Disease Suppression of Soils From the Valleys of Ecuador to *Fusarium oxysporum*, Causal Agent of Babaco Vascular Wilt

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Abstract

Studies to characterize suppression of *Fusarium oxysporum*, causal agent of babaco vascular wilt, in soils were conducted. Significant diversity of microorganisms was found in soils. Well known microbial antagonists such as *Fusarium* spp, *Trichoderma* spp., *Gliocladium* spp and *Phyalophora* spp were identified. Levels of suppression were high in soil A, in which suppression was due to microbiological, as well as non-microbiological factors. Both types of suppression may be beneficial for biological control of *F. oxysporum* in Ecuador.

Introduction

Carica stipulata (toronche), *Carica pubescens* (chamburo) and their hybrid, *Carica heilbornii* nm. *pentagona* (babaco) are the most promising caricaceae crops of Ecuador (1). *Fusarium oxysporum,* causing Babaco Vascular Wilt (BVW) is the main constraint to production of all these species. In babaco, yield losses caused by F. *oxysporum* can reach up to 100%. Plant resistance and chemical control methods have been developed for this disease by the CRSP-IPM project in previous years. However, use of disease suppressive soils could also be integrated into an IPM program to manage *F. oxysporum.*

In this study, soils from areas where babaco is regularly grown were studied in order to characterize suppression of *F. oxysporum.* Different levels of suppression to *F. oxysporum* have been identified in some soils.

Objectives

1. Identify and quantify the common microorganisms present in *F. oxysporum* suppressive soils from Ecuador.
2. Characterize the nature of suppression in soils.

Research Methods

Seventeen soil samples from babaco production areas with different soil characteristics were selected in order to characterize soil suppression (Table 1). Samples were analyzed at the Departament of Plant Protection of the Santa Catalina Experiment Station (EESC) of INIAP. Three soils with high levels of antagonistic microorganisms were selected to test for *F. oxysporum* suppression in the greenhouse.

In order to identify antagonistic microorganisms, a uniform sample of one gram of soil was suspended in 100 ml of sterile water. Three consecutive 1^{-10} dilutions were then conducted to get a 10^{-10000} dilution. From this soil suspension, 1 ml was placed on Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA). PDA was used to isolate most fungi, especially deuterimicetes, and CMA was used primarily to isolate oomycetes. Similarly one ml of soil suspension was also plated on BK (Kings B) medium to isolate *Pseudomonas* spp. Levels of antagonism to *F. oxysporum* were studied in three soils with high concentrations of microorganisms (Table 2).

Chlamydospores were produced from a single spore isolate of *F. oxysporum* according to Larkin, et al. Ten, 5 mm circular plugs of a 5-day-old *F. oxysporum* culture were transferred to 100 ml of Potato Dextrose liquid medium. After seven days, mycelium were blended for 1 minute and then the suspension inoculated to a sterilized sandy soil. Soil was stored at room temperature for two weeks. The concentration of chlamydospores was then calculated by plating soil onto PDA (one ml of a 1^{-1000} suspension of soil containing chlamydospores). Subsequently, the soil was mixed with the test soils to obtain 50000 chlamydospores per gram of soil. Inoculated soils were then incubated at field capacity for 30 days. Ten, 35-day old chamburo plants were transplanted in 200 gm of each soil treatment (sterilized and non-sterilized soil). In addition ten plants were transplanted to non-inoculated sterile and non-sterile soils (checks). Treatments were established in a completely randomized block design. Disease incidence and incubation period to the wilting stage were recorded. The wilting stage was considered chlorosis reaching the top of the plant. Rate of plant growth was also evaluated. Growth rate was calculated by subtracting plant height at the transplanting time (3 cm) from plant height at 30 days after inoculation.

Results and Discussion

In Table 1, microorganisms isolated from the soils are shown. Eighteen fungi in addition to *Pseudomonas* spp were isolated from soils. Different species of *Fusarium* spp., *Trichoderma* spp., *Gliocladium* spp., *Phyalophora* spp. were very frequent in soil samples. *Verticillium* spp., *Pythium* spp. and *Rhizoctonia* were also isolated at various frequencies.

Some soils such as numbers 6, 7 and 13 contained a considerable number of microorganisms.

In Table 2, the suppressive responses of three soils with significant levels of microorganisms are shown. Disease incidence was significantly reduced in soil B (40%) compared with soil A (20%) and soil C (13.3%). Similarly, disease incubation period was significantly higher in the non-sterilized soil B (48.67 days) compared to non-sterilized soils A (39.0 days), and C (40.3 days). Differences in incubation period between non-sterile and sterile soils for soils A, B and C were 10.6, 12.0 and 12.3 days, respectively, which suggest that biological antagonism in these three soils is similar. Differences in incubation period between soil B with soil A and C are apparently due to non-biological antagonism. Incubation period in sterile soils A and C were similar, 28.3 days and 28.0, days respectively, compared with an incubation period of 34.6 days for the sterile soil C. The contribution of non-biological antagonism in soil B was 14 days of incubation period compared to 6.6 days and 6.3 days for soil C and A, respectively.

The growing rates in non-sterile and non-inoculated soils A, B and C were similar at 5.0 cm., 6.4 cm. and 6.6 cm., respectively. These growing rates can be attributed to soil agronomic potential. Effect of sterilization on growth rate was high in soil A (8.4 cm.) compared to the non-sterile soil (5.0 cm.). In the other two soils, the effect of soil sterilization was not as high, but was present (Table 2). Pathogen inoculations slightly increased growth rate in sterile, as well as non-sterile soils except in soil A, in which inoculations considerably increased growth rate in non-sterile soils and considerably decreased growth rate in sterile soils. Inoculation caused an apparent increase in growth rate. In the case of soil A, soil fertility appears to be an important factor in the growth rate.

Considerable diversity of microorganisms was found in soils from the valleys of Ecuador. Many microorganisms may contribute to disease suppression in these soils. The fungi *Trichoderma* spp., *Gliocladium* spp. and *Fusarium* spp., and the bacteria *Pseudomonas* spp are considered to be good antagonists and were found consistently in many soils. Other fungi such as *Verticillium* spp., *Fusarium* spp. *Rhizoctonia* spp. and *Pythium* spp were also found in these soils.

In soils A and C, certain levels of disease suppression were observed. However, in soil B, levels of suppression were considerably higher. Biological suppression was similar in all three soils. High levels of non-biological antagonism in soil B might be explained by an alkaline pH, which is an important suppressive factor for *F. oxysporum.*

Growth of chamburo plants appears to be promoted by colonization of *F. oxysporum*. This might be due to plant response to *F. oxysporum* infection or to production of growth promoters during the infection process. Interactions between soil fertility and pathogen infection may also explain plant growth in infected plants, especially in soil A.

Natural suppression of *F. oxysporum* appears to be significant in some soils. Disease suppression could be maximized by the application of similar biological antagonists or by applying cultural practices to improve non-biological antagonism. Exploitation of both biological and non-biological antagonism may be possible.

Although, the use of suppressive soils alone is not the solution to management of BVW, it can contribute to BVW management by reducing disease incidence in babaco, chamburo and toronche orchards.

Impacts

The babaco vascular wilt is an important constraint to babaco, chamburo and toronche cultivation in Ecuador. Although chemical control and disease resistance developed in previous years in this project can be used to manage babaco vascular wilt, understanding the biological and non-biological nature of suppressive soils could help considerably in the management of *F. oxysporum* by reducing disease incidence and delaying development of BVW in babaco orchards, chamburo and toronche. These principles could also be applied to other *Fusarium* vascular wilt diseases, such as naranjilla vascular wilt.

Networking Activities

This study was conducted by a student of the Faculty of Agronomy of the Central University-Quito. Results have allowed us to establish a procedure to characterize antagonism in Ecuadorian soils. At present another student from the same university is characterizing other soils from Ecuador.

Training Output

The study was conducted in collaboration with the Agronomy faculty at the Central University. Students and teachers at this University have increased research capabilities and have directly benefited from the results of this research. In addition, results were also presented at a conference of the National symposium of Plant Protection held in Babahoyo-Ecuador.

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Table 1. Soil origin and population of microorganisms of 17 soils from the valleys of Ecuador.

Origin of soils	Microorganism populations																	
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**
1 Pastaza-Mera-Mera-Puyuyacu	20000	20000	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	140000
2 Pastaza-Mera-Mera-Puyuyacu.	20000	---	26667	30000	23333	---	---	---	---	---	---	---	---	---	---	---	---	---	141000
3 Pichincha-Quito-Tumbaco-CADET	10000	---	---	---	---	10000	---	---	---	---	---	---	---	---	---	---	---	---	64000
4 Pichincha-Quito-Tumbaco-CADET	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	82500
5 Pichincha-Quito-Tumbaco-Granja INIAP	---	---	---	---	---	---	19500	---	---	---	---	---	---	---	---	---	---	---	238888
6 Pichincha-Quito-Tumbaco-CADET	---	---	10000	23333	25000	---	20000	10000	---	---	---	---	---	---	---	---	---	---	98000
7 Pichincha-Quito-Tumbaco-Granja INIAP	20000	---	---	---	---	30000	40000	16667	---	---	---	---	---	---	---	---	---	---	173000
8 Pichincha-Mejía-Cutuglahua-INIAP	---	---	10000	---	---	---	---	31667	10000	10000	20000	10000	---	---	---	---	---	---	111000
9 Pichincha-Pedro Moncayo-Tabacundo-Finca	---	---	---	---	10000	---	---	20000	---	10000	---	---	---	---	---	---	---	---	162500
10 Pichincha-Pedro Moncayo Tabacundo-Finca	---	---	---	---	10000	---	---	---	---	---	---	---	---	---	---	---	---	---	75000
11 Pichincha-Mejía-Cutuglahua Lote raíces y tubérculos.	---	----	---	---	23333	---	---	---	---	---	---	---	---	---	---	---	---	---	177000
12 Tungurahua-Baños-Comun. Cacería-Sta Rosa de Runtum.	23333	---	---	---	16667	---	---	---	---	---	---	---	---	---	---	---	---	---	244500
13 Tungurahua-Baños-Comun. Cacería-Sta Rosa de Runtum.	30000	---	---	---	10000	---	---	10000	---	---	---	---	10000	---	---	---	---	---	130000
14 Tungurahua-Baños-Patate-Patate.	---	---	10000	---	10000	---	---	---	---	---	10000	---	---	10000	---	---	---	---	146500
15 Tungurahua-Baños-Comun. Cacería-Sta Rosa de Runtum.	---	---	---	40000	---	---	---	---	---	---	---	---	---	---	23333	---	---	---	209500
16 Pichincha-Mejía-Cutuglahua-CIP	---	---	---	---	10000	13333	---	9000	---	---	10000	---	---	---	---	---	---	---	114500
17 Carchi-Bolívar-San Vicente de Pusir	---	---	---	---	10000	10000	---	10000	---	---	---	---	---	---	---	10000	10000	10000	198000

A= *Trichoderma* sp. E= *Fusarium* sp. I= *Rhizoctonia* sp. M= *Cephalosporium* sp. Q=*Gliocephalis* sp.
B= *Geotrichum* sp. F= *Gliocladium* sp. J= *Gliomastix* sp. N= *Hendersonula* sp. R= *Streptomyces* sp.
C= *Phialophora* sp. G= *Pythium* sp. K= *Paecilomyces* sp. O= *Aureobasidium* sp. S= *Pseudomonas* sp.
D= *Verticillium* sp. H= *Cladosporium* sp. L= *Cunninghamella* sp. P= *Pithomyces* sp.

Table 2. Disease incidence and incubation period of babaco vascular wilt, and growth rate of chamburo in three soils suppressive to *Fusarium oxysporum* in Ecuador.

Soils/biological Condition	Disease Incidence (DI)		Incubation Period (IP)		Growing Rate (cm) (GR)	
	Inoculated*	check	Inoculated*	check	Inoculated	check
A						
Sterile	100	0	28.3	0	5.7	8.4
Non sterile	80.0	0	39.0 b	0	7.2	5.0
B						
Sterile	100	0	34.6	0	8.0	7.9
Non sterile	60.0	0	48.6 a	0	6.9	6.4
C						
Sterile	100	0	28.0	0	7.3	7.2
Non sterile	86.6	0	40.3 b	0	7.3	6.6

A: Central University Experimental Station. Tumbaco-Pichincha

B: Tumabaco Experimental station INIAP. Tumbaco-Pichincha
Sandy soil, Ph 7.9, Organic matter 3.10 %

C: Runtum-Baños-Tungurahua.
Sandy soil, Ph 5.4, Organic mater 5.3 %.

* Means were statistically different between sterile and non-sterile soils (T tests). Mean differences were calculated using the Duncan test at 0.5% confidence interval.