Tropentag 2010
ETH Zurich, September 14 - 16, 2010

Conference on International Research on Food Security, Natural Resource Management and Rural Development

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Fusarium Wilt (*Fusarium oxysporum* f. sp. cubense) in Gros Michel (AAA) bananas, the incidence at smallholder level of Nicaragua.

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Abstract

Research on Fusarium Wilt (Foc) went through two important eras. The "Gros Michel era" who discusses the possible origins and research associated to 'Gros Michel' epidemics in the American tropics. During the "Cavendish era" the research was focused on the diversity of the pathogen and not into the disease (Moore *et al.* 1999). Consequently Fusarium Wilt disease continued to be a problem in dessert bananas produced by smallholders (Buddenhagen 1990). Additionally, the last few decades have been characterized by the emergence of new infectious diseases and re-emergence of old infectious ones (Wilcox and Ellis 2006). Understanding the actual Fusarium Wilt status is important to the detection of free-disease areas and to avoid the entrance of new strains where a limited diversity already exist (Moore *et al.* 1999). The grower perception is a rich knowledge which permit to understand what are been the growers doing why they are doing, and what is required if a new technology is to be accepted by growers (Altieri, 1984), facilitating disease control strategies advocated by experts.

Materials and Methods

The study area included 30 farms equally distributed among two zones of northern Nicaragua (13º0' North, 85 º0' West), zones-1 in Jinotega and zone-2 in San Ramón. The farm selection began with a 200 growers list containing basic farms information, randomly 50 farms of up 15 ha and having banana and coffee growing under trees shade on at least 1 ha were chosen, from those 30 farms were selected by logistic convenience. Randomly each surveyed farm received 2 plots of 625 m^2 (25x25m) where boundaries were marked for better orientation. Quantitative measurement as: banana, coffee and trees density, Gros Michel (race 1 susceptible cultivar) density, number of banana pseudostem per plant, altitude, inclination and diseased plants for occurrence were registered in order to characterize the system. Diseased plants were indexed to incidence and severity. Incidence was determined by the number of Foc diseased Gros Michel plants by the total number of susceptible Gros Michel plants inside a plot (Subramanian *et al* 2006). Severity was graded according external and internal symptoms of yellowing, wilt and corm discoloration based on the International Network for Improvement of Banana and Platain (INIBAP) proposed by Orjeda (1998). Occurrence was determined by the ratio of Foc infected farms by the total number of surveyed farms. Pearson correlation coefficient test was used to identify relationship between the Foc disease incidence and farms measures. One way Anova was used to determine statistical difference among incidence of northern Nicaragua zones.
Grower perception was evaluated through a semi-structured questionnaire performed in each surveyed farm. A total of 26 questions were prepared to get their perception regard the banana and coffee crop importance, disease knowledge and management. Questions were analyzed by frequency of answers and statistical differences verified

by Pearson's Chi-Square Test. Pearson Correlation Coefficient Test was used to find relationships among answers on Foc disease topics and the current disease incidence status, demonstrated by Contingence Table and Correspondence Analyze Graphic. The software InfoStat version 2009e and SPSS 16.0 for windows were used for statistical verifications.

The vascular tissue sampling was carried on a maximum of 3 sub-samples per plot, whereby pseudostem and corm samples were taken separately. After segments sterilization on Sodium Hypochlorite solution at 2.5% (NaCl 2.5%) during 3 minutes, three consecutive rinsed wash in sterile water were performed (Pocasangre *et al.* 2000). Plant segments were placed on Potato Dextrose Agar media (PDA) half strength and incubated by 72 hours at 28ºC to 30ºC prior to purification. Randomly fungus colonies presenting *Fusarium* spp. primary characteristics (Leslie and Summerell 2006) were purified and incubated in PDA full strength during 15 days at 28ºC to 30ºC prior to gender morphological identification. Identified strains were stored in Eppendorf tubes by the filter paper technique for future pathogenicity test to be performed by local Nicaraguan staff.

Results and Discussion

Smallholders total farms size and associated banana and coffee area in northern Nicaragua were 5.5 and 4.2 ha^{-1} respectively, with altitude and inclination of 865 meters above sea level and 15%. Total Plant density was 5,357 plants per hectare, coffee:banana:trees ratio was 90% (4,818 plants/ha), 7% (355 plants/ha) and 3% (184 plants/ha) respectively. 'Gros Michel' represent 86% (305 plants/ha) of the total bananas grown. Foc diseased plants were detected in 7 of 30 surveyed farms, representing an occurrence of 23%. Occurrence in zone-1 was 7 of 15 farms (47%) whereas in zone-2 plants were Foc disease-free. The disease distribution map is presented in Figure 1A&B. Foc disease incidence was 1.12%, with 2.24% in zone-1 (Figure 1C). Foc incidence was not significantly ($p<0.05$) correlated to the field quantitative measures (Table 1A). Zone-1 and -2 differ statistically ($p=0.0073$) concerning Fusarium Wilt incidence (Table 1B). Foc severity at the time of sample collection was index as 2.6 for yellowing and 2.5 for wilt. Pseudostem discoloration was indexed at 1.2 (Figure 1D).

Banana was considered to be most important crop by 10% of the growers with significant difference ($p<0.0001$) whereas 76.7% named coffee as most important (Figure 2A). The reasons why banana is important was the additional income, mentioned by 36.7% of growers, monthly income, stable price and no reason correspond respectively to 3.3%, 6.7% and 53.3% (Figure 2B). Significant difference was found ($p=0.0001$) on banana production and disease control training where only 26.7% of growers has received (Figure 2C). Only 10% of growers stated to know Foc disease when asked (Figure 2D), additionally only 3.3% gave good descriptions and 90% could not describe it (Figure 2E). Control method is not take in place by 93.3% of growers with significant difference ($p<0.0001$), 3.3% cut and chop and 3.3% just grow a new planting material to replace the dead one (Figure 2F).

Answers to the questions: 1) have you received training on banana production and disease management 2) do you control Fusarium Wilt and 3) how do you control Fusarium Wilt disease, were correlated with Foc incidence using Contingence Tables and Correspondence Analyses Graphics. Significant differences were revealed concerning the fact as to whether growers had not been trained in banana production ($p=0.0068$, $r=0.45$), do not control disease ($p<0.0001$, $r=0.65$) and the method of control been 'do not control' ($p<0.0001$, $r=0.78$) as can be observed by the Figure 3.

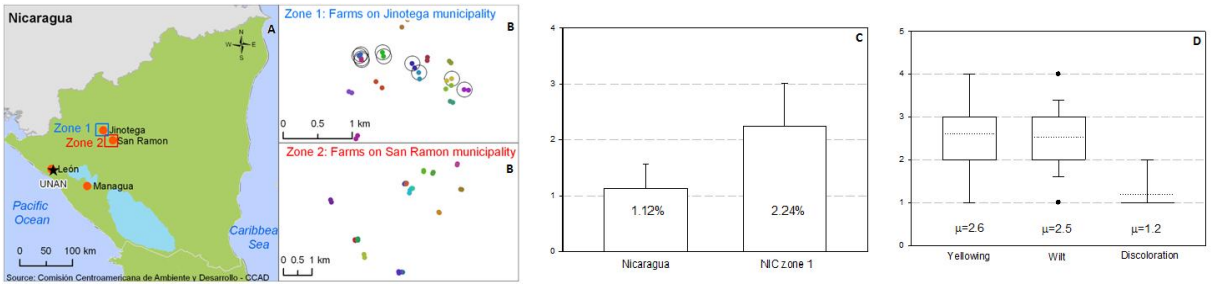

Figure 1: (A) Northern Nicaragua study zones identification. (B) Map up right: Jinotega (zone-1). Map lower right: San Ramón (zone-2). Color dots = farm plots. Black rings = Fusarium Wilt diseased plots. (C) Fusarium Wilt plant incidence in Nicaragua and zone-1. NIC=Nicaragua. Bars indicate Standard Error of the Mean. (D) Fusarium Wilt Severity.

Table 1: (A) Statistical correlation of Foc incidence and field data measures. (B) ANOVA Statistical analysis of northern Nicaragua zones.

A

	Incidence	
	(p)	(r)
Banana Density	0.414	0.155
Coffee Density	0.58	-0.105
Tree Density	0.307	0.193
Gross Michel Density	0.202	0.24
n° banana stems	0.678	0.079
no leguminous trees	0.289	0.2
Altitude	0.07	-0.34
Inclination	0.19	0.25

Pearson correlation (Significance 0.05)

B

Nicaragua Zones	n	Fusarium Wilt Incidence
Zone 1	15	2.24 a
Zone 2	15	0 b

Different letters means significative differences (p<=0.05)

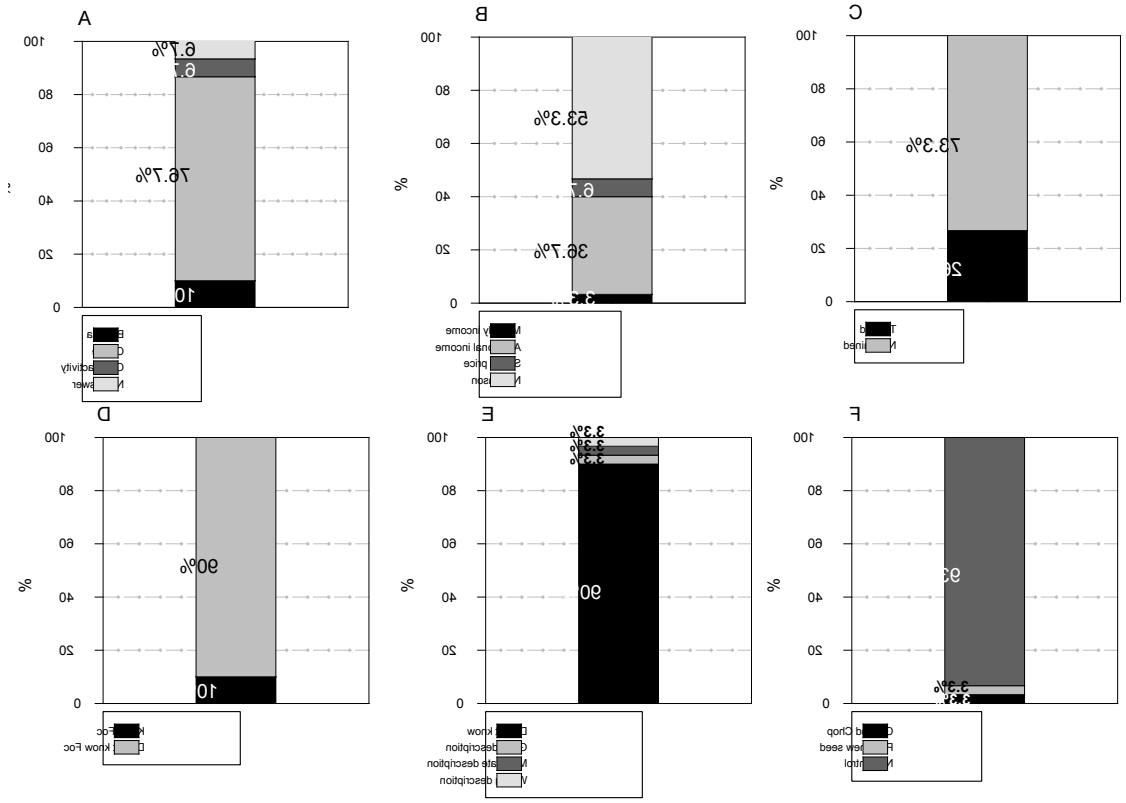

Figure 2: (A) What is the most important crop? (B) What is the banana crop importance? (C) Have you received training in banana production and disease management? (D) Do you know Fusarium Wilt disease? (E) Could you describe the symptoms of Fusarium Wilt disease? (F) What Fusarium Wilt control method is taken in place?

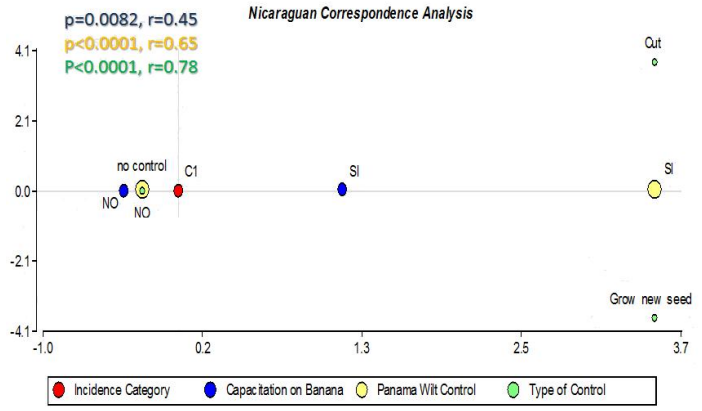

Figure 3: Nicaraguan correspondence analyses of Incidence and training, Control and Control type.

The presence of Foc in banana plants has been extensively reported for Central America (Stover and Simmonds 1987, Ploetz 2005). Nevertheless only a few studies examined Foc incidence in detail for these countries (Pocasangre 2009). In northern Nicaragua Foc disease occurred exclusively in zone-1, whereas zone-2 was disease-free. Cultural aspects might explain the differences. First of all banana is not the main crop and the exchange of planting material is not a common practice between farmers, second, the zone-2 have greater diversity of banana cultivars in fields with geographical isolation from zone-1, facts that limit Foc dissemination. Historically

banana production is relatively new in northern Nicaragua (Aguilar 1995, Soto 2008). In 1935, Foc destroyed a huge amount of susceptible banana in Central America plantations and during the same period banana industries closed their doors for approximately 20 years because of the Sandinist Revolution (Soto 2008). This interruption in expansion of the banana industry certainly affected disease dissemination. In addition, the northern region of NIC is not considered to be a traditional banana production region (Aguilar 1995). There are no reports of past surveys at the study area, however, it has been reported that in the southwest Pacific region Foc disease incidence in 'Bluggoe' (race 2) cultivars averaged 9.9% of sampled plants (Gongora and Narvaes 1994), 2.4% to 6% during the wet season in December 2001, increasing to 8.4% in dry season, February 2002 (Dolmuz *et al.* 2002). In the same southwest region in Rivas Department, 36% of sampled farms were Foc disease positive (Rodriguez *et al.* 2002). Even when considering differences in epidemiology among Foc races the data shows similar incidence values during these years. The level of Foc severity in NIC demonstrated extensive yellowing and wilting for external symptoms and corms completely clean with no vascular tissue discoloration for internal symptoms. These results are only preliminary base line data since it is important to consider that severity varies according inoculum density in soil in a particular region, plant age and period of incubation (Ploetz and Pegg 2000). It seems that the lack in training is related to incidence and would be a menace for Foc dissemination in the region.

Conclusions

(1) In northern Nicaragua Foc is occurring in 23% of sampled farms with 2.24% of incidence in zone-1 where disease was found.
(2) Zone-2 was Foc disease-free and represents an opportunity for the seed production.
(3) There are statistical differences of incidence among zones.
(4) In Nicaragua studied zones, the low rate of banana production and disease control training seems to have a strong effect on Fusarium Wilt incidence and would be a future constrain to 'Gros Michel' banana production if expanded.
(5) 18 isolates were recovered and identified morphologically as *Fusarium* spp. to be tested their pathogenicity in the next future by local Nicaraguan partners.

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