D		E		F	
				T	R	T	R	T	R
0.005	25-32	31	-	0	7	12	48	5	N
0.01-0.05	29-68	40-44	17	2-3	13-21	2-20	51-57	2-32	47
0.1-1.0	58-82	63-72	27	63-67	23-33	24-54	57-79	28-37	58-77
5.0	-	-	51	81	74	67	95	65	N
10.0-50.0	87-95	93-97	82-97	82-89	83-94	86-93	93-100	82-95	100

Notes - A : Calixin Bulletin data (Anon., 1988) B : Average of 9 areas this publication (table 4)

C : de Lapeyre de Bellaire, 1990, using conidia of *Pseudocercospora musae*

D : Honduras E : Panama, Farm 76 F : Panama, Farm 33 N : no ascospore discharge

T : first laboratory R : second laboratory processing same sample.

APPENDIX 1 - Average germ tube length in microns and percentage reduction of germ tube length of ascospores of *Mycosphaerella fijiensis* in response to 0.1 ppm of tridemorph (45 tests).

Test N°	Control	Tridemorph	Reduction
1	215.8	116.1	46.2
2	201.8	124.3	38.4
3	173.7	64.2	63.0
4	250.0	116.5	53.4
5	160.1	37.6	76.5
6	235.1	54.2	76.9
7	192.6	97.2	49.5
8	287.7	124.3	56.8
9	222.8	170.5	23.5
10	199.1	76.4	55.5
11	176.8	66.1	47.0
12	215.3	92.6	56.2
13	176.8	117.5	33.5
14	181.1	119.0	34.3
15	213.5	117.7	44.9
16	231.8	150.8	34.9
17	265.4	134.2	49.4
18	218.0	132.5	39.2
19	291.3	145.2	50.1
20	231.8	167.2	27.9
21	255.2	136.6	46.5
22	195.9	136.4	30.1
23	214.1	135.2	36.9
24	260.5	91.1	65.0
25	176.7	95.2	46.0
26	200.4	138.9	30.7
27	187.7	59.8	68.1
28	204.7	61.6	69.9
29	175.6	41.4	76.4
30	161.6	42.1	73.9
31	167.8	47.2	71.9
32	179.3	41.4	76.9
33	164.1	26.4	83.9
34	178.3	42.2	76.3
35	195.7	62.0	68.3
36	208.0	32.9	84.2
37	168.4	49.5	70.6
38	224.6	55.0	75.5
39	227.5	81.4	64.2
40	222.9	68.5	69.3
41	187.8	66.5	64.6
42	206.3	85.9	58.4
43	222.0	81.5	63.3
44	190.8	71.4	62.6
45	157.5	31.1	80.3

Notes : Banana leaf samples were collected on different dates from several locations in Honduras, Belize, Costa Rica, Panama, Ecuador, Mexico and Philippines. Incubation temperature  $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 48 hr in Calixin amended water agar. Each reading is an average of 50 ascospores. Control average is  $206.1\mu \pm 4.9\mu$ ; 0.1 ppm is  $89.0\mu \pm 6.0\mu$ . Average percent reduction is 56.8.

APPENDIX 2 - Average germ tube length in microns and percentage reduction of germ tube length of ascospores of *Mycosphaerella fijiensis* in response to 10.0 ppm of tridemorph (45 tests).

Test N°	Control	Tridemorph	Reduction
1	215.8	27.5	87.3
2	201.8	21.9	89.1
3	173.7	0	100.0
4	250.0	19.0	92.4
5	160.1	11.4	92.9
6	235.1	0	100.0
7	192.6	10.1	94.8
8	287.7	N.D.	N.D.
9	222.8	37.7	83.1
10	199.1	21.5	89.2
11	176.8	0	100.0
12	215.3	21.2	90.2
13	176.8	23.1	86.9
14	181.1	N.D.	N.D.
15	213.5	0	100.0
16	231.8	0	100.0
17	265.4	20.8	92.2
18	218.0	18.7	91.4
19	291.3	16.2	94.0
20	231.8	43.0	81.4
21	255.2	0	100.0
22	195.9	25.4	87.0
23	214.1	17.2	92.0
24	260.5	13.9	94.7
25	176.7	16.1	90.9
26	200.4	30.5	84.8
27	187.7	18.6	90.1
28	204.7	0	100.0
29	175.6	9.8	94.4
30	161.6	0	100.0
31	167.8	0	100.0
32	179.3	0	100.0
33	164.1	N.D.	N.D.
34	178.3	0	100.0
35	195.7	0	100.0
36	208.0	0	100.0
37	168.4	0	100.0
38	224.6	18.7	91.7
39	227.5	0	100.0
40	222.9	0	100.0
41	187.8	0	100.0
42	206.3	29.0	85.9
43	222.0	44.5	80.0
44	190.8	29.8	84.4
45	157.5	0	100.0

Notes : Banana leaf samples were collected on different dates from several locations in Honduras, Belize, Costa Rica, Panama, Ecuador, Mexico and Philippines. Incubation temperature  $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 48 hr in Calixin amended water agar. N.D. = No Discharge of ascospores. Each reading is an average of 50 ascospores. Control average is  $206.1\mu \pm 4.9\mu$ ; 10.0 ppm is  $13.0\mu \pm 2.1\mu$ . Average percent reduction is 93.7.

ranged from 0.006 to 0.04 with an average of 0.023. Molina and Salas (1990) using similar techniques but different concentrations found a wide range of variability among samples with the ED<sub>50</sub> ranging from 0.03 down to 0.00005 ppm. To calculate the ED<sub>50</sub> probit values were regressed against Log<sub>10</sub> of the propiconazole concentration. They suggest that a more meaningful measure of sensitivity is the slope of the regression line.

As a result of the wide range of ED<sub>50</sub> values, the frequency distribution of a range of germ tube inhibition values was plotted using 0.1 and 10.0 ppm of tridemorph. The results were similar using individual measurements from a large population of 1,800 ascospores or from 45 populations (leaf samples) averaging 50 ascospores each. However, results with 14 random samples of 50 ascospores each were more variable. Inhibition levels were much higher at the 46-55% inhibition level. However, at inhibition levels of 16 to 30% and 76 to 85% results were similar for all

three sampling methods. About 7% of the ascospores fell into the 16-30% inhibition level and about 16% of the ascospores in the 76-85% inhibition level. These inhibition levels are the least variable and should be the most indicative of any shifts in sensitivity to tridemorph. At 10.0 ppm of tridemorph inhibition levels were similar for all three sample sizes.

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#### ACCION IN VITRO DEL TRIDEMORFE (CALIXINA) SOBRE MYCOSPHAERELLA FIJIENSIS AGENTE CAUSAL DE LA ENFERMEDAD DE LAS RAYAS NEGRAS.

R.H. STOVER.

Fruits, Mar.-Apr. 1992, vol. 47, n° 2, p. 291-301.

RESUMEN - La germinación de las ascosporas no es afectada cuando las muestras foliares son conservadas durante 28 días a 22-27°C. Contrariamente, ninguna germinación fue observada después de 42 días. Ningún cambio significativo es observado tanto para la germinación como para el crecimiento de los tubos germinativos cuando las muestras se conservan 42 días a 11°C. La Cl 50 (reducción de 50 p. 100 del largo de los tubos germinativos) fue determinada sobre 40 muestras después de la liberación de ascosporas sobre medios conteniendo diferentes concentraciones de tridemorfe ; 0, 0.005, 0.01, 0.05, 0.1, 1.0, 10, 25 50 ppm. La Cl 50 fue evaluada a 0.079 + 0.014 ppm. La mayor parte de los valores se

situan entre 0.05 y 0.1 ppm. A 0.1 ppm la reducción de los tubos germinativos es de 57 p. 100 y de 94 p. 100 a 10 ppm. Una gran variabilidad fue observada para las Cl 50 y un análisis más fino sobre el crecimiento se realizó sobre 1800 ascosporas obtenidas a partir de 31 muestras foliares. 66 p. 100 de las ascosporas presentan una reducción de crecimiento de 31-65 p. 100 a 0.1 ppm. A 10 ppm más de 90 p. 100. Una distribución similar se observó con 45 muestras foliares (50 ascosporas por muestra). Diferentes resultados se obtienen con solamente 14 muestras y siempre 50 ascosporas por muestra. En concentraciones de tridemorfe inferiores a 1.0 ppm, se observan diferencias entre los resultados obtenidos en los diferentes laboratorios. Para evaluar las diferencias de sensibilidad al tridemorfe, se recomienda evaluar la inhibición de crecimiento de los tubos germinativos de un número suficiente de muestras foliares en las concentraciones 0.1 y 10 ppm que, aproximadamente, corresponden a las Cl 50 y Cl 90 de *Mycosphaerella fijiensis*.

