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Award Number: 2014-70004-22033

Dr. Gladys González Martínez, P.I.
Professor
Department of Agricultural Economics and Rural Sociology

Dr. Ángel González, Co P.I.
Professor
Department of Crops and Environmental Sciences

Dr Esbál Jiménez Cabán, Co P.I.
Associate Professor
Department of Animal Science

May, 2016

Resident Instruction Grant Program for Institutions of Higher Education in Insular Areas
Undergraduate Research Experience 2015-2016
Participating Students and Faculty

Student	Mentor	Area of Expertise
Camila V. Elías Arroyo camila.elias@upr.edu	Alexandra Gregory alexandra.gregory@upr.edu	Agricultural Economics
Austin Hullquist Jayne austin.hullquist@upr.edu	Luis E. Solórzano luis.solorzano1@upr.edu	Ruminant Nutrition
Marangely Santiago Sanabria marangely.santiago1@upr.edu	Jaime E. Curbelo jaimee.curbelo@upr.edu	Dairy Cattle Mastitis
Pedro Olivencia Morell pedro.olivencia1@upr.edu	Paul Randel Folling paul.randel@upr.edu	Animal Nutrition
Robert Ryan Torres robert.ryan@upr.edu	Leyda Ponce de León leyda.ponce@upr.edu	Dairy Products
Valerie Morales Coll valerie.morales3@upr.edu	Abner Rodríguez Carías abner.rodriguez3@upr.edu	Ruminant Nutrition Forage Conservation
Santiago Acosta González santiago.acosta@upr.edu	María Libran Salas maria.libran@upr.edu	Horticulture
Rey Cotto Rivera rey.cotto@upr.edu	Lydia Rivera lydiai.rivera@upr.edu	Tropical Plant Phytopathology/Mycology
Stephanie M. Plaza Torres stephanie.plaza2@upr.edu	Dra. Merari Feliciano Rivera merari.feliciano@upr.edu	Phytopathology
Llelenys Sanoguet Crespo llelenys.sanoguet@upr.edu	Pablo Morales Payan morales.payan@upr.edu	Organic Agriculture
Noelymar González Maldonado noelymar.gonzalez@upr.edu	David Sotomayor david.sotomayor@upr.edu	Soil and Water Quality Soil Fertility

Abner A. Rodríguez Carías, Ph.D.
Professor
Department of Animal Science
Project Coordinator

Orisnela M. Solano Pelaez, M.S.
Graduate Student
Department of Agricultural Education
Project Coordinator

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AVAILABILITY OF SHEEP AND GOAT MEAT AT FARMERS' MARKETS IN PUERTO RICO

Camila V. Elías Arroyo and Alexandra Gregory
Department of Animal Science and Agricultural Economics and Rural Sociology

Abstract

Import and production data for small ruminant meat, goat and sheep meat (G&SM), can indicate that there is an opportunity for local producers in Puerto Rico to increase production. This research studied the supply of the small ruminants' meat at Farmers' Markets with Meat Markets (FMwMM) in PR and determined the availability of G&SM. In addition, we determined if there was a statistical difference between meat markets that supply G&SM and those who do not versus operator's characteristics and type of operation by using a chi square test. A questionnaire was designed to gather information about availability of small ruminant meat at the FMwMM that included the following: country of origin of the meat sold, sales price, value of sales comparison between beef, goat, and sheep sales, type of small ruminant meat, among other variables. Twenty, 20, participants from the FMwMM were interviewed with the instrument and results showed that: 70% offered small ruminant meat of which 50% offered both G&SM, 43% only offered goat meat, 7% percent only offered sheep meat, the average sales price was $4.80 for goat meat and $4.75 for sheep meat. The majority indicated that they sell locally produced sheep and goat meat. FMwMM sold more kid and lamb meat, 53.8% and 62.5% respectively. These results might indicate that consumers might have some preference for kid meat over lamb.

Keywords: Availability of sheep and goat meat, consumer preference for sheep and goat meat, small ruminant meat production, chi square test

Resumen

Según los datos de importación y producción de carne de pequeños rumiantes existe una oportunidad para los productores en Puerto Rico a aumente la producción. En esta investigación se estudió la oferta y disponibilidad de carne de pequeños rumiantes en las carnicerías de las típicas plazas del mercado de Puerto Rico. Además, se estudió si había una relación estadística entre disponibilidad de carne de pequeños rumiantes y país de origen con educación y edad de los operadores, utilizando la prueba de chi cuadrado. Se diseñó un cuestionario para obtener información de la disponibilidad de la carne de pequeños rumiantes en las plazas del mercado con la siguiente información: origen de la carne, precio, comparación entre las ventas de res, cabro y ovejo, tipo de carne, entre otros. Se entrevistó en 20 carnicerías en las plazas del mercado utilizando el instrumento y se encontró que: 70% ofrecían carne de pequeños rumiantes del cual 50% ofrecía ambas carnes, 43% sólo ofrecía cabro y 7% solo ofrecía carne de ovejo, el precio promedio fue de $4.80 para cabro y $4.75 para ovejo. Las carnicerías de las plazas del mercado vendieron más cabrito y cordero, 53.8% y 62.5% respectivamente. La mayoría indicó que venden carne de pequeños rumiantes producida en el país. Estos resultados probablemente indican que los consumidores pueden tener preferencia por carne de cabro sobre la de oveja.

Palabras Claves: Disponibilidad de carne de cabro y ovejo, preferencias de los consumidores de carnes de cabros y ovejos, producción de carne de pequeños rumiantes, prueba de chi cuadrado

Introduction

The industry of animal husbandry in Puerto Rico which includes poultry, beef, pork, rabbits, sheep and goat meat has great economic importance. In 2013/2014, animal products contributed $400.1 million to the Gross Farm Income (GFI) (Agricultural Statistics Office, Department of Agriculture in Puerto Rico, 2013/2014). GFI for small ruminants is a very small when compared to beef, GFI for beef in 2014 was $29.2 million while small ruminant was $462 thousand. There has been a slow but steady rise for the demand for sheep and goat meat (Planning Board of Puerto Rico, 2015). In May 2015, Puerto Rico imported 130,900 pounds with a sales value of $330,917 at the port (Planning Board of Puerto Rico, 2015). Almost all of the small ruminant meat consumed here in the island is imported. Although demand is rising local goats and sheep producers in Puerto Rico do not have a high market share (González, E. E. et al., 2014). The Agricultural Census in 2012 reports that number of farms was 425 for sheep and 470 for goats (USDA-NASS, 2012). In 2012 there were 62 municipalities, which are more than half of the country, had sheep farms and 57 municipalities had goat farms. The GFI reports that Puerto Rico (PR) produced 147,000 pounds of small ruminant meat for the year 2014. If we compare what we produce locally in a year with what we import in a month, we can notice that what we produce in a year is more or less than what we import in a month. It's important that producers know if small ruminant meat is being paid at a premium price by consumers, so that they can make the necessary adjustments to increase production.

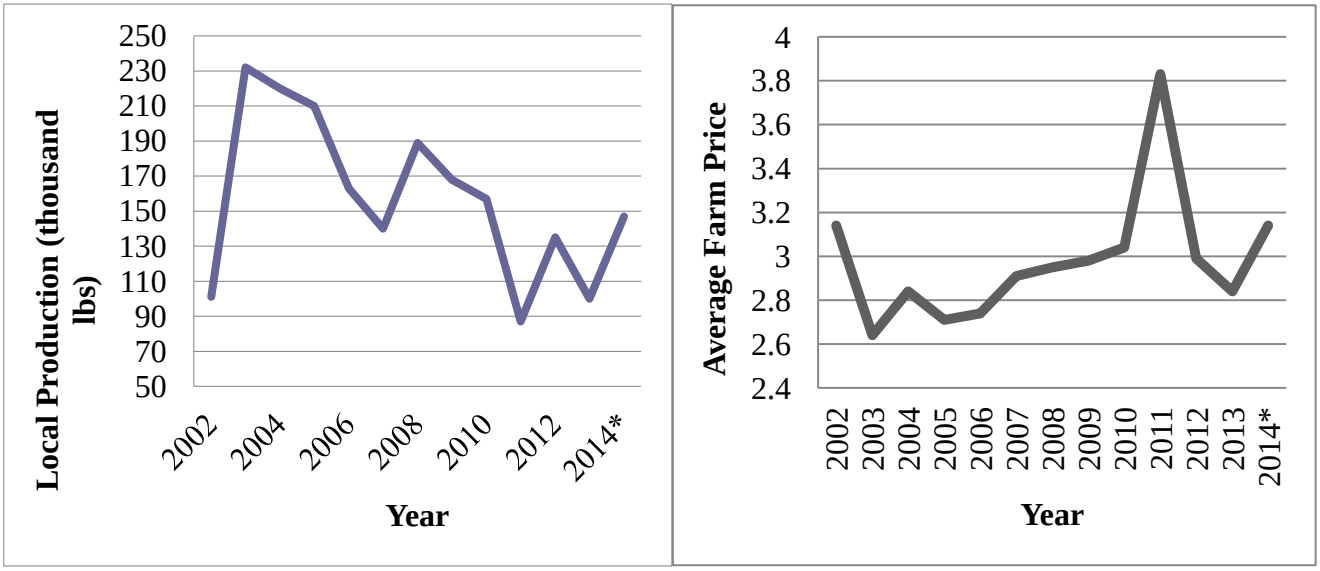
The Census of Agriculture provides us an insight on PR's inventory and value of sales for small ruminant meat in 2002, 2007, and 2012 (Table 1). Statistics shows that for both goat and sheep the number of farms and inventories are declining, with the exception of sheep inventory in 2012. We can notice that the value of sales of sheep is higher than the value of goat sales. One reason for sheep been preferred over goat is because lamb meat has a more pinkish color and is tenderer than goat meat (Curbelo, 2008). One possible explanation for this is that goat meat is usually a darker color, so consumers can be more attracted to lamb meat because of its appearance when being bought. One can appreciate that value of sales for both, goat and sheep, have increased.

Table 1: Livestock, Poultry, and Their Products – Inventory and Sales

Commodity			**2002**	**2007**	**2012**
Inventory	Sheep	Farms	641	568	425
		Number of Animals	19,749	11,137	12,539
	Goats	Farms	541	664	470
		Number of Animals	8,110	7,359	5,655
Sales	Sheep	Farms	309	220	276
		Number of Animals	7,742	4,934	7,519
		Value (dollars)	453,413	(NA)	645,730
	Goats	Farms	208	221	131
		Number of Animals	2,614	3,126	1,579
		Value (dollars)	226,690	(NA)	287,550

Source: Census of Agriculture

Figure 1, Panel a shows local production of small ruminant meat from years 2002-2014. We can appreciate that in 2003 production was at its maximum and after that year shows a downward trend, with a slight increase from 2013 to 2014. Figure 1b presents average price for small runiant meat in Puerto Rico, were in 2011 it reached its maximum. In terms of the Average Farm Price (AFP), 2011 has the maximum price reaching to $3.83 per pound which explains the highest GFI for that year.



Panel a Panel b

Figure 1: Production of Small Ruminant Meat (Panel a) and Average Price (Panel b) in Puerto Rico
Source: Office of Agricultural Statistics

The Planning Board of Puerto Rico (2014-2015) provides us with monthly data on the import of goat and sheep meat (Figure 2, Panel a). In Puerto Rico, goat and sheep meat are mostly imported. Small ruminant meat mostly comes from United States, but the second country of origin with the highest imports is Australia. The import value of goat and sheep meat was on its peak on August 2014, but it shows a downward trend since then. The total imports value from June 2014 to May 2015 was 3.2 million dollars. In Figure 2, Panel b we can notice that the average price was relatively high on November 2014, but it has started decreasing since that date. Production of small ruminant meat was high in August 2014 which complements the value on the same date, but it also decreased afterwards (Figure 3).

Panel a Panel b

Figure 2: Goat and Sheep Imports and Average Price in Puerto Rico, June 2014-May 2015
Source: The Planning Board of Puerto Rico, External Trade Data

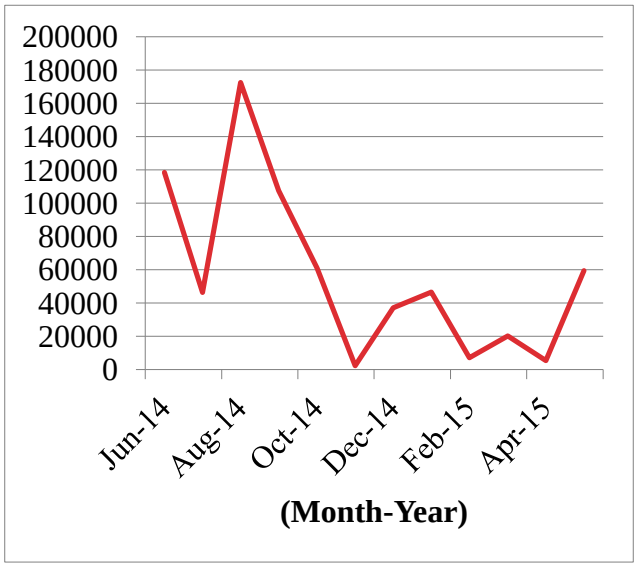


Figure 3: Production of Goat and Sheep Meat
Source: The Planning Board of Puerto Rico, External Trade Data

Table 2 presents the municipalities that have more than 20 farms of sheep and goats in 2012 (USDA NASS, 2012). Data shows that Añasco, Cabo Rojo, Coamo, Lajas, and Orocovis are the municipalities with more sheep and goat farms, were Lajas is the municipality with more farms: 115 farms of sheep and 65 farms of goats. These five municipalities make up a 42.6% of sheep farms and a 32.8% of goat farms in all of Puerto Rico in 2012.

Table 2: Location of Farms in Puerto Rico

Municipalities with more than 20 farms	Number of Farms 2012	
	Sheep	**Goats**
Añasco	24	-
Cabo Rojo	42	42
Coamo	-	22
Lajas	**115**	**65**
Orocovis	-	25
Total	**181**	**154**
Puerto Rico	**425**	**470**
Percentage	**42.6%**	**32.8%**
Number of Municipalities with Farms	**62**	**57**

Source: USDA-NASS 2012

In 2009, a census about sellers of goat, sheep, and rabbit meats in the farmers' markets of Puerto Rico was made (Cortés & Gayol, 2009). According to the Department of Agriculture of Puerto Rico, GFI for small ruminant meats hold a positive economic impact. This Census included information for 25 butcheries in 15 municipalities of PR about sales, perceptions of the meats, disposition to have variety in quantity to buy, positive and negative factors, among other information. Results showed that 59% of the participants sold only goat meat produced in Puerto Rico, while 86% sold only sheep meat. They also found that 68% and 43% were willing to pay more for goat and sheep meat from Puerto Rico respectively. This clearly tells us that the majority of small ruminant meat sold in Farmers' Markets of Puerto Rico for the year 2009 was locally produced while the rest of the meat comes from Australia and Central America (Cortés & Gayol, 2009). In another study, regarding meat availability of goat and sheep meat at grocery stores around PR between 2007 and 2010 (González, E. E. et al., 2014). They found that for that period more than 79% of the grocery stores offered goat and sheep meat, and that in 2010, 47% of the grocery stores in San Juan Region had available goat and sheep meat.

Goat meat production in the United States has increased due to growing populations of different ethnicities that consume this type of meat (Solaiman, 2007). This study also indicates that the top 3 producers of goats are: China, India and Pakistan but Australia and New Zealand are two major exporters which are not within the major producers. Something really relevant for this study is that Hispanics are among the largest populations of ethnicities that eat goat meat in the US (Solaiman, 2007). Given that the population of Hispanics in the US increased by 57.94% in 2000 compared to 1990, represents an opportunity for goat producers in Puerto Rico to star exporting the meat. In addition, consumption had increased in the US mostly because of Muslim population and celebration of their holidays (Solaiman, 2007).

Sheep production, specifically lamb, in the US is declining, but imports are increasing (Jones, 2004). Lamb Meat demand is highest in Easter/Passover (Jones, 2004). Compared to other meats, lamb is not eaten much due to cultural differences of consumers in the US. Wool production is affected by prices, which also affects lamb and mutton supply for meat. High prices of wool will give a lower supply of lamb and mutton. This also represents an opportunity for local producers to start exporting lamb given that there is no formal market for wool.

The purpose of this study is to provide a descriptive analysis of the availability of small ruminant meat at farmers' markets in PR. Puerto Rico's traditional farmers' markets are declining or they are being replaced by other types of farmers' markets such as urban markets or organic markets. In this study we define a traditional farmers' market as a typical market usually in a closed building open every day or almost every day of the week, that is located in the middle of downtown in which local or imported products are sold and sellers are not necessarily farmers. This study excludes family markets, urban markets, and road stands. Our theory is that consumers that buy from these farmers' markets are people of old age with low income given that the majority of the people prefer to buy everything at grocery stores for the convenience in hours, thus we do not expect farmers' markets to sell their products to people in the work force. The objectives of this study are to study the availability and type of sheep and goat meat in the farmers' markets in Puerto Rico, to know the quantity of the small ruminant meat being sold at the butcheries of the farmers' markets, identify if the small ruminant meat is imported or locally produced, determine the price of sheep and goat meat in the farmers' markets in Puerto Rico, and consider the demographic aspects of the operators of the butcheries that sell goat or sheep meat.

Methodology

In this project we performed an assessment to identify the farmers markets that are within our definition. After identifying the farmers markets with butcheries the operator was surveyed. An instrument was designed to gather information about availability of small ruminant meat, country of origin of these meats, sales price, quantity sold, meat cuts, knowledge about different meat cuts, type of consumers, value of sales comparison between beef, goat, and sheep sales, seasonality, social and demographic characteristics, among others. A pre-test to the questionnaire was made at two grocery stores to see if the questionnaire was coherent and fluent.

A χ^2 test was performed to study if there is some statistical relationship between the availability of sheep and goat meat and country of origin with the age of the operator, and education of the operator. The χ^2 test formula (Sullivan, 2012) is as follows:

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

H_0 : The row and column variables are independent

H_A : The row and column variables are dependent

Where: O_i = represents the observed number of counts in the *i*th cell
E_i = represents the expected number of counts in the *i*th cell

With an alpha (α) of 0.05 significance and (r-1) (c-1) degrees of freedom, where c is the number of columns and r is the number of rows.

Results and Discussion

We identified twenty butcheries from fourteen different farmers' markets that fit into our definition which all of them were surveyed. We collected data between June 2015 to February 2016 and we used Stata software package to analyze results and perform statistical tests. Figure 1

presents the results from the assessment we performed to find the farmers' markets that were within our definition. The municipalities painted in green represent the farmers' market with no butchery and yellow represents no farmers' market at all. The areas in red and both shades of blue presentas the municipalities that we included in our study.

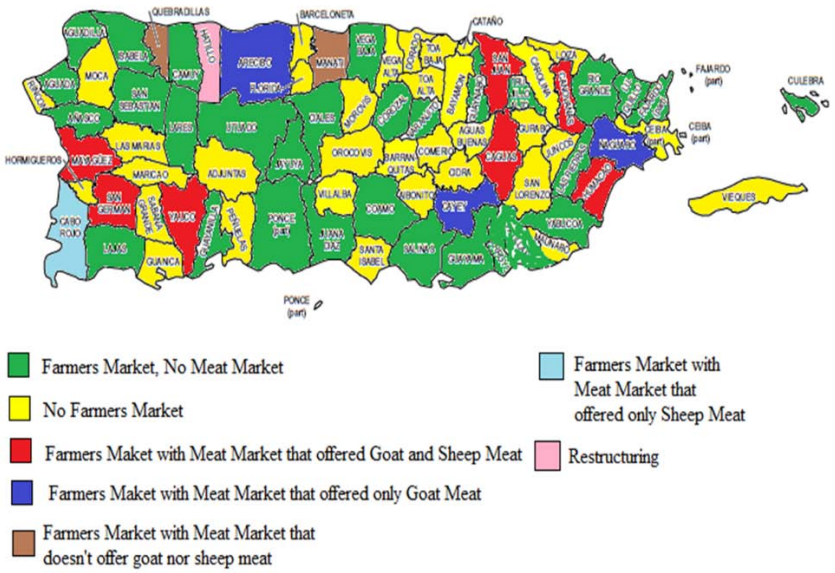


Figure 1: Map of Puerto Rico with information about farmers' markets

Twenty butcheries were surveyed in this study. Table 1 show us what type of meat is being sold at those butcheries. Most of the meats sold at the butcheries are beef, calf, pig and poultry. Of the twenty butcheries, fourteen, 70%, sold small ruminant meat, Figure 2.

Table 1: Farmers' Market Information with Meat Market

Town	Number of Butcheries	Type of Meat Sold					
		Beef	Calf	Pig	Poultry	Fish/Seafood	Other
Canóvanas	1	✓	✓	✓	✓	✓	
Mayagüez		✓	✓	✓			rabbit
Mayagüez	4	✓	✓	✓	✓		
Mayagüez		✓	✓	✓			
Mayagüez		✓	✓	✓			rabbit
Río Piedras		✓	✓	✓	✓		
Río Piedras	4	✓	✓	✓	✓		
Río Piedras		✓	✓	✓	✓		
Río Piedras		✓	✓	✓	✓		
Naguabo	1	✓	✓	✓	✓	✓	
Santurce	2	✓					
Cabo Rojo	1	✓	✓	✓		✓	
Manatí	1	✓	✓	✓	✓	✓	
Cayey	1	✓	✓	✓	✓	✓	

Town	Number of Butcheries	Type of Meat Sold					
		Beef	Calf	Pig	Poultry	Fish/Seafood	Other
Caguas	1	✓	✓	✓	✓	✓	
Yauco	1	✓	✓	✓	✓		
San Germán	1	✓	✓	✓	✓		rabbit; guinea hen
Quebradillas	1	✓	✓	✓	✓		
Humacao	1	✓	✓	✓	✓	✓	rabbit; guinea hen
Arecibo	1	✓	✓	✓	✓	✓	

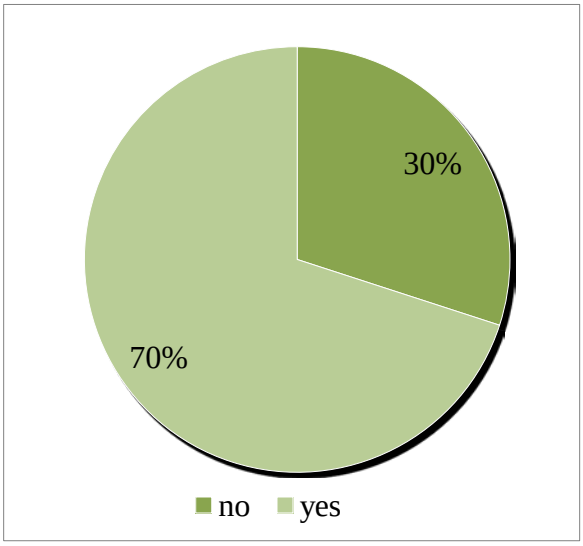


Figure 2: Butcheries that sell Small Ruminant Meat (Goat and Sheep)

Seven butcheries sold both small ruminant meats, six butcheries sold only goat meat and one sold only sheep meat (Figure 3). The small ruminant meats mostly sold in these butcheries were kid and lamb (Figures 4 & 5). Kid and lamb are the young meats, kid being the young meat of goat and lamb being the young meat of sheep. Table 2 shows us the origin of the meat, and Table 3 specifies from which country the meat is from. The majority of the small ruminant meats being offered at the butcheries are locally produced. The small portion of the meat that is being imported is from USA or Australia (Table 3).

Table 2: Meat Origin

	Local	Imported	Both
Goat	8	1	4
Sheep	7	0	1

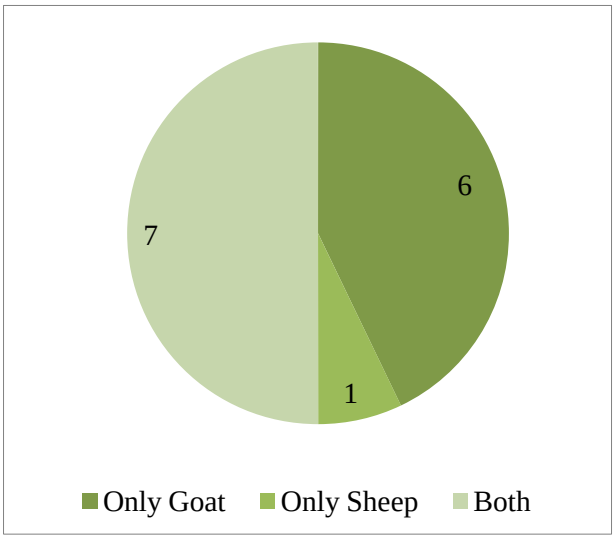


Figure 3: Number of Butcher shops that Supply Small Ruminant Meat

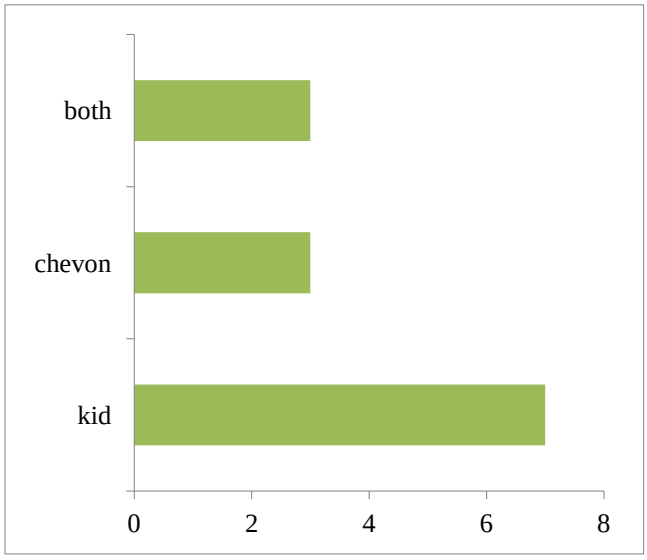


Figure 4: Type of Goat Meat

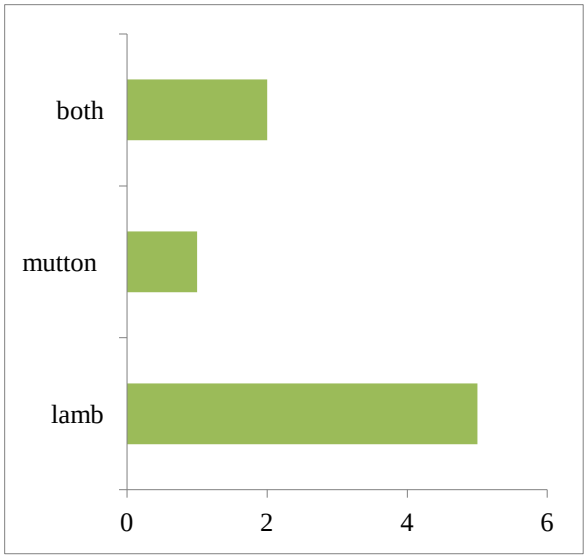


Figure 5: Type of Sheep Meat

Table 3: Meat Origin in terms of the country

	Goat	**Sheep**
Australia	3	0
USA	1	0
PR	10	6
doesn't know	1	1
everywhere	1	1

The average age of experienced years for goat meat of the operator was 38 years and 28 years for sheep. Various meat markets were family businesses passed down from generation to generation, so they have a long time working at the market. Figure 6 shows the average price per pound of

goat and sheep meat and their respective maximum and minimum prices. The average price for goat was $4.80 for goat and $4.75 for sheep. Figure 7 shows the different meat cuts being offered at the butcheries. The meat cut mostly being offered is fricassee, which are cubed pieces used for stewing.

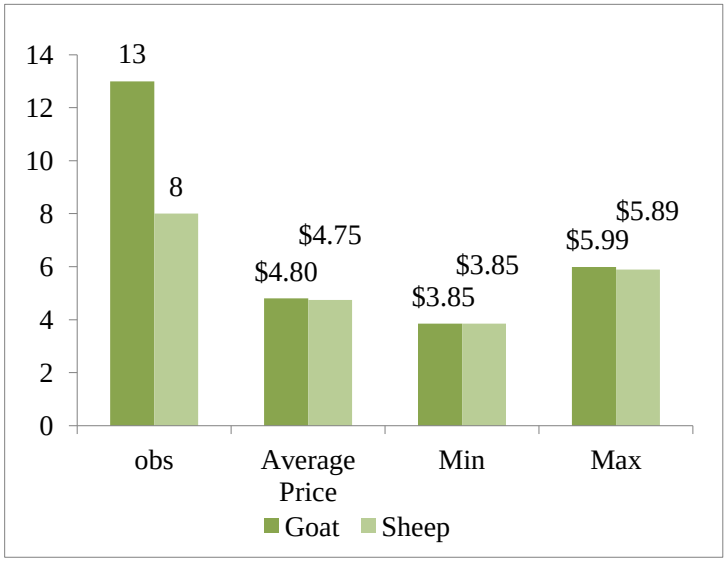


Figure 6: Summary Statistics of Sales Price

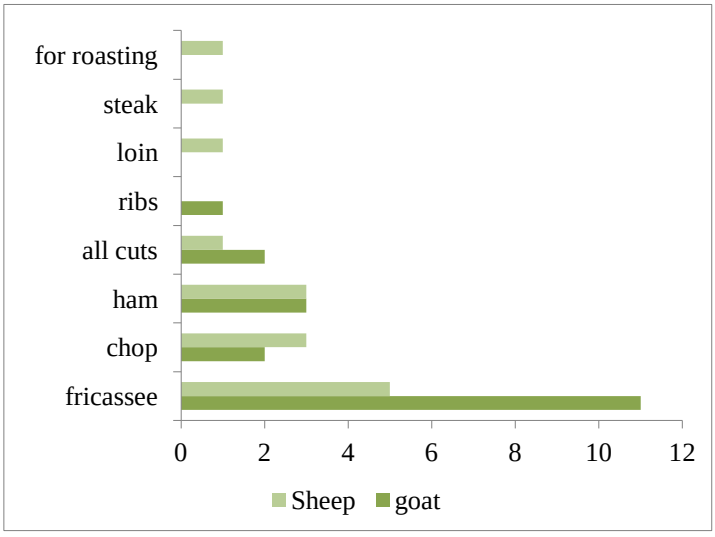
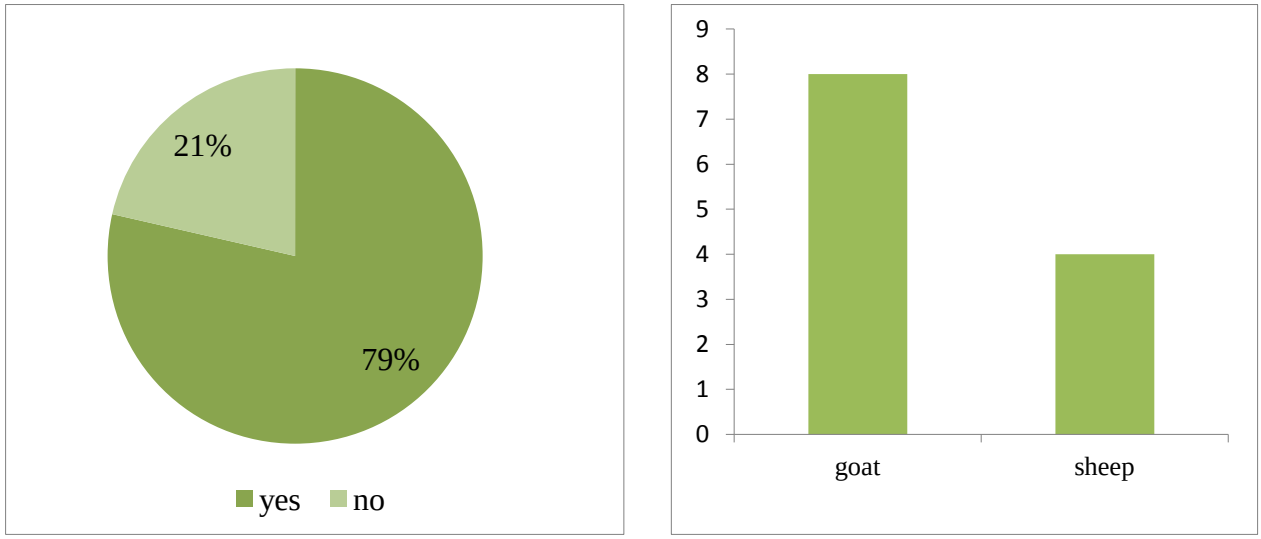


Figure 7: Types of Meat Cuts

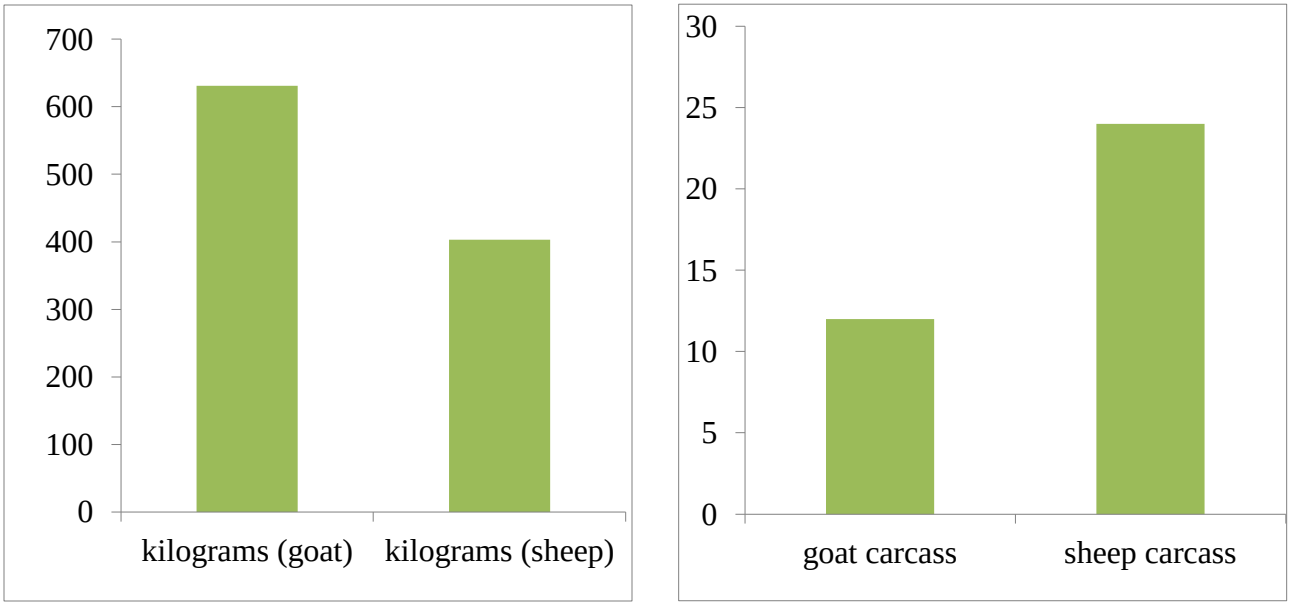
Knowledge about different meat cuts was asked to operators, and the majority did know about other meat cuts that weren't fricassee (figure 8, Panel a). Figure 8, Panel b presents the number of operators that sells meat to restaurants. Also, small ruminant meat is being sold in fewer quantities than beef meat when being compared. These butcheries sell round 1,034 kilograms of goat and sheep meat, and 12 carcasses of goat per year and 24 carcasses of sheep per year (figure 9a & b). The seasons from the butchers' perspective in which they sell more of this meat is in Mother's and Father's Day (Figure 10). Prices between beef and goat/sheep meat were compared

(Figure 11, Panel a and b). Results show that beef meat has a lower price than sheep and goat meat.



Panel a Panel b

Figure 8a: Operators' Knowledge of Different Meat Cuts (Panel a) and Operators sell to Restaurants (Panel b)



Panel a Panel b

Figure 9: Quantity Sold Monthly

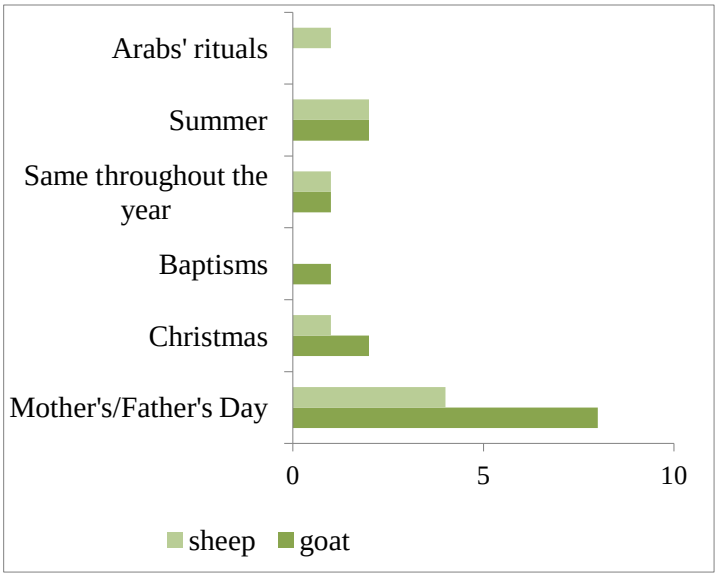
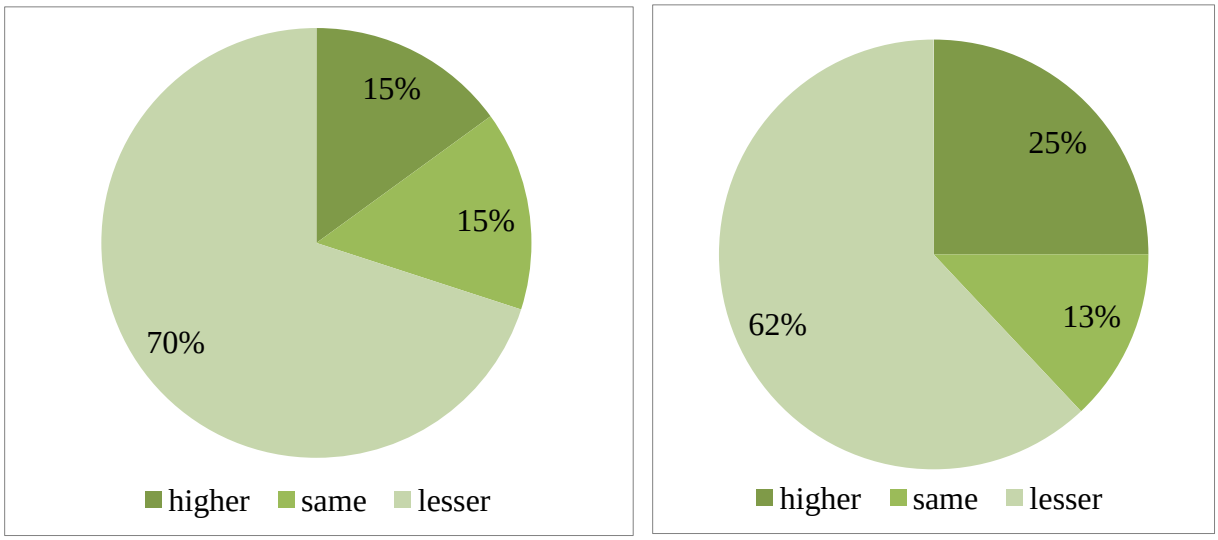


Figure 10: Season in which Meat Market Sells More



Panel a Panel b

Figure 11: Beef Meat Prices Compared to Goat Meat Prices (Panel a) and Beef Meat Prices Compared to Sheep Meat Prices (Panel b)

Figures 13 and 14 Panels a and b provide us with the profile of the participants. The operators were mostly male having 51 or more years of age, have obtained a high school diploma or have some studies at the university.

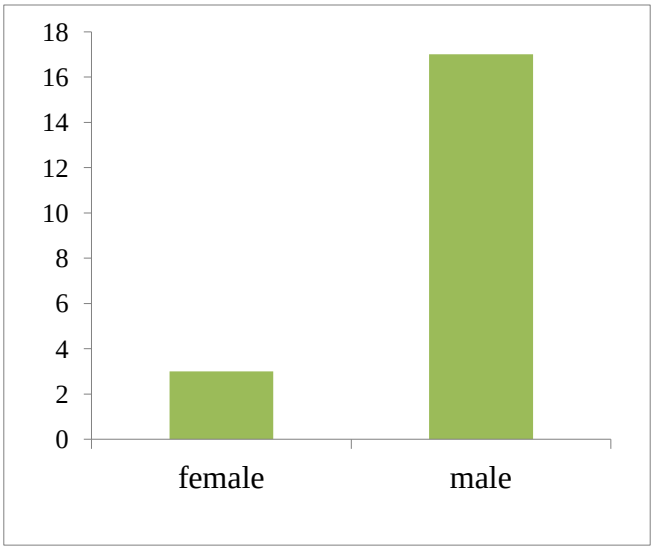
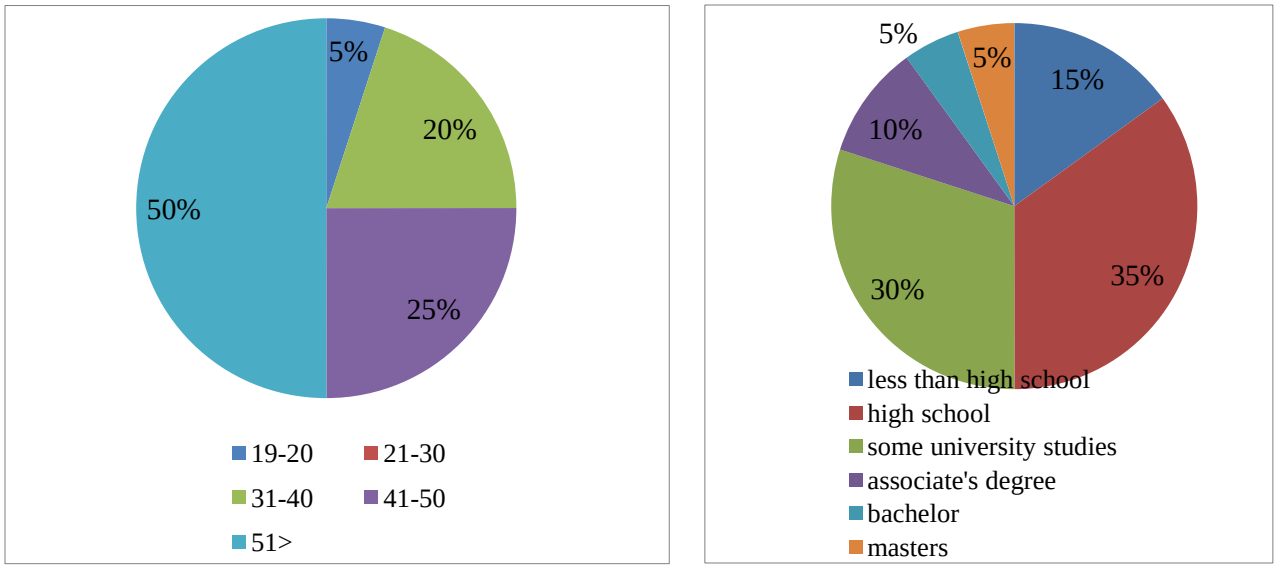


Figure 13: Gender of the Operators



Panel a Panel b

Figure 14a: Age of the Operators (Panel a) and Level of Education of the Operators (Panel b)

A chi square test was performed using STATA to study the statistical relationship, dependence, for: availability of goat and sheep meat and country of origin between the level of education and age of the operator. Results showed that the only variables which were statistically dependent were the age of the operator and the availability of goat meat, Table 4.

Table 4: Chi Square Test

Variables	Age	Education
Availabilty of goat meat	χ^2= 6.46 Pr = 0.040	χ^2 = 1.44 Pr = 0.838
Availabilty of sheep meat	χ^2 = 1.75 Pr = 0.417	χ^2 = 3.79 Pr = 0.435
Origin of goat meat	χ^2 = 5.28 Pr = 0.260	χ^2= 10.40 Pr = 0.238
Origin of sheep meat	χ^2 = 1.14 Pr = 0.565	χ^2 = 1.14 Pr = 0.767

Conclusions

In this study we defined farmer's market as: a typical market usually in a closed building open every day or almost every day of the week, that is located in the middle of downtown in which local or imported products are sold and sellers are not necessarily farmers. We performed an assessment to identify the butcheries within our definition of farmers market. We found 20 butcheries in 14 different farmers' markets that offer small ruminant meat. Results also showed that most butcheries sell locally produced goat and sheep meat instead of imported, and they sold more goat meat than sheep meat. Given the low amounts being sold at these butcheries located at these farmer's markets we recommend to study where are local producers selling these small ruminants. In addition, we recommend to do a study of the consumers profile that buy at these farmer's markets. We theorized that must take into consideration that the consumers that buy in farmers' markets are old people with a low income and that these farmers' markets are not being considered as a principal place to buy fresh groceries which are being replace by new types of farmers' market such as urban markets, family markets, and organic markets. Small ruminant meat is consumed in Puerto Rico, but in fewer quantities than beef meat, we recommend to do a study consumers profile of sheep and goat meat in Puerto Rico.

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THE EFFECT OF LACTIC ACID BACTERIA ON THE FERMENTATION PRODUCTS, AEROBIC STABILITY, AND VOLUNTARY CONSUMPTION OF FORAGE SORGHUM SILAGES

Austin Hallquist and Luis Solórzano
Department of Animal Science

Abstract

Advances in the preservation of the nutritional quality of silage represent significant economic savings in animal production due to the extensive use of silage as a feed ingredient. Current evidence shows improvements in silages treated with microbial inoculants in the following areas: fermentation products, aerobic stability, and voluntary consumption by livestock. The objective of this study was to determine the effects of treating whole plant sorghum silage with a product containing *Lactobacillus plantarum*, *Enderococcus faecium*, *Pediococcus acidilactic*, and *Pediococcus pentosaceus* compared to a non-inoculated control. Whole plant sorghum silage was chopped and ensiled in twenty silos per treatment. Silos had a capacity of 18.9 L that were maintained in anaerobic conditions for a period of 47 d. At the end of the ensiling period, the silage was sampled and analyzed for their chemical composition. Additionally, an aerobic stability test was carried out by monitoring the temperature of five samples from each treatment at six hour intervals during 7 d. The voluntary consumption was recorded in a switch-back experimental design with two feeding periods lasting 7 d. Total Mixed Rations (TMR) were offered to eight mixed race Pygmy goats (four per treatment period combination). A chemical analysis revealed a tendency ($p<0.09$) of decreased lactic acid production and an increase in pH ($p<0.09$) in the inoculated silage utilized during the experiment. While the aerobic stability remained unaffected, the voluntary consumption of the inoculated TMR increased numerically.

Keywords: Microbial Inoculants, Lactic Acid Bacteria, Sorghum Silage

Resumen

Avances en la preservación de la calidad nutricional del ensilaje representan ahorros económicos significativos en la producción animal debido al uso extensivo del ensilage como ingrediente alimenticio. Actualmente, hay evidencia sobre mejoramientos en ensilajes tratados como inóculos microbianos en las siguientes áreas: productos de fermentación, estabilidad aeróbica y consumo voluntario. El objetivo de este estudio fue determinar los efectos de tratar el ensilaje de sorgo con un producto que contiene *Lactobacillus plantarum*, *Enderococcus faecium*, *Pediococcus acidilactic* y*Pediococcus* pentosaceu, comparado con el control no inoculado. El ensilaje de sorgo fue picado y ensilado en veinte silos por tratamiento. Estos tenían una capacidad de 18.9 L que fueron mantenidos en condiciones anaeróbicas por 47 d. Al finalizar el período de ensilaje, muestas fueron recolectadas y analizada para determinar su composición química. Adicionalmente, se realizó una prueba de estabilidad aeróbica por medio del monitoreo de la termperatura de las muestras en intervalos de seis horas durante 7 d. El consumo voluntario fue recolectado utilizando un modelo experimental reversible en dos períodos de alimentación con duración de 7 d. Las raciones totales mixtas fueron dadas a ocho cabras pigmeas de raza mixta (cuatro por tratamiento). El análisis químico reveló una tendencia ($p<0.09$) de una disminución en la producción de ácido láctico y un aumento en pH ($p<0.09$) en el ensilaje

inoculado utilizado en el experiment. A pesar de que la estabilidad aeróbica no fue afectada por el inóculo microbiano, el consumo voluntario de la dieta inoculada incrementó numericamente.

Palabras Claves: Inóculos microbianos, bacterias productoras de ácido lacto, ensilaje de sorgo

Introduction

Microbial inoculants are one of the most common silage additives that are used to preserve forages. These additives typically consist of bacteria, such as lactic acid bacteria (LAB) that act by converting water soluble carbohydrates (WSC) into organic acids which lower the pH of the silage. Due to the fact that LAB inoculants constitute a broad category of diverse bacteria that are employ many distinct functions, they may be further classified as either homofermentative or heterofermentative according to the fermentation pathway that each one undergoes during the ensiling process.

Homofermentative LAB typically convert WSCs into lactic acid which is a relatively strong acid that provokes a rapid drop in pH needed in order to inhibit the proliferation of undesired microorganisms. Heterofermentative LAB, on the other hand, are capable of producing acetic acid and, to a lesser degree, propionic acid which confer the silage a better aerobic stability during the feeding phase. Aerobic stability is a measure of the overall resistance against decomposition that inevitable occurs once the silage is exposed to oxygen. Research investigations (Keles and Demirci, 2011; Mohammadzadeh et al., 2012; Nkosi and Meeske, 2010) have shown that the addition of certain heterofermentative bacteria such as *Lactobacillus buchneri* (LB), inhibits the growth of yeast present in the silage during the anaerobic phase via the production of acetic acid among other possible fermentation products (Comino et al., 2014).

Apart from improvements in the fermentation characteristics and aerobic stability of silages inoculated with LAB, there is also evidence that they improve animal performance (e.g. voluntary intake, milk production, increases in live weight). Muck (2010) reported that approximately half of all research investigations involving homofermentative microbial inoculants that were surveyed showed improvements in areas related to animal production. Despite the large volume of evidence indicating improvements in animal production, Muck, R. (2010) affirms that the exact cause for such improvements is still to be determined by further research.

The three primary objectives of this research investigation include the following: to determine the effect of the LAB inoculant on the fermentation products of the silage, to establish the effectiveness of the microbial inoculant to preserve the silage once exposed to oxygen utilizing aerobic stability as a criterion, and to measure the effect of feeding the inoculated silage on the voluntary consumption in mixed race Pygmy goats.

Materials and Methods

The first phase of the investigation began on 30th of September in 2015 in which approximately one metric ton of forage sorghum was transported from Araus Agro Inc. located in Salinas, Puerto Rico to the Alzamora Farm located on the campus of the University of Puerto Rico at Mayagüez (UPRM) in the city of Mayagüez, Puerto Rico. There, four samples from different sections of the forage load were taken and preserved in an industrial freezer for posterior analysis

of its initial chemical composition. The forage sorghum was then divided into two groups in the Small Ruminant Pavilion at the Alzamora Farm in preparation for the application of the two different treatments that were used. The first treatment was a control that consisted of one liter of water that was applied via a standard hand sprayer while the second treatment consisted of one liter of water in which 0.5 grams of the microbial inoculant. The microbial inoculant provided $>9.1 \cdot 10^{10}$ colony forming unit per gram (CFU/g) containing *L. plantarum*, *E. faecium, P. acidilactic*, and *P. pentosaceus*. Each treatment was manually ensiled and sealed into 20 individual 18.9 L buckets made of high density polyethylene (HDPE) immediately after the application of each treatment. All 40 silos were transported from the Small Ruminant Pavilion to an adjacent laboratory where they were stored at room temperature for a total of 47 d.

After 47 d, 5 silos from each treatments, 10 silos total, were randomly selected, opened, and samples of approximately 1 kilogram (kg) were taken from each one. These samples were sent to the Dairy One Forage Testing Laboratory (Ithaca, New York) in order to determine the chemical composition. Statistical analysis was performed using a completely randomized experimental design via the software program SAS.

Additionally, 1 kg samples from each of the five silos of both treatments, 10 in total, were taken and individually placed into polystyrene chambers (Figure 1). Once accommodated into each slot, a thermometer was inserted into the center of each sample. The temperature of each of the ten samples was measured and recorded every 6 hours for a total of 168 hours.

For the final phase of the investigation, both silages were fed to mixed race Pygmy goats in the Small Ruminant Pavilion at the Alzamora Farm on the UPRM campus. There were a total of eight goats allocated to a switch-back experimental design. In said model, there was a total of two feeding periods of one week each in which the first four days of each period were allotted to diet adaptation while the final three days were allocated to data collection. As part of the reversible experimental model, goats that were assigned to the control diet during the first feeding period switched to the inoculated diet during the second feeding period. Likewise, the goats assigned to the inoculated diet during the first feeding period switched to the control diet during the second feeding period. The silage was offered to each goat in the form of total mixed rations (TMR) in a silage to concentrate ratio of 1:1 (DM basis). Each goat was offered 4.5% (DM basis) of their body weight. The amount of TMR offered and rejected were recorded to determine the amount consumed by each animal. Statistical analysis was performed using a switch-back experimental design utilizes the SAS software.

Results

Table 1. Initial Chemical Composition of the Forage Sorghum

Variable	Forage	Standard Error
DM[1], %	18.95	0.34
CP, %	10.43	0.082
ADF, %	46.35	0.089
ADF (LC), %	62.58	3.52
Lignin, %	7.55	0.37
Starch, %	0.80	0.54
WSC, %	4.28	0.29
Simple sugars, %	4.68	0.23
Ash, %	14.62	0.78

[1] As fed; all other on a DM basis. [2] WSC = water soluble carbohydrates.

Table 2. Effect of inoculating forage sorghum on the fermentation characteristics after 47 d of ensiling

Variable	Control	Inoculated	S. E.	P
pH	4.66	4.82	0.031	0.09
Acetic Acid, %	4.69	3.95	0.018	0.21
Lactic Acid, %	1.14	0.63	0.031	0.09
L : A	0.24	0.15	0.033	0.08
Propionic Acid, %	0.40	0.41	0.030	0.19
Butyric Acid, %	0.28	0.85	0.021	0.19
Total Acid, %	6.52	5.91	0.025	0.14
NH_3/N	10.60	11.20	0.002	0.73

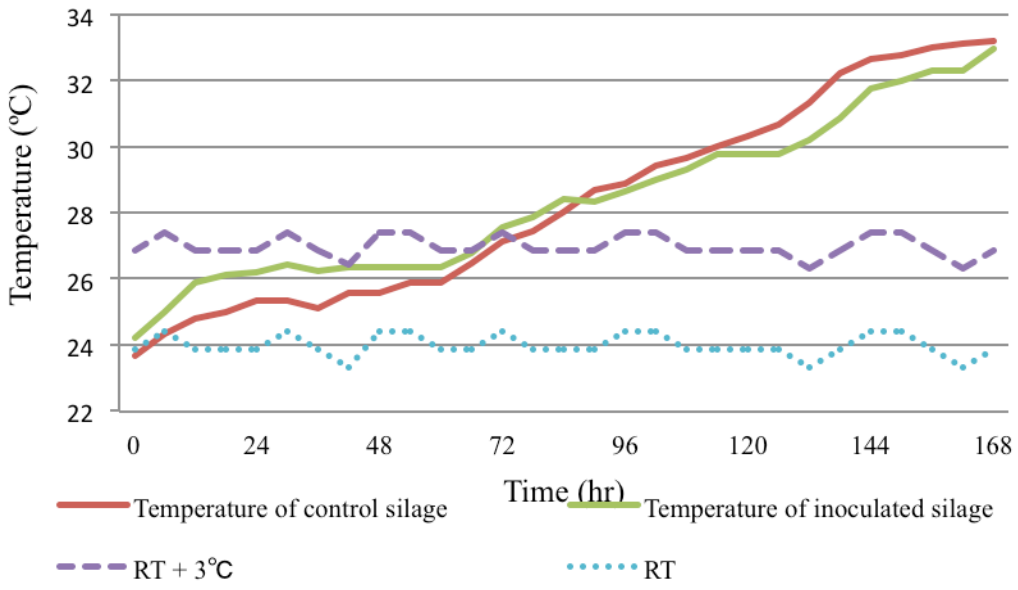
L:A = ratio of lactic acid to acetic acid; NH_3/N = ammonia nitrogen

Table 3. Effect of inoculating forage sorghum silage on the voluntary consumption of TMR

Treatment	Consumption (kg)
Control TMR	1.33
Inoculated TMR	1.52
P =	0.458

TMR = total mixed rations

Graph 1. Effect of silage inoculation of sorghum on silage on temperature post-aerobic exposure



RT = room temperature; RT + 3ºC = room temperature plus three degrees

Figure 1. Silage samples placed into polystyrene module utilized to measure temperature during the second phase of the experiment

Discussion

Contrary to what had been previously expected, the value established for the pH of the control silage was found to be 0.16 higher ($p<0.09$) than that of that which had been inoculated with the microbial inoculant. Additionally, the lactic acid present within the inoculated forage was 0.51 percent units lower than that of the control. These results were atypical of homolactic fermenters (Muck, 2010) and there was not any heterofermentative bacterium present within the microbial inoculant. Probably the effectiveness of the inoculant was affected due to the high moisture content (>80%) of the sorghum forage.

The microbial inoculant utilized in the second phase of the investigation did not influence the aerobic stability of the silage once exposed to oxygen. Despite the slightly higher acetic acid content in the control silage, it did not influence the aerobic stability. This finding suggests that factors other than acetic acid may be involved in determining the aerobic stability of silages. This outcome was obsered (Meeske et al., 2002) in research conducted with microbial inoculants containing exclusively homofermentative LAB.

Although the voluntary consumption of mixed race Pygmy goats used did increase in the diet containing the inoculated silage, the difference was found to not be statistically significant. It is possible that the relatively small sample size could have influenced this outcome. Furthermore, the initial quality of the forage sorghum that was utilized was found to be rather poor. As shown in Table 1, the Dry Matter (DM) content was determined to be 18.95% which indicates that forage was too moist upon harvest. In addition, the lack of starch and the high ash content (14.62%) which is indicative of the presence of contaminants (e.g. soil and/or sand) within the forage. In future trials, forage sorghum should be harvest at DM content of >30% and starch level >20%. Also, more animals added to each treatment and a higher quality initial forage been used, would have been statistically significant as reported by Nkosi et al. (2016) who found increases in the voluntary intake of soybean silage in Damara rams.

Conclusion

The value for the pH of the inoculated silage tended to be higher ($p<0.09$) than that of the control. Additionally, the lactic acid content, as well as the ratio of lactic acid to acetic acid, was found to be higher ($p<0.09$ and $p<0.08$, respectively) in the control. Silages had similar aerobic stability. There was a numerical increase in voluntary consumption for the silage treated with the microbial inoculant compared to the control, but these findings were not statistically significant.

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INFRARED THERMOGRAPHY AS A TOOL FOR BOVINE TUBERCULOSIS DETECTION

Marangely Santiago-Sanabria, Jaime E. Curbelo-Rodríguez and Yomar R. Vélez
Department of Animal Science

Abstract

Many control measures have been established in order to eradicate bovine tuberculosis (BT). This infectious disease is caused by *Mycobacterium bovis* (*M. bovis*) and is related with significant losses in the dairy industry. The most common method for BT prevention is continuous screening of animals using the tuberculin antigen test (CFT). Suspected cows are then re-tested comparing skin thickness, post antigens injections, to quantify inflammation (CCT). However, cross-reactions during CCT testing can occur in animals that have been previously sensitized by non-pathogenic environmental strains of mycobacteria, such as *Mycobacterium avium subspecies paratuberculosis (Map)*. Both tests require animal restrain and a period of 72 hours in order to read the results. Infrared thermography (IRT) may represent a noninvasive alternative tool for detection of BT. The objective of the present study was to use IRT as a tool for early detection of BT during veterinary BT readings. Ultimately, it is expected to provide veterinarians in Puerto Rico a supportive tool for routine evaluation of BT. In order to achieve this, an approach verification trial was performed in 87 lactating cows to determine if IRT could be used to detect changes in temperature associated with inflammation post CCT. Using a thermography camera, these cows were monitored for 48 hours post injection at 24-hour intervals. Suspected cows post CFT testing (n=11) underwent IRT imaging at 0 and 72 hour. On both tests, baseline IRT images were collected pre and 72 hours post injection in order to be used for IRT comparisons. In the CFT test, treatment by time did not interact to affect TIR (P=0.63). No differences in IRT between the injection site and control site were found at time 0 (P= 0.08). However, differences in IRT were observed 24 (P=0.01) and 48 hours (P<0.001) post injection, relative to the control site. In the CCT, treatment by time did not interact to affect TIR (P=0.61) or skin thickness (P=0.31). In addition, there was a no correlation between the TIRmax temperature and thickness difference between time 0 and 72 for *M. avium* (P=0.35) or *M. bovis* (P=0.64; Figure 7).. Further research is required to improve IRT application on BT screening. The lack of differences in IRT among treatments could be attributed to the limited amount of animals included in the study and to the fact that BT is eradicated in Puerto Rico.

Keywords: Bovine Tuberculosis, thermography imaging, *Mycobacterium bovis*, *Mycobacterium avium spp. paratuberculosis*, Johne's disease

Resumen

Se han establecido muchas medidas de control con el fin de erradicar la tuberculosis bovina (TB). Esta enfermedad infecciosa es causada por *Mycobacterium bovis* (*M. bovis*) y se relaciona con pérdidas significativas en la industria lechera. El método más común para la prevención de BT es el monitoreo continuo de los animales mediante la prueba de antígeno de tuberculina (CFT). Vacas sospechosas son re-evaluadas comparando el grosor de la piel, luego de inyección con antígenos, para cuantificar la inflamación (CCT). Sin embargo, las reacciones cruzadas durante la prueba CCT pueden ocurrir en animales previamente sensibilizados con cepas de micobateria ambientales no patogénicas, como *Mycobacterium avium subespecie*

paratuberculosis (*Map*). Ambas pruebas requieren restricción de animales y un período de 72 horas para leer los resultados. La termografía infrarroja (IRT) puede representar una herramienta alternativa no invasiva para la detección de laTB. El objetivo del presente estudio fue utilizar IRT como herramienta para la detección temprana de TB durante las lecturas de BT por veterinarios. En última instancia, se espera proporcionar a los veterinarios en Puerto Rico una herramienta de apoyo para la evaluación rutinaria de BT. Con el fin de lograr esto, un ensayo de verificación se realizó en 87 vacas lactantes para determinar si IRT se podría utilizar para detectar los cambios en temperatura asociados con a inflamación post inyección con CCT. Usando una cámara termográfica, estas vacas fueron monitoreadas por 48 horas luego de la inyección de CFT a intervalos de 24 horas. Vacas sospechosas según la CFT (n = 11), se sometieron a imagen IRT a 0 y 72 horas. En ambas pruebas, imágenes IRT base se tomaron antes y 72 horas después de la inyección CCT con fines comparativos de IRT. En la prueba de CFT, tratamiento por tiempo no interactuaron para afectar la TIR (P = 0.63). No hubo diferencias en IRT entre el sitio de inyección y el control en el tiempo 0 (P=0.08). Sin embargo, se observaron diferencias en IRT 24 (P=0.01) y 48 horas (P<0.001) post inyección, relativo al sitio control. En el CCT, tratamiento por tiempo no interactuaron para afectar la TIR (P=0.61) o el grosor de la piel (P= 0.31). En adición, no s observó correlación entre TIRmax y grosor de la piel pre y post inyección en *M. avium* (P=0.35) o *M. bovis* (P=0.64). Se requieren investigaciones adicionales para mejorar la aplicación de IRT durante monitoreo de TB. La falta de diferencias en IRT entre tratamientos se podría atribuir a la cantidad limitada de animales incluidos en el estudio y al hecho de que TB está erradicada en Puerto Rico.

Palabras Claves: Tuberculosis bovina, termografía infrearoja, *Mycobacterium bovis*, *Mycobacterium avium spp. paratuberculosis*

Introduction

Bovine tuberculosis (BT) is an infectious disease, caused by *Mycobacterium bovis* (*M. bovis*) that affects domestic and wild animals. Infected cattle can remain asymptomatic for long periods of time with a small proportion of animals developing granular nodes, a characteristic symptom of bovine tuberculosis. Other non-specific symptoms that may develop in infected animals with *M. bovis* are cough, weakness and ultimately death. Currently there is no cure for this disease; therefore, culling animals upon detection is highly recommended. Bovine tuberculosis can affect people through the ingestion of unpasteurized milk and by inhaling infective droplets (World Organization for Animal Health [OIE], 2011). According to the OIE, “Many developed countries have reduced or eliminated BT from their cattle population; however, significant pockets of infection remain in wildlife in Canada, United Kingdom, United States and New Zealand.” Effective control practices have been achieved due to continuous screening of animals using tuberculin antigen test, which is based on purified protein derivatives (PPD).

The caudal fold tuberculin test (CFT) is the official routine examination required for all herds in the U.S. (USDA, 2005). In the CFT test, a bovine PPD is injected in the caudal fold and skin fold thickens is measured after 72 hours. If the site of injection exhibits a significant increase in thickness, the individual is then classified as a suspect for bovine tuberculosis. These suspect animals are then re-tested by the Animal and Plant Health Inspection Services (APHIS) to certify if the animal is infected or not. However, cross-

Figure 2. FLIR-E8 thermography camera

reactions can occur in animals that have been sensitized by non-pathogenic environmental strains of mycobacteria, due to the presence of antigens common to virulent and non-virulent mycobacterial strains (Pollock et al. 2005). According to Jungersen et al. (2002), the mycobacteria usually responsible for cross-reaction with *M. bovis* is *Mycobacterium avium spp. paratuberculosis (Map).* This infectious bacterium has not yet been eradicated in US herds, being frequently diagnosed by local veterinarians in many dairies in Puerto Rico. This disease is responsible for significant losses at the farm level. In fact, during 2001, the US reported an estimated annual loss of $150 million due to this disease (Méndez-Olvera, et al., 2013). However, recent reports indicate higher losses (over $200 million; Groenendaal et al. 2015). The most common effects of *Map* infections on cattle are chronic diarrheas and weight loss. This disease is commonly referred as Johne's disease and it is a suspect causative agent of Crohn's disease in humans (Greenstein, 2003). Up to date there is no information regarding CFT results and cross reactivity between *M. bovis* and *Map*. This information may provide important information about the prevalence of Johne's disease in Puerto Rico.

In order to confirm if suspect individuals with BT (identified with CFT) are infected with *M. bovis* or if it is a cross-reaction with *Map*, suspects must go through a cervical comparative tuberculin test (CCT). In this test, two intradermal injections with *M. bovis* PPD and *M. avium* PPD are applied at two different sites at the cervical area (neck; Figure 1). Skin thickness in each injection site is measured with a caliper before and 72 h post CFT injection. The difference in thickness is then plotted in a CCT scattergram to determine if the animal is positive to *M. bovis.* Both the CCT test and the CFT test are invasive procedures, which require animal restrain and a period of 72 hours in order to read the results.

Figure 1. Injection sites at the cervical area (neck). Superior and inferior shaved squares correspond to M. avium and M. bovis PPD injection sites, respectively.

Infrared thermography (IRT) may represent an opportunity to reduce time and make the procedure less invasive. This technology is extensively researched for veterinary applications (Johnson and Dunbar, 2011). It provides a noninvasive way to determine the superficial temperature of biological and non-biological surfaces, which can then be correlated to an inflammatory related disease. Johnson & Dunbar, (2008) demonstrated that IRT could be used to discriminate between positive and negative cows in the CCT test. These researchers used the localized heat at the injection site to classify *M. bovis* positive animals with 86% accuracy within 24 hours, instead of the typical 72 hours. According to Rekant et al. (2016), "Perhaps the most promising application of IRT in individual animal medicine is the early identification of an increase in body temperature that is indicative of the development of a fever or local inflammation." This provides a method for early isolation of possibly ill animals and assists in controlling the transmission of the disease. The objective of the present study was to use IRT as a tool for early detection of BT during veterinary CCT readings and identify if the cross-reactions observed frequently observed in the CFT in Puerto Rico are due to the presence of *Map*. Ultimately, it is expected to provide veterinarians in Puerto Rico a supportive tool for routine evaluation of bovine tuberculosis preventive measures in cattle.

Materials and Methods

This study was conducted in three private dairy farms on the northwestern region of Puerto Rico. An approach verification trial was performed in 87 lactating cows to determine if IRT could be used to detect changes in temperature associated with inflammation post CFT injection in the caudal folds. In order to accomplish this, images were collected using the FLIR-E8 thermography camera (FLIR Systems Inc.; Figure 2) at 24 hour intervals for a 48 hour period. Cows classified as suspect for BT (according to caudal thickness measured by a veterinarian) were subjected to an additional IRT image at 72 hour. In a different trial, IRT images were collected in 11 lactating cows during the comparative CCT test at 0 and 72 hours relative to PPD injections. On both test, the baseline IRT temperature was collected (Time 0) in order to be used for IRT comparison post injection.

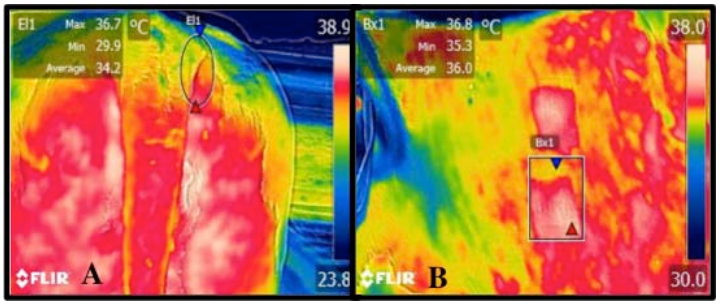

Figure 3. Infrared thermography imaging of the left (M. bovis PPD injection site) and right (control side) caudal folds (Panel A) and IRT of the CCT upper injection site (M. avium PPD) and lower injection site (M. bovis PPD; Panel B).

The FLIR Tools Plus program (FLIR Systems Inc.) was used to quantify IRT images using as objective the injection sites and the contralateral site (no injection) in the CFT test and the *Map* injection site as control in the CCT test. The IRT max temperature (IRTmax) was used for statistical analysis. For the CFT test and CCT, both caudal folds (Figure 3A) and PPD injection sites (Figure 3B). were imaged simultaneously. All IRT and skin thickness data were analyzed using the SAS University Edition. A PROC GLIMMIX was employed, using each cow as random effect, to determine the effect of treatment, time and their interactions on IRTmax and skin thickness.

Results and Discussion

Caudal fold tuberculin (CFT) test

During the verification trial, out the 87 lactating cows that evaluated, two were classified as suspicious to BT. The IRTmax temperature 72 hours post injection in these cows were 35.2° C and 36.8° C at the injected caudal fold and 35.2° C and 36.1° C at the non-injected caudal fold, respectively. These temperature values were not considered different according to previous studies (Johnson & Dunbar, 2008). Therefore, even though the cows were suspects to the CFT test, IRT was not able to classify them as such. Treatment by time did not interact to affect IRTmax ($P=0.63$). In addition, no differences in IRTmax between injections sites in time 0 were observed ($P=0.08$; Figure 4). However, differences were observed at 24 ($P=0.01$) and 48 hours ($P<0.001$) post injection relative to the control site. The observed increment in temperature in the injection site could be attributed to a non-specific inflammatory response caused by the PPD injection since all the animals tested negative to *M. bovis* in the CCT. It remains to be determined if the increase in temperature could be attributed to cross reactivity with *Map* antibodies. A limitation observed during collection of caudal folds IRT images in the CFT test was the variation in the injection sites, which in numerous occasions were beneath the tail base, limiting the feasibility of this approach in detecting inflammation.

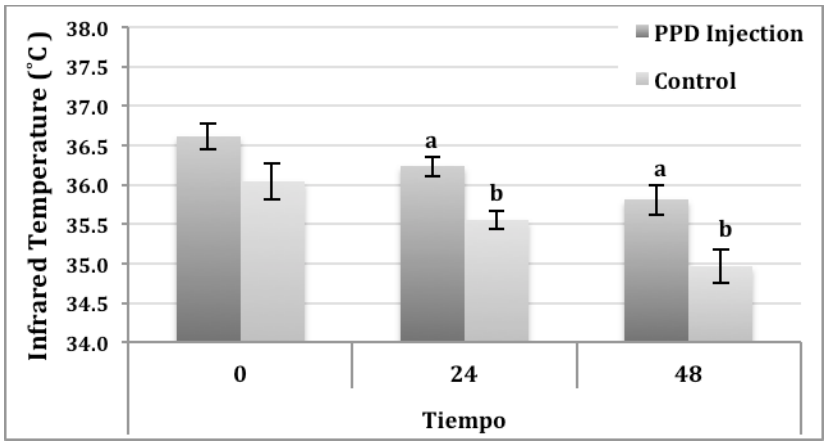

Figure 4. Infrared thermography max temperature of caudal folds at the PPD injection and control site. Every time point represents the mean IRTmax ± SEM (°C; n=87). Different letters indicate statistically significant differences between treatments ($P < 0.05$).

Comparative cervical tuberculin (CCT) test

None of the eleven CFT suspect cows were classified as suspect to *M. bovis* or *Map* by the APHIS veterinarians during the CCT test. Treatment by time did not interact to affect IRT (P=0.61) or skin thickness (P=0.31). The IRTmax and skin thickness did not differ between pathogen injection site (P=0.94 and P=0.13, respectively; Figure 5 and 6). The max temperature of the area injected with *M. avium* PPD showed no differences in temperature relative to the area that was injected with *M. bovis* PPD. This could be attributed to the fact that none of the animals tested were positive to neither of these pathogens. In addition, there was a no correlation between the TIRmax temperature and thickness difference between time 0 and 72 for *M. avium* (P=0.35) or *M. bovis* (P=0.64; Figure 7). During analysis of IRT images, it could be appreciated that the syringe puncture area presented lower temperature relative to the surrounded area, which could be caused by edema development.

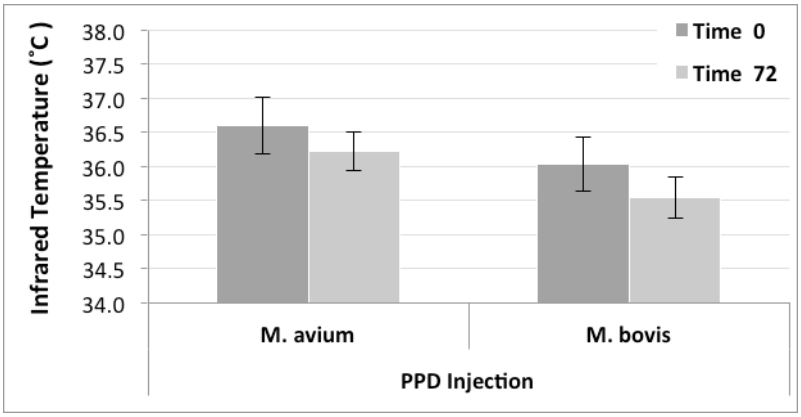

Figure 5. Infrared thermography max temperature of cervical injection sites with *M. avium*-PPD and *M. bovis*-PPD. Every column represents the mean maxIRT at its corresponding time point ± SEM (°C; n=11). No differences between treatments were observed ($P < 0.05$).

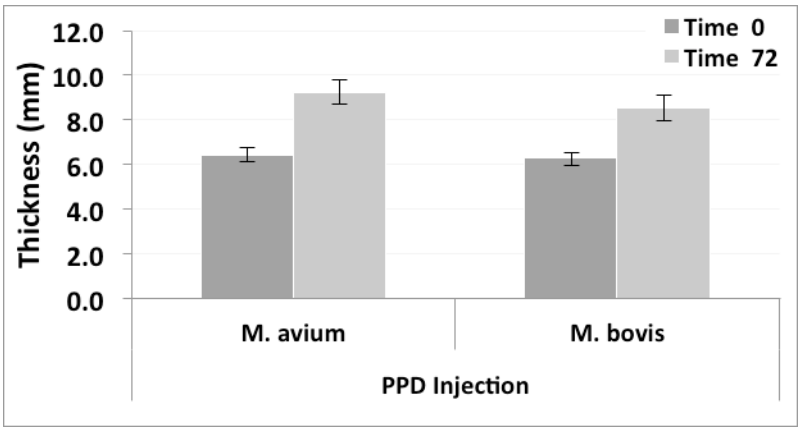

Figure 6. Skin thickness of cervical injection sites with *M. avium*- and *M. bovis*-PPD. Every column represents the mean maxIRT at its corresponding time point ± SEM (mm; n=11). No differences between treatments were observed (P < 0.05).

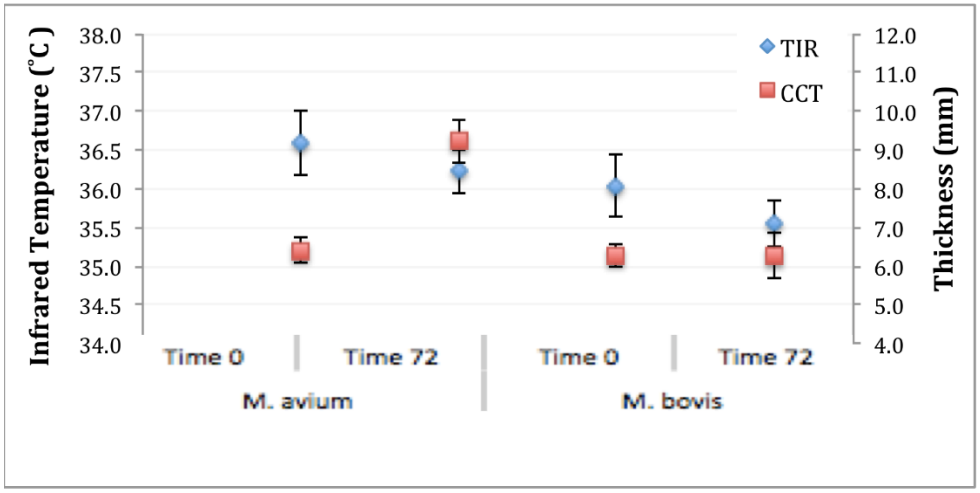

Figure 7. Association between TIR and skin thickness of cervical injection sites with *M. avium*- and *M. bovis*-PPD. Every point represents the mean maxIRT or thickness of n=11 cows at its corresponding time point ± SEM. No association between the difference TIRmax and thickness pre and post injection between groups was found (P < 0.05).

Conclusion

Due to the fact that none of the animals included in the study were pre-sensitized with *M. bovis* or *Map*, no differences in IRT were found between groups pre vs post injections. However IRT was capable to detect differences in temperature in the CFT injection site, which could be attributed to a non-specific inflammatory response caused by the PPD injection. It remains to be determined if the increase in temperature in the CFT test could be attributed to cross reactivity with *Map* antibodies. Serum samples from suspected cows (n=11) were collected and will be screened for *M. bovis* and *M. avium* using molecular tests. Regardless, IRT is a very useful technology and should be studied further, such as in experimentally infected animals with *Map*.

Acknowledgments

The authors want to acknowledge the USDA/APHIS veterinary service personnel, especially Drs. Fred Soltero, Noelia Moyeno and José Acosta for their assistance in this research trial.

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ADDING A SOURCE OF FAT AT DIFFERENT LEVELS DURING THE STAGE OF FATTENING ON INTAKE, GROWTH, CARCASS YIELD AND LIVER FAT CONTENT OF MEAT TYPE RABBITS

Pedro Olivencia, Gianhisy Díaz, Paul F. Randel, Luis C. Solórzano and Abner A. Rodríguez
Department of Animal Science

Abstract

The raising of meat type rabbits in Puerto Rico occurs in three stages: nursery, growing and fattening. The fattening stage usually is carried out between 60-90 days of age using a commercial feed (CF) low in energy density, which results in a slow rate of gain and a poor feed conversion ratio. In previous research adding fat sources at 4% of the total diet increased rate of gain and carcass yield, but the rabbits presented symptoms of fatty liver. The present experiment was conducted to evaluate the effects of different levels of dietary addition of a single fat source containing palmitic acid (PA) feed to meat type rabbits during the fattening stage on growth, carcass yield, and liver fat content. Thirty-two New Zealand white rabbits (60 days old, 1796g LW) were placed in individual elevated cages and distributed by live weight (LW) into four groups and heaviest rabbits were assigned to the control diet (T1, 100% CF); intermediate animals to treatment 2 (T2, 98% CF, 2% PA), treatment 3 (T3, 96% CF, 4% PA); while the lightest rabbits received treatment 4 (T4, 94% CF, 6% AP). Diets were offered daily at 5% of rabbit LW on a dry matter basis during 59 days. Data collected on productive performance, carcass yield and liver fat content were statistically analyzed according to a completely randomized experimental design with 10 replicates per treatment and use of Bonferroni-test for mean separation. Feed intake was higher ($P<0.05$) in control rabbits (69.29 g/d) and those fed the 2% fat diet (68.22g./d) than in animals fed 4% (62.50 g/d) or 6% (63.54 g/d) of the fat supplement. However, daily gain (g) was higher in rabbits fed with 6% fat (17.02) than in those fed 4% (14.73), 2% (15.59) or 0% (13.26) fat addition in the diet. Feed conversion ratio (g/g) was better ($P<0.05$) for T4 (3.94) than T1 (5.23), T2 (4.56) and T3 (4.64). The carcass yield percentage and liver weight were similar for all treatments. However, liver fat percentage (11.7%) of rabbits fed with 6% added fat was higher ($P<005$) than in those receiving 0% (9.1%), 2% (9.7%) and 4% (9.1%) of fat addition. In summary, partial substitution of a commercial concentrate basal diet with 6% of the fat supplement, improved rabbit daily LW gain and feed conversion ratio, but not carcass yield; and it increased the liver fat content. However, the treatment with 4% of added fat did not have this undesirable effect

Keywords: Rabbits, Fat, Intake, Performance, Carcass, Liver

Resumen

La crianza de conejos tipo carne en Puerto Rico se divide en las tres etapas: iniciadora, crecimiento y engorde. La etapa de engorde generalmente ocurre entre los 60-90 días de edad y utiliza un alimento concentrado (AC) bajo en densidad energética, lo que resulta en bajas ganancias de peso y una pobre conversión alimenticia. En investigaciones previas la adición de una fuente de grasa al 4% de la dieta total aumentó la tasa de ganancia y el rendimiento de la canal, pero los conejos presentaron síntomas de hígado graso. El presente experimento buscó evaluar el efecto de la adición de una fuente de grasa conteniendo ácido palmítico (AP) a distintos niveles dietéticos sobre la ganancia en peso, rendimiento de la canal y contenido de grasa del hígado de conejos tipo carne durante la etapa de engorde. Treinta y dos conejos blancos

de la raza Nueva Zelanda (60 días de edad, 1796g PV) en jaulas individuales elevadas se distribuyeron en base al peso vivo (PV) entre cuatro grupos; los más pesados se asignaron al control (T1, 100% AC), los intermediarios al tratamiento 2 (T2, 98% AC, 2% AP) y tratamiento 3 (T3, 96% AC, 4% AP) y los conejos menos pesados recibieron el tratamiento 4 (T4, 94% AC, 6% AP). Se ofrecieron las dietas diariamente al 5% del PV en base seca durante 59 días. Los datos sobre desempeño productivo, rendimiento de la canal y contenido de grasa en el hígado se analizaron estadísticamente según un diseño experimental completamente al azar con 10 repeticiones por tratamiento y la prueba de Bonferroni para la separación de medias. El consumo fue mayor ($P<0.05$) en conejos del grupo control (69.29 g/d) y en los del tratamiento con 2% de la grasa añadida (68.28 g/d) que en los alimentados con adiciones de 4% (62.50 g/d) o 6% (63.54 g/d) de grasa. Sin embargo, la ganancia en peso diaria (g) fue mayor en conejos alimentados con 6% de grasa añadida (17.02) que aquellos que aquellos que recibieron dietas con 4% (14.73), 2%(15.59) o 0%(13.26) de grasa suplementaria. La conversión alimenticia (g/g) fue mejor ($P<0.05$) con T4 (3.94) que con T1 (5.23), T2 (4.56) y T3 (4.64). El porcentaje de rendimiento de la canal y el peso del hígado no difirió entre los tratamientos. Sin embargo, el porcentaje de grasa del hígado (11.7%) de conejos alimentados con adición de 6% de grasa fue mayor ($P<005$) que aquellos que recibieron 0% (9.1%), 2% (9.7%) y 4% (9.1%) de grasa suplementaria. En resumen, la sustitución parcial de una dieta basada de concentrad comercial por 6% de grasa, mejoró la ganancia en peso diaria del conejo y la conversión alimenticia, pero no así el rendimiento de la canal y además aumentó el contenido de grasa en el hígado. En cambio, el tratamiento con 4% de grasa añadida no tuvo este efecto indeseable.

Palabras Claves: Conejos, Grasa, Consumo, Rendimiento, Canal, Hígado

Introduction

The raising of meat type rabbits in Puerto Rico is approximately of 90 days with slaughter weights of 5-6 lb. This type of rabbit is raised in three stages: nursery (1-30 days old), growing (31-60 days old) and fattening (61-90 days old). Usually the same commercial concentrate feed (CF) is used in the three stages and it is too low in energy density for the optimal growth. In previous research adding fat sources at 4% of the total diet increased animal performance and carcass yield, but the rabbits presented symptoms of fatty liver (Glass et al., 2015). The fat sources used in that experiment was a free fatty acid and a triglyceride. The present experiment was conducted to evaluate the addition at different percentage levels of a single fat source containing palmitic acid (PA) in the diet of meat type rabbits during the fattening stage on growth, carcass yield, and liver fat content.

Materials and Methods

The experiment was conducted in a rabbit pavilion of the Alzamora Laboratory Farm (Figure 1), located at the University of Puerto Rico, Mayaguez Campus. The animals used were 32 New Zealand White rabbits, 12 males and 20 females, 60 days of age. The rabbits were placed in elevated cages (Figure 2) and divided in four groups of 8; the heaviest rabbits were assigned to treatment 1 (T1, 100% CF), those of intermediate size to treatment 2 (T2, 98% CF, 2% PA) and treatment 3 (T3, 96% CF, 4% PA); and the lightest rabbits to treatment 4 (T4, 94% CF, 6% AP). Diets were offered daily at 5% of rabbit live weight on a dry matter basis during 36 days to evaluate feed intake, growth and feed conversion responses. On the 37th day they were transported to a slaughter plant at the Agricultural Experimental Station in the municipality of

Lajas (Figure 3). Data obtained at slaughter included carcass weight and yield and weight of the following organs stomach, cecum, kidneys and liver. In addition the coloration and fat percent of the liver were evaluated.

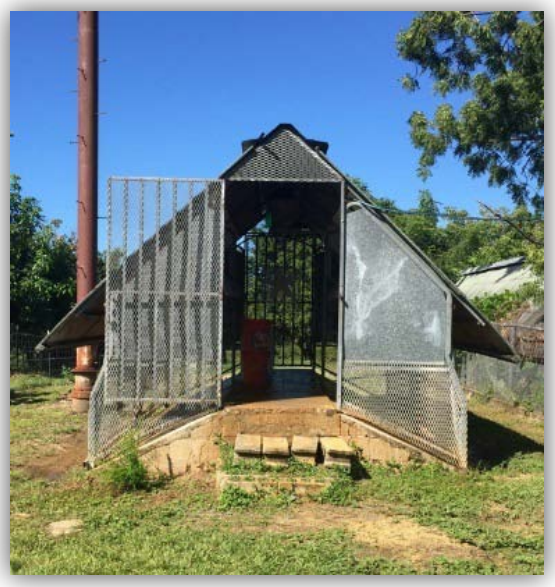


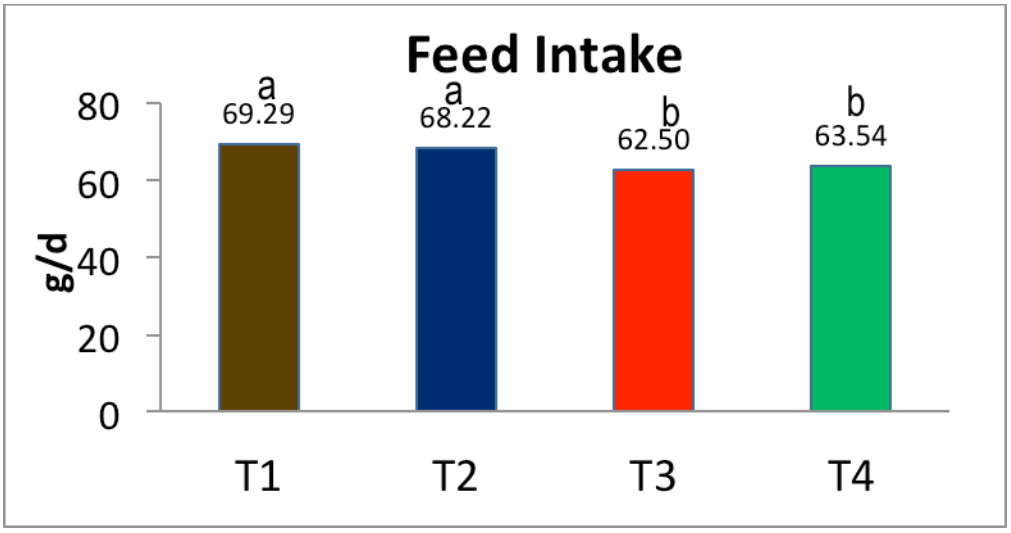
Figure 1- Rabbit Pavilion

Figure 2- Elevated Cages

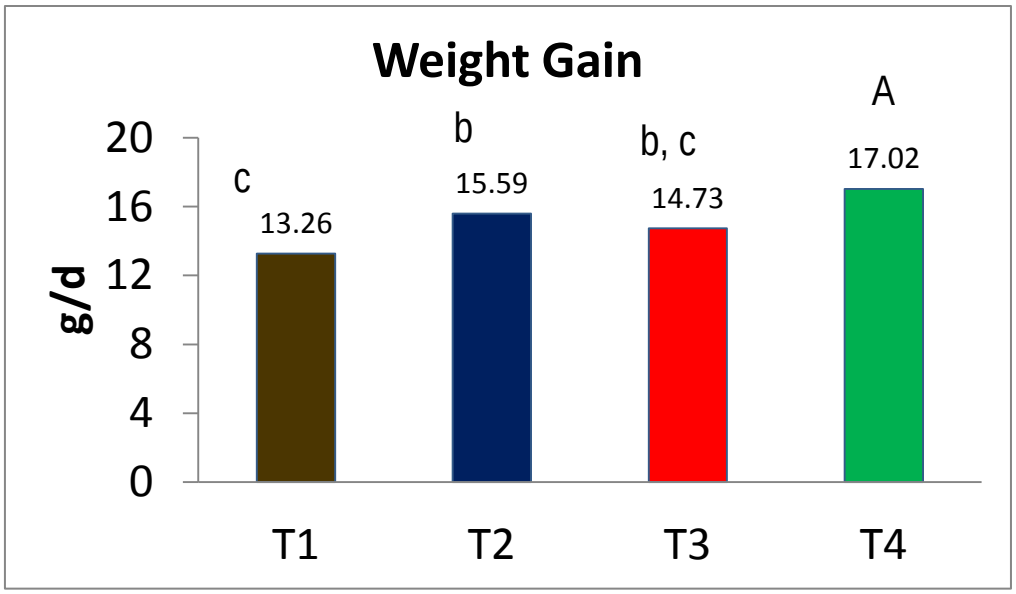
Figure 3- Slaughter Plant

Statically analysis of the data was according to a completely randomized design with 10 replicates per treatment and Bonferroni test for mean separation (SAS, 2004).

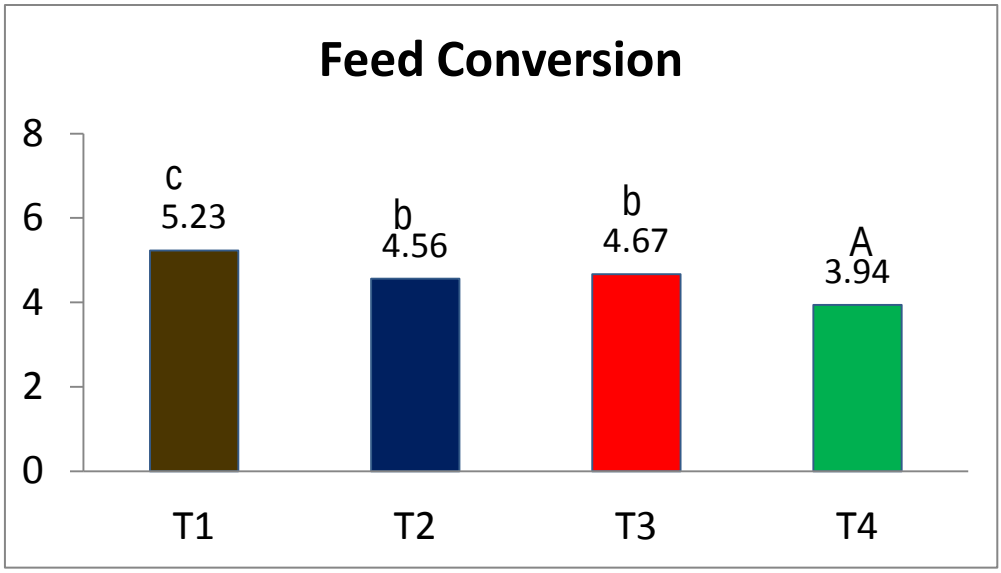
Results



Graph 1- Feed Intake was higher for T1 than T2, T3 and T4 because the heaviest rabbits were assigned to treatment 1.



Graph 2- Daily weight gain was highest in the rabbits of smallest size assigned to T4.



Graph 3- Feed Conversion is the proportional relationship between food intake and body weight gain of the rabbit. The lower the numerical value the better feed conversion obtained. Rabbits in T4 had the best feed conversion in comparison to those of the other treatments

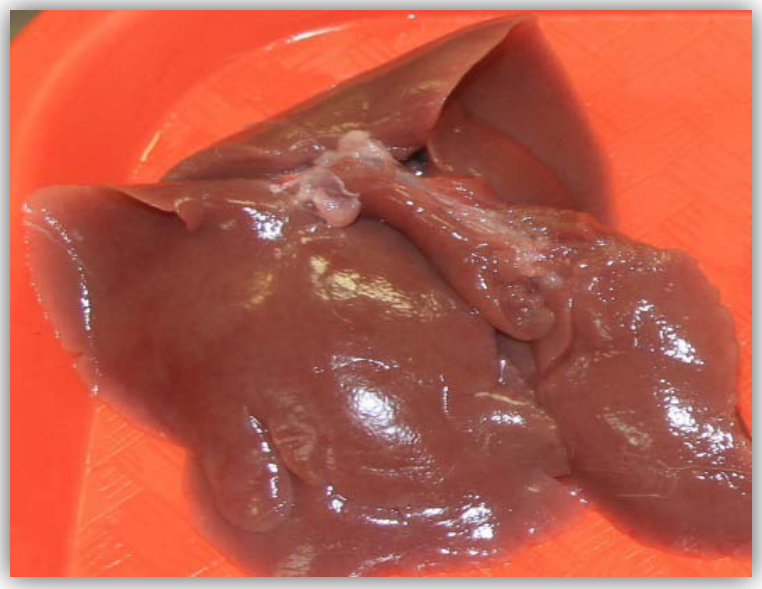
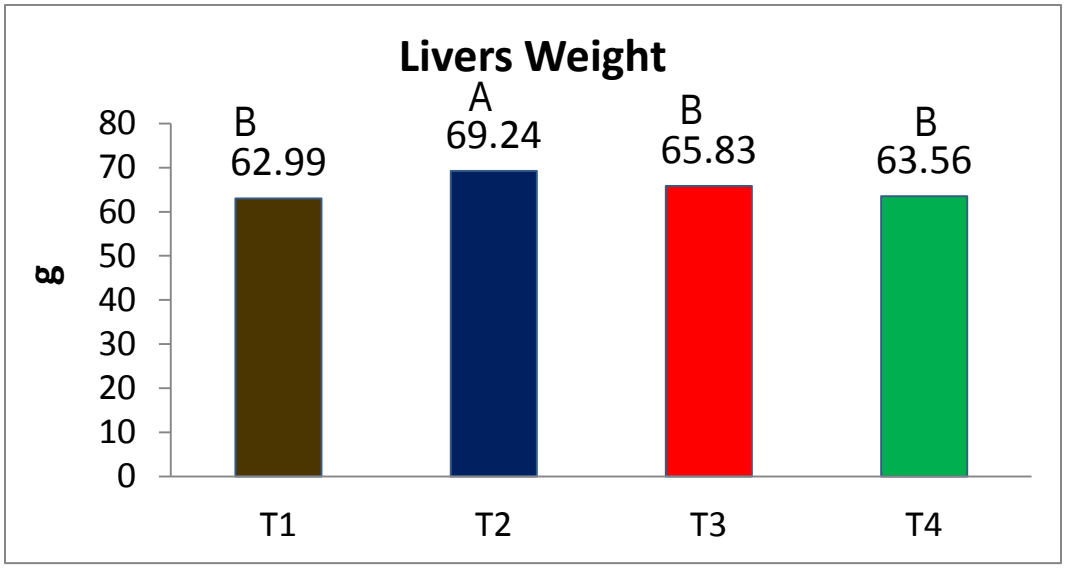


Figure 4- The liver coloration did show yellowness, a clinical sign of fatty liver



Graph 4- Liver weight showed to have consistent trend with increasing added fat level across the four treatments

TRT	Fat %
1	**9.1[b]**
2	**9.7 [b]**
3	**9.0 [b]**
4	**11.7[a]**

Table 1- The liver fat % was higher for rabbits inT4 than for those in T1, T2 and T3

Conclusion

The partial substitution of a commercial concentrate basal diet with 6% addition of the fat supplement daily weight gain and feed conversion ratio, but not carcass yield while also increasing the liver fat content in fattening rabbits, whereas the 4% level of addition was not reflected in higher liver fat content.

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ORGANOLEPTIC PROPERTIES OF ICE CREAM ELABORATED WITH BOVINE AND CAPRINE MILK MIXES.

Robert K. Ryan and Leyda Ponce de León
Department of Animal Science

Abstract

Four types of hard serve vanilla ice cream were manufactured using goat milk at 100%, 50% and 25%, and 100% cow milk with a standard percentage of total solids (11%), sugar (13%), fat (10%), stabilizers (0.25%). These were also evaluated for textural and sensory characteristics. A sensory test was held to determine publics preference with the four ice cream mixes. The samples were ranked on preference with different numbers from 1 to 4 (one being the most preferred and four the least). The goal of this study is to analyze the different organoleptic properties (smell, taste, texture) of four mixes of caprine milk with bovine milk to find the optimal amount of caprine milk to make an ice cream with the same organoleptic qualities of one made with 100% goat milk and reduce production cost. Preliminary results show ice creams made with 50% caprine milk had higher acceptability between volunteer tasters, and had a lower melting rate. Conclusion, thus it is concluded that the acceptable quality of ice cream similar to 100% goat milk can be prepared by using 50% goat milk and 25% cow milk.

Keywords: Goat, Ice Cream, Composition, Sensory Analysis

Resumen

Cuatro tipos de mantecados “hard serve” de vainilla fueron fabricados a partir de leche de cabra al 100%, 50% y 25%, y leche de vaca a 100% con un porcentaje de nivel de sólidos totales (11%), azúcar (13%), grasa (10%), estabilizadores (0.25%). Estos fueron evaluados para características de textura y sensoriales. Se realizó una prueba sensorial para determinar la preferencia del público con las cuatro mezclas de mantecado. Las muestras se clasificaron de acuerdo a la preferencia con números del 1 a 4 (uno siendo el más preferido y cuatro el menos). El objetivo de este estudio es analizar las diferentes propiedades organolépticas (olor, sabor, textura) de cuatro mezclas de leche de cabra con leche bovina, para encontrar la cantidad óptima de la leche de cabra que tenga las mismas cualidades organolépticas de un mantecado manufacturado con 100% leche de cabra y reducir los costos de producción. Los resultados preliminares muestran que el mantecado hecho con un 50% de leche caprina tuvo mayor aceptación entre los panelistas voluntarios y obtuvo una tasa de fusión más bajo que los otros mantecados. Se concluye que la calidad aceptable para elaborar un mantecado similar a 100% de leche de cabra se puede preparar mediante el uso de 50% de leche de cabra y leche de vaca 25%.

Palabras Claves: Cabra, Mantecado, Composición, Análisis sensorial

Introduction

Organoleptic properties are defined as the aspects of food or water that individuals experience via the senses (taste, touch, smell and sight). The particular taste, smell and color of goat milk comes from higher numbers of three fatty acids (caproic, caprilic and caprine) which are present in lower quantities in cow’s milk (Yanguilar, 2013).

The demand of goat milk increases thanks to its capability of being a possible alternative to bovine milk, due to people with cow milk allergies and other gastro-intestinal illnesses (Haenlein et. al., 2004). Although there is still much research needed to find the exact reason for cow milk allerginicity, researches have correlated many allergic reactions to the presence of a-s-1-casein which is one of the major proteins in cow milk and there's very few found in goats (Haenlein et. al., 2004). Another reason for its growing demand is because goat milk has shown to possess better health values than cow milk (McGhee, 2015), and the use of goat milk products such as cheeses and yogurts are branded as "gourmet" in developed countries.

The production of goat milk has become a growing industry, according to survey by the USDA (2016) milk goat inventory has increased a 3% from 2015 to 2016 in the United States. Although it has increased production over the years, caprine milk still needs further scientific research on its sensorial, texture and nutritional characteristics, since its essential for the future of the dairy goat industries. The goal of this study is to find the optimal amount of goat milk to manufacture an ice cream with similar organoleptic qualities as a 100% goat milk ice cream and lower goat ice cream production cost.

Materials and Methods

Obtaining the goat milk

The goat milk was obtained from "Aprisco Lechero Mercado & Crespo" farm in Aguada. Milk was transported in an ice chest and pasteurized upon arrival at 64 Celsius (°C) for 30 minutes prior to mixing (Figure 1).

Ice Cream Mixes

The ingredients for the mixes were goat and cow milk, sugar, unsalted butter, dry skim milk and gelatin. The standard value we want for each ice cream is 13% sugar, 11% total solids, 10% fat and .25% stabilizers for each mix. Composition analysis of the milk, butter and dry skim were determined prior formula calculation. To determine the pounds needed of the different ingredients we used the Serum Point Method (Goff, 2013)). As flavoring, we used vanilla bean strands.

Ingredients were mixed and heat treated at 64°C for about 20 minutes. All ingredients were weighted and mixed in a stainless steel container. Later on, the ingredients are heated and mix until they dissolve completely. Once uniformed, the ice cream mix was processed through a HC-5000 homogenizing machine (Figure 2) with a force between 2000-5000psi. Mixes are held for 24hrs in a freezer at -5°C, then frozen for 20 minutes in an ice cream machine (Figure 3) Emery Thompson® (Model 20NW) and stored in plastic containers for 24hrs in an ultra-low freezer (VWR Model 5728) at -39°C. Ice cream will be analyzed for composition and melting and sensory properties.

Figure 1. *Milk Pasteurization.*

***Figure** 2. Mixes being homogenized without sugar.*

Figure 3. *Ice cream machine.*

Compositional Analysis

Mojonnier Analysis

This analysis consists of three consecutive extractions with Ammonium hydroxide (NH4OH), Petroleum ether and Ethylic ether, and propyl alcohol (95%) (Werh, 2004). Between each extraction, mixtures are left standing for 30 minutes. Subsequently, the organic layer of the extraction was decanted into glass dishes. The organic layer left on the glass dish is then heated for 15 minutes to evaporate the solvent. Finally, the product is placed in the oven for 15 minutes and weighted afterwards. This weight is the fat content from the ice cream and represents its fat percentage.

CEM SNG by subtraction

The milk and ice cream were analyzed to obtain its Fat Solid content. This is done with the CEM-Smart System 5. In this system the sample type to be analyzed is selected and the machine is calibrated with fiber-glass pads placed inside (Figure 4). The ice cream is heated by the CEM microwave Smart System 5, the water is evaporated and difference in weight is the total solids percentage. The difference between the percentage and 100 will be the total solid percentage value.

Figure 4. *CEM calibrating pads prior to analysis.*

Melting Test

For this test four samples of 1 ounce were prepare with 2 replicas per sample. The fusion test consists of accumulating the melted ice cream in 10 minute intervals for an hour in a controlled room temperature of 23-24˚C. Coffee filters were used with Erlenmeyer bottles to filter and collect the melting ice cream (Ohmes et al, 1998 y Abd El-Rahman et al, 1997).

Ranking Test

For the sensory analysis a ranking test was held at the Sensory Analysis Lab in the Center of Investigation of Food and Technology in the University of Puerto Rico at Mayagüez. Thirty Non-trained panelist were offered four different ice cream samples with aleatory 3-digit code and order. They had to write each code in the order given and evaluate for preference. Each sample was ranked from one to four on preference. One being the most preferred and four the least. All data is then tabulated in Excel Microsoft.

Results and Discussion

Composition

The following values were calculated to verify that the ice cream mixes were made properly. Fat and nonfat solid are approximately in the standard values selected. This is done to make sure mixes are not adulterated.

Table 1. *Composition of four different ice cream samples manufactured with bovine and caprine milk.*

Ice Cream	Total Solids (%)	Fat (%)	Nonfat Solids (%)
100% Goat	22.37	9.87	12.5
25% Goat	22.40	9.68	12.72
50% Goat	22.04	9.58	12.46
100% Cow	20.74	9.22	11.52

Fusion

In this test the ice cream manufactured with 25% goat milk had the highest melting weight with a total of 5.52g, shown in Table 2. Secondly, the ice cream with 100% goat milk had a difference of 0.16g lower than the previous ice cream mentioned. On the other hand, the ice cream with 50% had the lowest melting rate (Figure 5), while the ice cream with 100% cow milk had the second lowest meting rate of all.

It can be observed in figure 5 how the ice cream with 25% of goat milk increased melting rate significantly compared to other ice creams between 20 and 30 minutes. This behavior is due to a cover of ice crystals and/or free water over the ice cream, which provokes a faster melting rate. According to Muse (2004), melting rate increases as ice crystal size increase, as flow path becomes less obstructed. On the contrary the other ice creams did not form as many crystals and show a much steady increase in melting rate.

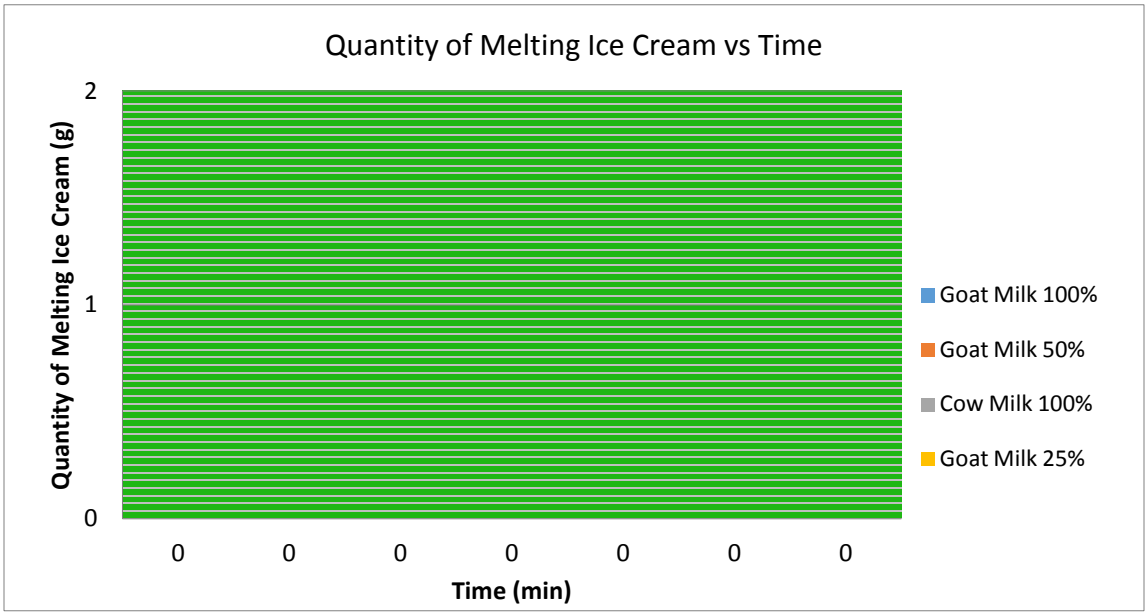

Figure 5. *Relation between melted ice cream and time in 10 minute intervals for an hour for each ice cream with 100%, 50%, 25% caprine milk and 100% bovine milk.*

Table 2. *Total melting weights of four different ice cream samples.*

Ice Cream	Total Melting Weight(g)
100% Goat	5.36
25% Goat	5.52
50% Goat	3.66
100% Cow	4.45

Sensory Test

Thirty volunteering students and faculty members of the University of Puerto Rico were given four samples of ice cream made with bovine and caprine milk. According to 30 volunteer testers the ice cream with the most balance mix of both milks was the ice cream made with 50% goat milk with an intensity of 2, shown in Table 3. Testers described the ice cream to be smooth and delicious. Most volunteers found the ice creams made with goat milk more flavorful than the one made with only cow's milk. This helps determine the acceptability of the ice cream as a possible added value product.

Table 3. *Average intensity values of four different ice creams manufactured with caprine and bovine milk. This were ranked for preference by 30 Volunteers in a tasting panel. One being the most preferred and four being the least.*

Ice cream	Intensity
100% Goat Milk	2.12
25% Goat Milk	2.125
50% Goat Milk	2
100% Cow Milk	3.333

Conclusion

All four ice creams were analyzed for composition, sensory and melting rate. Fat and nonfat solid percentages are approximately close to standard values, which shows ice cream were made properly with no signs of adulteration.

Results show that the ice creams manufactured with 25% and 100% goat milk had the highest melting rate and fat content. On the contrary, ice creams made with 50% goat milk and 100% cow milk had the least fat content and melting rates.

The overall acceptability was higher in the ice cream made with 50% goat milk, which volunteers could not find a difference in flavor between this one and the 100% goat milk ice cream. Thus it is concluded that the acceptable quality of ice cream similar to 100% goat milk can be prepared by using 50% goat milk and 25% cow milk.

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EXERCISE INDUCED PULMONARY HEMORRHAGE IN RACE HORSES IN PUERTO RICO

V. Morales[1], S. Glass[1], J. De Angel[2], B. Vallejo[2], and A. A. Rodríguez[1]
University of Puerto Rico, Mayagüez Campus[1] and Equus Center of Veterinary Medicine[2]

Abstract

Exercise induced pulmonary hemorrhage (EIPH), a very common disease in race horses, is characterized by an alteration of the respiratory system, which depending on the magnitude, causes bleeding in lung passages making it difficult for the horse to breathe. In Puerto Rico the only partially effective defense against this disease is the preventive administration of a diuretic. EIPH is evaluated subjectively by endoscopies on a scale from 0-5; 0 corresponding to no hemorrhage and 5 to a severe condition. A data set was analyzed to determine factors associated with the incidence of EIPH in race horses in Puerto Rico and the preventive effect of the diuretic. The data were supplied by Equus Company and the factors to be considered were time of the year (month), sex of the animal, and distance of the race. The randomly sampled data represented 20% of the endoscopies recorded in 2014 or about 2,632 observations. Of this number of endoscopies 1,377 were from racing horses, representing 52% of the total. Of the 1,377 race horses, 488 presented some degree of EIPH, equivalent to 35% of the population. The frequency of EIPH was basically equal during the 12 months of the year, showing that factors like weather and length of daylight have little effect. Similar lack of effect was observed for sex, as 243 of the 488 horses with the condition were females and 245 were males. Horses participating in shorter races had a higher incidence of EIPH than those in longer races. Results also showed that 426 of the 488 horses that were administrated the diuretic still manifested some degree of hemorrhage. In summary 35% of the race horses studied presented pulmonary hemorrhage; time of year and sex did not alter the incidence, while races of shorter distance showed more tendency to promote the disease. The diuretic was not effective as a preventive method for EIPH.

Keywords: Race horses, Exercise Induced Pulmonary Hemorrhage, Diuretic

Resumen

Hemorragia Pulmonar Inducida por Ejercicio (HPIE), es una condición común en caballos de carrera. La condición, se caracteriza por la alteración del sistema respiratorio del animal y dependiendo del nivel causa sangrado en los pasajes del pulmón complicando la respiración del caballo. En Puerto Rico el único método parcialmente efectivo contra esta condición es la administración de un diurético. HPIE es evaluado subjetivamente por endoscopias en una escala de 0-5; 0 correspondiendo a ningún sangrado y 5 la condición en su fase más severa. Se analizó una data para determinar factores asociados con la incidencia de HPIE en caballos de carrera en Puerto Rico y evaluar el efecto preventivo del diurético. La data fue suministrada por Equus Centro de Medicina Veterinaria y los factores evaluados fueron mes del año, sexo y distancia de la carrera. Se seleccionó aleatoriamente 20% de las endoscopias llevadas a cabo en el 2014 o un total de 2,632 observaciones. De esta cantidad de endoscopias 1,377 eran caballos de carrera, representando 52% del total. De los 1,377 ejemplares 488 presentaron algún grado de HPIE, equivalente a un 35% de la población. La frecuencia de HPIE fue similar durante los 12 meses del año, demostrando que el clima y la duración de luz no afecto la incidencia. Asimismo se

observó poca diferencia entre al evaluar el efecto sexo, ya que 243 de los 488 fueron hembras y 245 fueron machos. Caballos participando en carreras de distancias cortas presentaron mayor incidencia de HPIE que ejemplares en carreras largas. Los resultados también mostraron que 426 ejemplares de los 488, fueron tratados con el diurético, sin embargo presentaron algún tipo de grado de HPIE. En conclusión 35% de los caballos estudiados presentaron algún grado de HPIE; el mes del año y el sexo no son factores que afectan la incidencia de HPIE mientras que carreras de distancias cortas mostraron más tendencia a la condición que las carreras largas. La administración del diurético no funciona de manera efectiva como método preventivo de HPIE

Palabras Claves: Hemorragia Pulmonar Inducida por Ejercicio, Caballos de Carrera, Diurético

Introduction

Most race horses in Puerto Rico are thoroughbreds, a breed known for its speed and agility, and commonly used for racing. However these animals are more likely to suffer several health conditions than those of other breeds. The main cause of this condition is the methods being used to raise, train and feed these horses. Some symptoms of this condition are; fractured joints, colics and pulmonary hemorrhage (EIPH). EIPH usually causes bleeding in the passages of the lungs of the horse. EIPH was first identified as epistaxis (bleeding from the nostrils) after intense exercise, and a decrease in performance (Tobin, 2012). But later studies showed that the bleeding originates in the alveoli. If the bleeding is intense it can cover the alveoli space, also bleeding in the nose sometimes leads to sudden death. Annually EIPH causes horse owners to spend heavily on medical treatment, special training and veterinary services. Therefore this illness represents a medical and economical hazard to the local racing industry.

There is no cure for EIPH or for the specific factors that causes the bleeding. The only partial remedy available in Puerto Rico and the U.S.A is the administration of a diuretic called furosemide. The diuretic draws the water away from the lungs and keeps the blood pressure from getting too high (Tobin, 2012). However administration of the diuretic has been a very controversial topic in the racing industry. Some experts believe the drug does not function properly while others consider it the solution for EIPH. This experiment was conducted to evaluate the different factors that may induce EIPH and to evaluate the preventive efficiency of the diuretic in the race horse industry.

Methodology

This experiment sought to evaluate the effect of time the year, sex, and the distance of the race on EIPH, as evaluated by endoscopies carried out in horses shortly after finishing a race. Endoscopies are a common technique used to evaluate the pulmonary tract and different degrees of EIPH. The latter are expressed on a scale from 0-5, 5 being the most severe in which epistaxis is present. In Puerto Rico the main veterinary company attending the racing industry is Equus “Centro de Medicina Veterinaria”. This company has several veterinarians that perform the endoscopies and fill out a data sheet for each patient of the information to be evaluated. Random selection of 20% of the endoscopies carried out in 2014 and chi square testing was performed to determine the frequency of EIPH as affecting by the factors under study. A significant value in a chi square test was defined as the $P < 0.05$ level. After the chi square testing, charts and tables were created to help reach clear conclusions as to which factors influence EIPH.

Results and Discussion

We collected data on a total of 2632 individuals yet only 52% of these were horses that were racing. Out of the 1377 that were racing 889 did not present any degree of EIPH, while the remaining 488 horses did present the condition, which is equal to 35% of the racing horses. These were evaluated for EIPH by degrees as shown in Figure 1. Degree 1 was the one of highest incidence. This 488 horse data set was statistically analyzed by month. It was expected that during warmer months of the year the frequency of EIPH would be higher. However, as Figure 2 shows EIPH was found to be randomly distributed throughout the year, with no higher frequency months of note. The number of cases was about 40-50 per month, June being the month of lowest incidence and November the highest. The value of the chi square was 0.4359 which confirms that there was no significant difference of EIPH between months of the year. It was also expected that male horses would have a higher tendency towards EIPH than mares, yet Figure 3 shows no association of EIPH frequency with the sex of the horse. Of the 488 affected animals, 245 were males and 243 were mares. The chi square by sex test gave a not significant value of 0.5879. However with regard to distance of the race the chi square test did yield a significant value of 0.02. The opposite result, that longer races would provoke more hemorrhage had been expected. As shown in Table 1 shorter races had a higher tendency to cause EIPH. Races of 400-1200m (Distance 1) presented a total of 189 cases with different degrees of EIPH whereas the races of 1300-1400m (Distance 2) and >1600m (Distance 3) presented 155 and 122 respectively. The efficiency of the diuretic in preventing EIPH was supported by these data. Figure 4 shows that of the 488 race horses that exhibited some degree of EIPH, 426 were administrated the diuretic before the race and only 51 were not. The value of the chi square test was 0.3759, nearly a significant value. Therefore the diuretic is dubious worth as a preventive method.

Figure 1

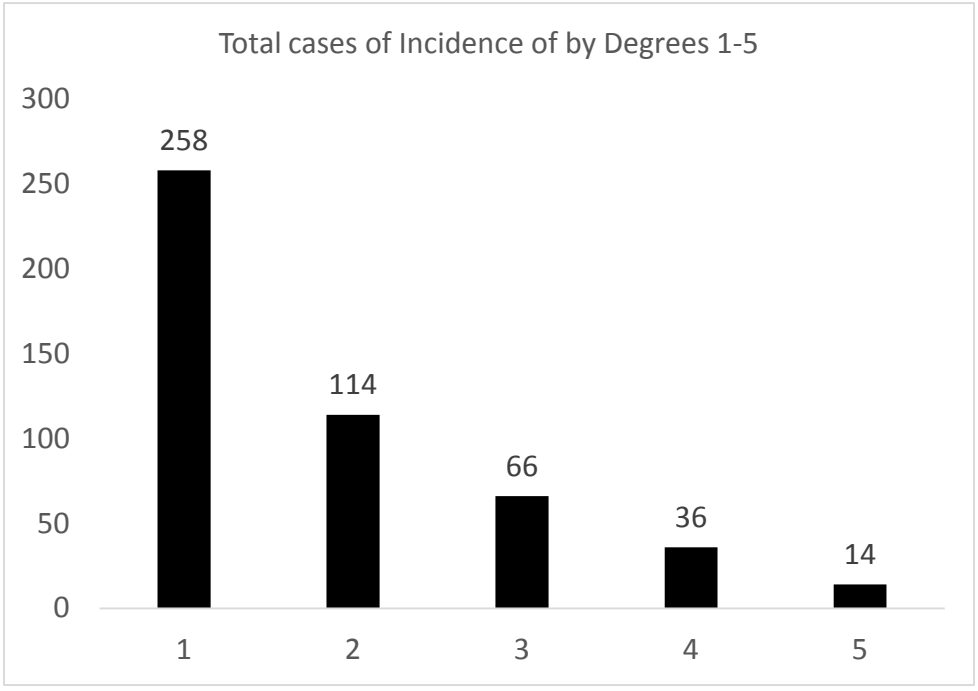

Figure 2

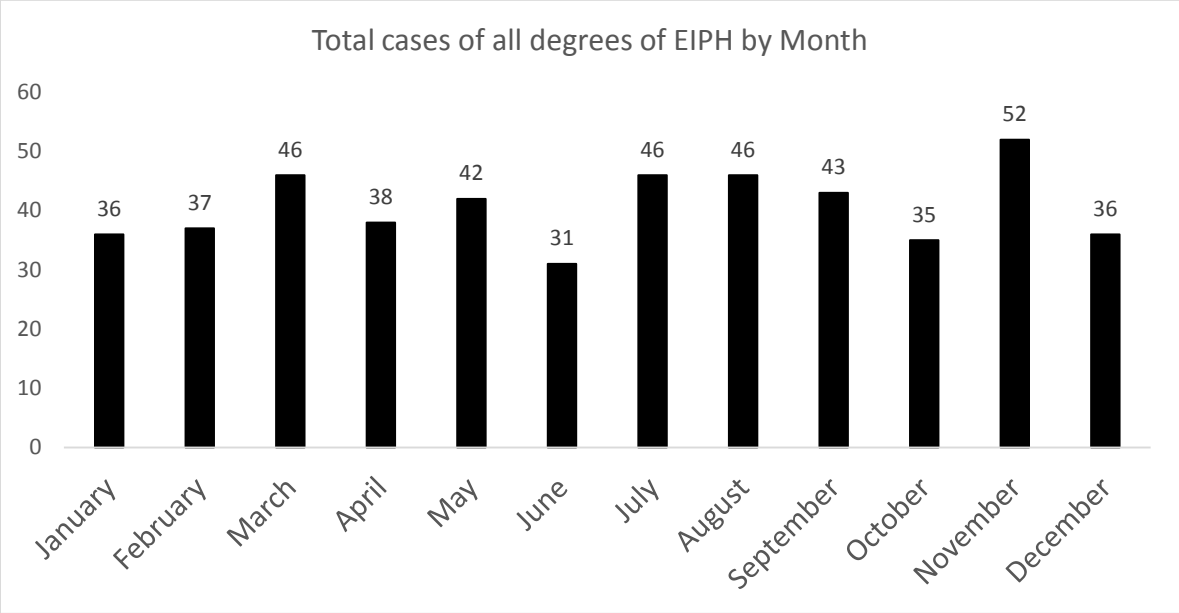

Figure 3

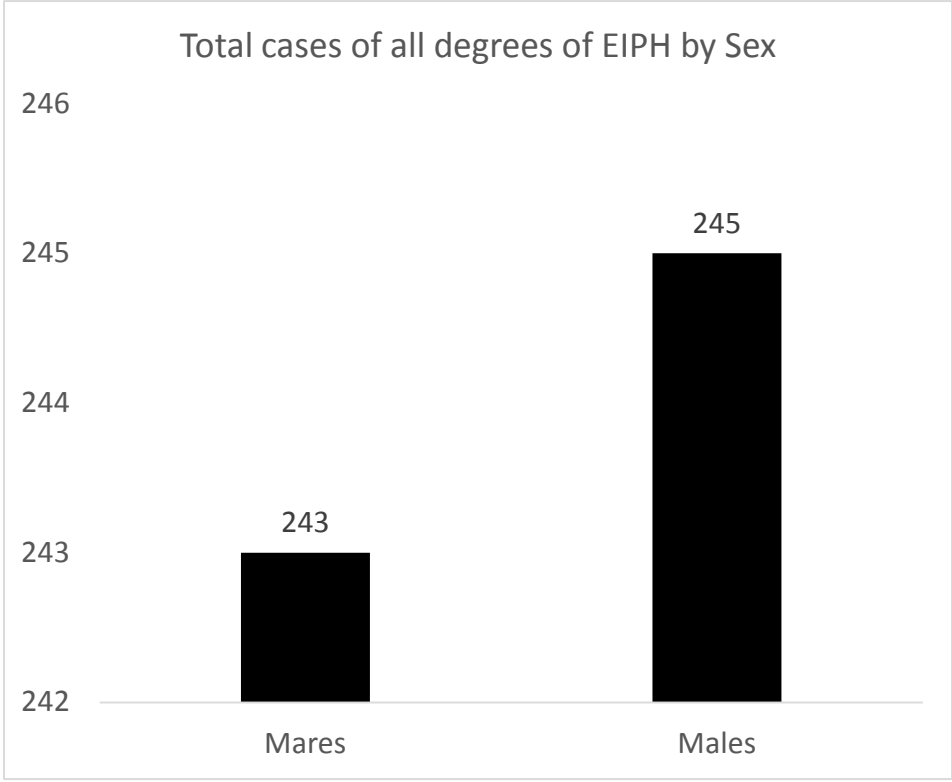

Figure 4

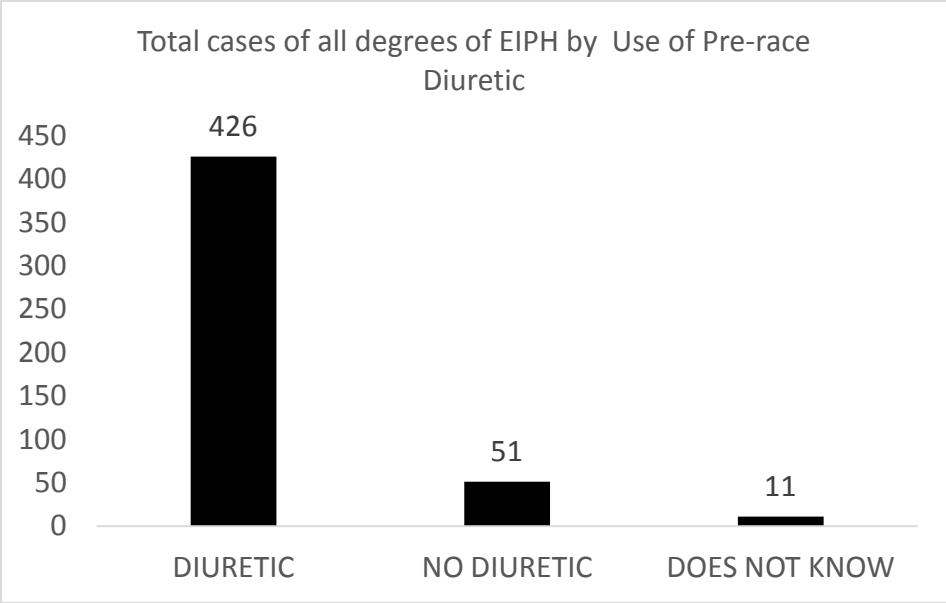

Table 1: Incidence of EIPH by Distance of the Race

400-1200m	93	54	23	14	5
1300-1400m	92	24	22	12	5
>1600	66	31	12	10	3
Does not know	5	7	8	0	0

Conclusions

Of the local racing horse popularity sampled 35% presented some degree of EIPH. EIPH frequency showed as relatively even distribution during all months of the year. Both sexes were affected with nearly the same frequency. Participation in shorter races resulted in a higher tendency toward EIPH rather than longer races. Perhaps in shorter races horses are more strongly impacted by the change in speed and strength in much less time, thus producing more bleeding. The diuretic treatment showed no sign of effectiveness as a preventive method in the present data. This may be because the diuretic is of no real usefulness for this purpose or because it's not being administrated correctly.

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EFFECT OF PLANT EXTRACTS IN THE CONTROL OF DISEASES CAUSED BY PHYTOPATHOGENIC FUNGI

Santiago Acosta and M. Librán
University of Puerto Rico-Mayagüez, Mayagüez, PR 00680

Abstract

In recent decades the use of harmful chemicals in conventional food production has been cause for health and environmental concern among the general population. Researchers are looking to find organic alternatives to substitute harmful chemicals practices. The objectives of this study, is to identify phytopathogenic fungi in crops and to develop treatments by using plants with fungistatic properties. Cultivar of lettuce (*Lactuca sativa*) "Black Seed Simpson" and culantro (*Eryngium foetidum*) were selected as the crops to be evauated. Crops selected to develop treatments were, Garlic (Allium sativum), ginger (*Zingiber officinale*) and basil (*Ocimum* spp.). Damaged tissues, from lettuce and culantro, were dissected and placed in PDA culture. Fungi present were isolated, purified, and identified. Crops were inoculated with fungi identified to determine pathogenicity. Nine treatments under concentrations of 25, 50, and 75% of plant extracts were formulated, applied, and evaluated in vitro. The fungi identified were *Fusarium* spp. and *Rhizoctonia* spp. The pathogenicity tests show necrotic spots developed on the most outer leaves of culantro plants inoculated with *Rhizoctonia* spp. No symptoms of pathogenicity were observed on lettuce plants inoculated with *Fusarium* spp. The in vitro test of the treatments showed that garlic had the most inhibitory effect over the fungi's radial growth and normal morphological development on both. Ginger was the second most inhibitor while the basil treatment aided the growth and development of the fungi.

Keywords: Plant extracts, *Fusarium*, *Rhizoctonia*

Resumen

En las últimas décadas el uso de productos químicos nocivos en la producción convencional de alimentos ha causado preocupaciones relacionadas al bienestar del ambiente y la salud de la población general. Los investigadores buscan encontrar alternativas orgánicas para sustituir el uso de sustancias químicas nocivas. Los objetivos de este estudio son identificar hongos fitopatógenos en cultivos y desarrollar tratamientos empleando el uso de plantas con propiedades fungistáticas. El cultivar de lechuga (*Lactuca sativa*) "Black Seed Simpson" y el recao (*Eryngium foetidum*) se seleccionaron como los cultivos a ser evaluados. Los seleccionados para desarrollar los tratamientos fueron ajo (*Allium sativum*), jengibre (*Zingiber officinale*) y albahaca (*Ocimum* spp.). Tejidos dañados, de la lechuga y el culantro, se diseccionaron y colocaron en cultivo PDA. Se aislaron, purificaron e identificaron los hongos presentes. Los cultivos se inocularon con hongos identificados para determinar su patogenicidad. Nueve tratamientos, en concentraciones de 25, 50, y 75% de extractos de plantas, se formularon, aplicaron, y evaluaron in vitro. Los hongos identificados fueron *Fusarium* spp. y *Rhizoctonia* spp. Las pruebas de patogenicidad mostraron desarrollo de manchas necróticas en las hojas más externas de las plantas de culantro inoculadas con *Rhizoctonia* spp. No se observó síntoma de patogenicidad en plantas de lechuga inoculadas con *Fusarium* spp. La prueba in vitro de los tratamientos mostró que el ajo tenía el efecto más inhibitorio sobre el crecimiento radial y

desarrollo morfológico normal de ambos hongos. El jengibre fue el segundo más inhibidor mientras que el tratamiento de albahaca ayudó al crecimiento y desarrollo de los hongos.

Palabras Claves: Extractos de plantas, *Fusarium*, *Rhizoctonia*

Introduction

The health and environmental concerns caused by the use of harmful chemicals in conventional food production have lead investigators to research on organic alternatives that substitute these harmful chemicals. Pesticides in plants may be absorbed, stored, metabolized, and/or released to the environment. These processes determine both the pesticide's impact on the plant and its residue characteristics (Norris, 1974). Leaf crops, such as lettuce (*Lactuca sativa*) and culantro (*Eryngium foetidum*) are consumed fresh and retain chemicals applied on its tissues. In Puerto Rico, these two crops are fundamental food in peoples' diet. The Puerto Rico Department of Agriculture reported 2.7 million dollars between 2013-2014 in its Agricultural Gross Income (Puerto Rico Department of Agriculture, 2015), combined from both crops. Interest in organic food has grown remarkably as consumers and marketers react to popular media about health and environmental effects of pesticides, genetically-modified organisms, and food safety (Hughner, 2007). Consumers buy organic because of their desire to avoid the chemicals used in conventional food production (Ott, 1990; Jolly, 1991; Wilkins and Hillers, 1994). It is imperative to develop research conducted to evaluate alternatives system in pest management without harmful substances that affect the environment as well the human being health. Some studies, such as "Evaluation of plant-derived products against pests and diseases of medicinal plants: A review" by R.T. Gahukar (2012) and "Efecto toxicológico de extractos vegetales sobre *Fusarium oxysporum* bajo condiciones controladas." by Wilder Lenin Yugcha Quintana (2015), have studied and evaluated the effects of plant extracts in pest control. The objectives of this study are to identify phytopathogenic fungi in economically significant crops in Puerto Rico (such as lettuce and culantro) and to develop treatments by using extracts from plants with reported fungistatic properties.

Methodology

Cultivar of lettuce (*Lactuca sativa*) "Black Seed Simpson" and culantro (*Eryngium foetidum*) were selected as the crops to be evaluated. Damaged tissues, from lettuce and culantro obtained by local farmers in Mayagüez, P.R., were dissected and placed in PDA culture. Fungi present were isolated, purified, and identified. Various media, such as malachite green agar, *Rhizoctonia* selective media, V8 culture media, were selected and used to isolate fungi based on a preliminary morphological identification. The fungi were identified taxonomically by the key of Barnett and Hunters in the "Ilustrated Genera Of Imperfect Fungi (1998). Crops were inoculated, (Fig.1.), with the isolated fungi to determine pathogenicity. Colonies were increased in liquid and solid media following the procedure from Vasquez et. al. (2009) and Castellanos et. al (2011). Nine treatments, replicated 3 times, containing three different plant extracts were evaluated following the procedure from. These were , concentrations of 25, 50, and 75%, of Garlic (*Allium sativum*), ginger (*Zingiber officinale*) and basil (*Ocimum* spp.). The treatments were evaluated in vitro.

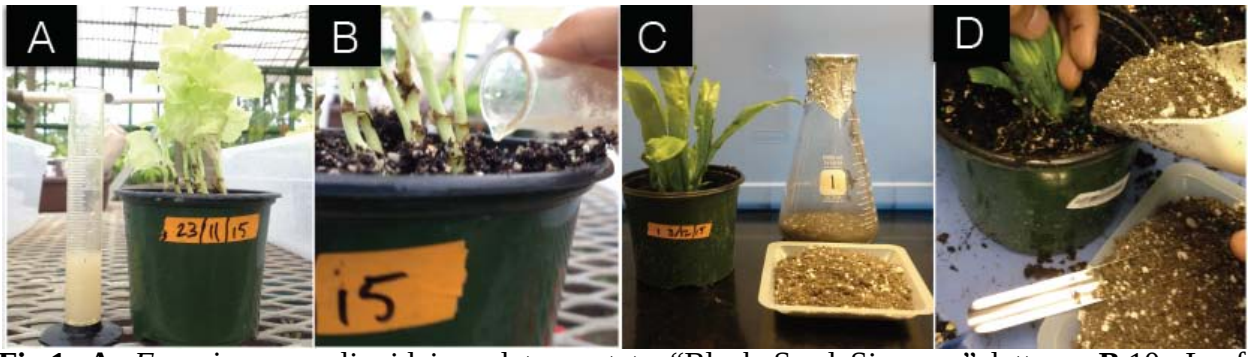

Fig.1. A. *Fusarium* spp. liquid inoculate next to "Black Seed Simpson" lettuce. **B.**10mL of media containing *Fusarium* spp. inocule applied on lettuce's rhizosphere. **C.** *Rizoctonia* spp. solid inoculate alongside culantro. **D.**100g of growing media containing *Rizoctonia* spp. inocule applied on culantro's rhizosphere.

Results

The fungi isolated and identified from lettuce and culantro, were *Fusarium* spp.(Fig.2.) and *Rhizoctonia* spp.(Fig.3.). The pathogenicity tests shown necrotic tissue developed on the most outer leaves of culantro plants inoculated with *Rhizoctonia* spp. (Fig.4.). No symptom of pathogenicity were observed on lettuce plants inoculated with *Fusarium* spp. Through the in vitro treatments, results shown that garlic had the most inhibitory effect over the fungi's diameter growth. A normal morphological development was observed on the colonies of both fungi under the garlic treatment. Ginger was the second most inhibitor, weak and deficient morphological growth was observed on colonies of both fungi. The basil treatment promoted the growth and development of the fungi. Results shown that both treatments, garlic and ginger, delayed the growth and development of both fungi as compared with the control treatments. Garlic extract treatments proved to be the most effective during the first six to eight days in both *Rhizoctonia* spp. and *Fusarium* spp.(Fig.6. and Fig.7.)

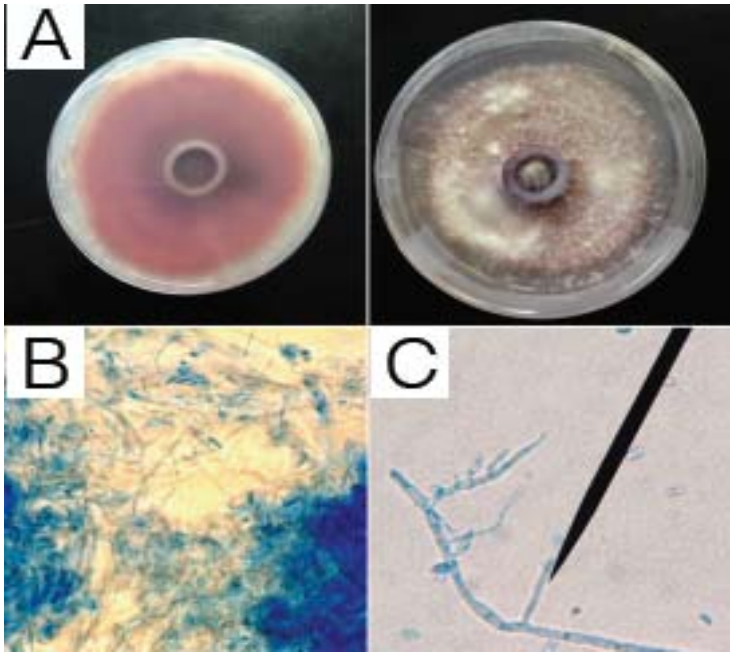

Fig.2. A. Colony of *Fusarium* spp. **B.** Mycelium and conidias **C.** Conidia formation

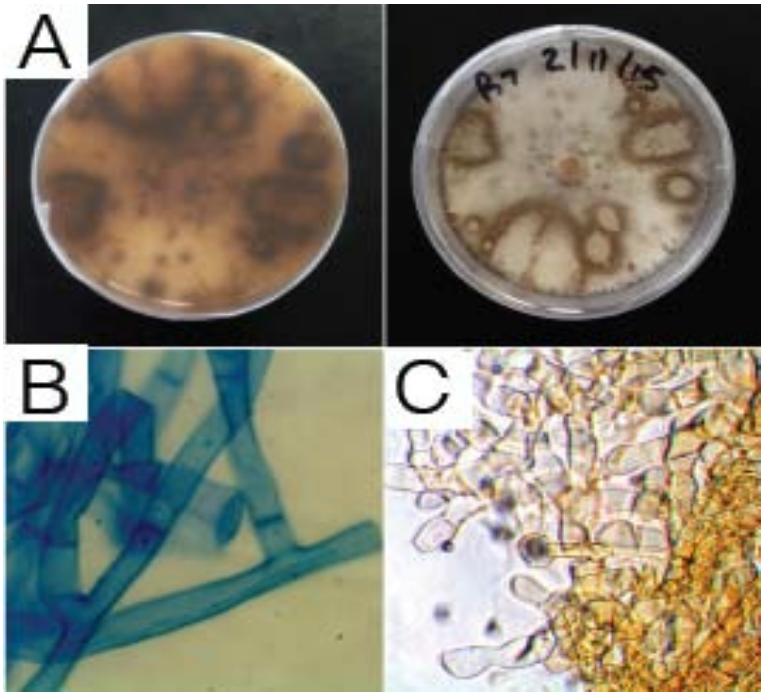

Fig.3. A. Colony of *Rhizoctonia* spp. **B.** *Rhizoctonia* spp. distinctive hypha branching at 90o **C.** Section of loose sclerotium.

Fig.4. Necrotic tissue developed on culantro plants after inoculation with *Rhizoctonia* spp.

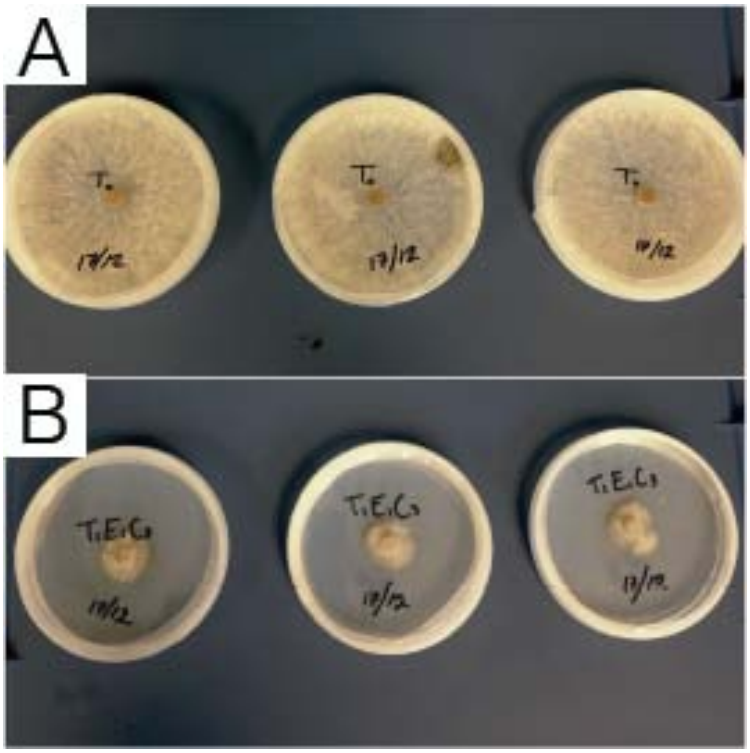

Fig.5. A. *Rhizoctonia* spp. control treatment colonies after four days of inoculation. **B.** *Rhizoctonia* spp. colonies scarce growth on PDA media containing 75% garlic extract after four days of inoculation.

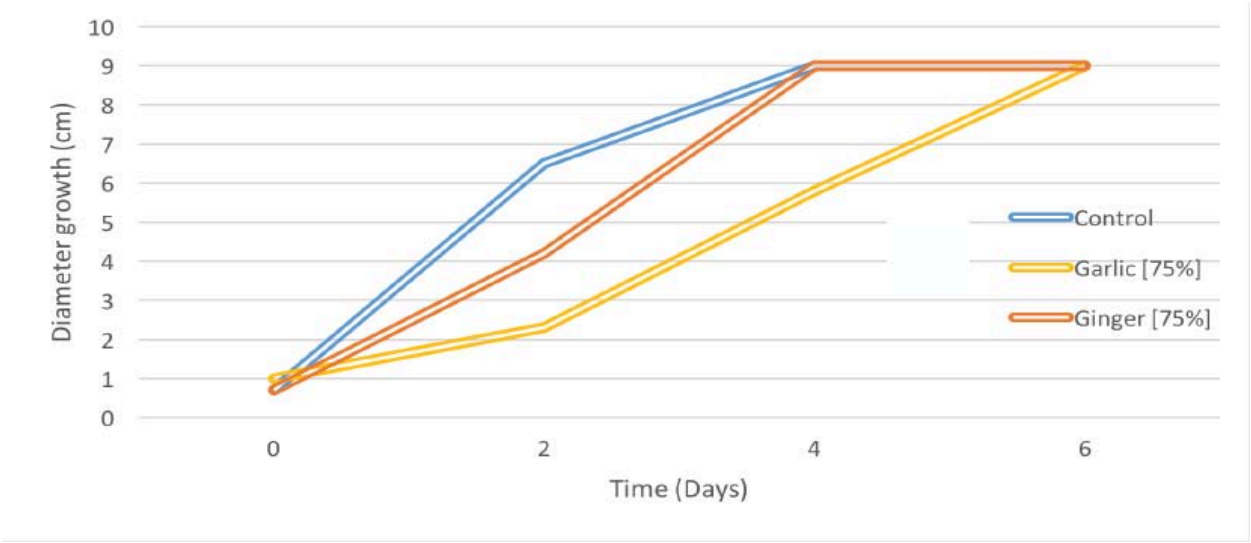

Fig.6. Effects of garlic and ginger extract treatments on diameter growth of *Rhizoctonia* spp.

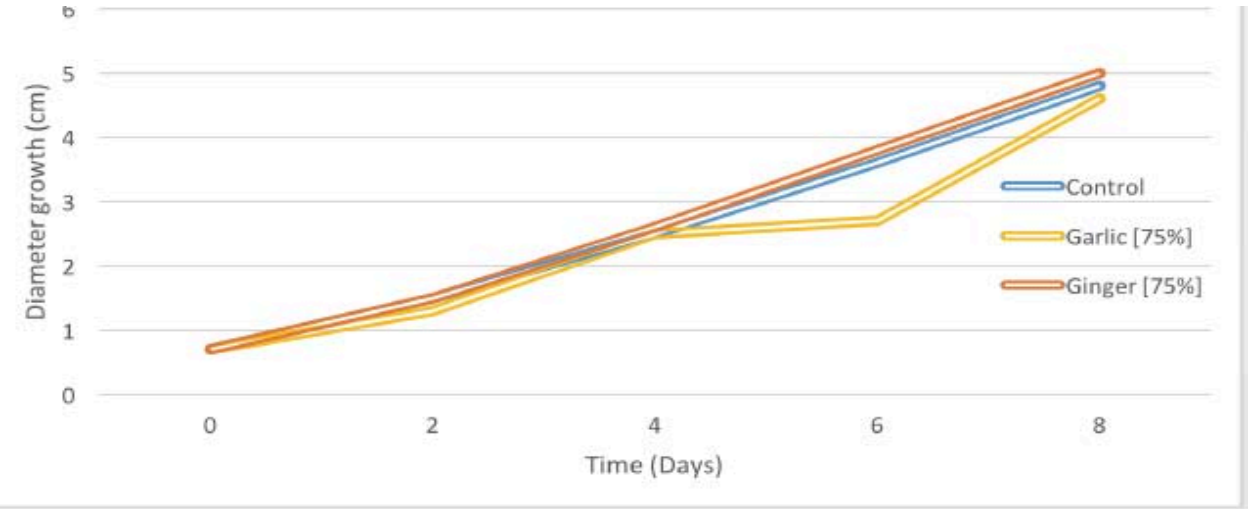

Fig.7. Effects of garlic and ginger extract treatments on diameter growth of *Fusarium* spp.

Conclusions

According with the results of this study, garlic extract at 75% concentration and ginger extract at 75% concentration could both be effective in reduction or inhibition of *Fusarium* spp. and *Rhizoctonia* spp. colonies growth and normal morphological growth. These could be an alternative to reduce the infestation caused by these two fungi on plant tissues. The optimum treatment in this study could be a combination of the 75% garlic and 75% ginger extracts, applied every 4 to 6 days.

Acknowledgement

This research was possible thanks to the funding of CariPac. The work done in this project couldn't have been possible without Dr. Maria del Carmen Libran's mentorship and guidance, graduate student Lilliam I. Cardona's help at every phase of the project, sub-graduate student Rey Cotto, Dr. Lydia Rivera, and lab technicians Victor Gonzalez and Luis Collazo for always being willing to share their knowledge and equipment.

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PHYTOPLASMAS, BACTERIA AND FUNGI FROM THE VASCULAR TISSUE OF TROPICAL PALMS IN PUERTO RICO

Rey Cotto, L. Simbaña and L. I. Rivera Vargas
Department of Agro-Environmental Science, Crops Protection Program
University of Puerto Rico at Mayagüez, Mayagüez, PR 00680
rey.cotto@upr.edu

Abstract

Microorganisms represent a unique range of life forms that invade and colonize plant vascular tissues. Of these, pathogens might occurs in the xylem and phloem inducing symptoms commonly known as wilt, necrosis and chlorosis. These diseases are generally caused by bacteria and fungi that infect through the roots and enter the plant vessels where they proliferate and obstruct water, minerals and cell products transport. This lead to impairment of the whole plant with eventual death. Thus it is important to study vascular pathogens to be able to know their diversity and plant host range. This information will allow us to develop solutions to these important agricultural problems. Palms are essential tropical ecosystems and are an important agricultural commodity. In 2012, Puerto Rico had about 255 palm tree farms with 1039 acres of production and annual revenues of 6 million dollars. This research seeks to identify potential vascular pathogens associated with symptoms observed in palms throughout the island. A total of 12 palms were sampled. DNA extracted from vascular tissue of *Roystonea borinquena* and *Cocos nucifera* was tested for phytoplasmas. In addition, 15 vascular fungi and 25 bacteria were isolated in culture media. DNA extracted from palm vascular tissue resulted negative for phytoplasmas. Molecular characterization of bacterial DNA showed that only 33% of the isolated bacteria have homology to previously reported species in databases. These were: *Bacillus subtilis*, a biological control agent, *Myroides odoratus* and *Sphingomonas* spp. A 22% of the sequences were associated with non-culturable bacteria and 67% of the isolated bacteria DNA have no homology in the databases. Fungal isolates were morphologically identified as *Fusarium oxysporum and Pestalotiopsis microspora*, this last species identification was confirmed by DNA analysis. Some microorganisms identified are commonly present in soil and are potential pathogens of plants. Pathogenicity tests will be conducted on greenhouse palms to confirm their association with observed symptoms.

Keywords: Palms, Lethal Yellowing, Fungi, Vascular Tissue, Bacteria, Arecaceae

Resumen

Los microorganismos representan una gama única de formas de vida que invaden y colonizan los tejidos vasculares de las plantas. De éstos, los patógenos pueden ser encontrados en el xilema y floema, induciendo síntomas que comúnmente son conocidos como marchitez, necrosis y clorosis. Estas enfermedades son causadas generalmente por bacterias y hongos que infectan a través de las raíces y entran en el sistema vascular de la planta en el que proliferan, obstruyen agua, minerales y el transporte de productos celulares. Esto puede conducir a un deterioro de toda la planta, con la eventual muerte. Por lo tanto, es importante estudiar los patógenos vasculares, para poder conocer su gama de huéspedes y su diversidad entre las plantas. Esta información nos permitirá desarrollar soluciones a estos problemas agrícolas. Las palmeras son parte esencial de los ecosistemas tropicales y son un producto agrícola importante. En 2012,

Puerto Rico tuvo alrededor de 255 fincas de palmeras, con 1039 acres de producción e ingresos anuales de 6 millones de dólares. Esta investigación busca identificar potenciales patógenos vasculares asociadas con los síntomas observados en las palmas a lo largo de la isla. Se tomaron muestras de un total de 12 palmas. El ADN extraído del tejido vascular de *Roystonea borinquena* y *Cocos nucifera* fue muestreado para fitoplasmas. Además, 15 hongos vasculares y 25 bacterias fueron aisladas en medios de cultivo. ADN extraído a partir del tejido vascular de palmas resultó negativo para fitoplasmas. La caracterización molecular del ADN bacteriano mostró que sólo el 33% de las bacterias aisladas tienen homología con especies previamente reportadas en las bases de datos. Estos fueron: *Bacillus subtilis*, un agente de control biológico, *Myroides odoratus* y *Sphingomonas* spp. Un 22% de las secuencias se asociada con bacterias no cultivables y 67% del ADN de las bacterias aisladas no tienen homología en las bases de datos. Los hongos aislados se identificaron morfológicamente como *Fusarium oxysporum* y *Pestalotiopsis microspora*, esta última identificación de las especies se confirmó por análisis de ADN. Algunos microorganismos identificados están comúnmente presentes en el suelo y son potenciales agentes patógenos de plantas. Las pruebas de patogenicidad se llevarán a cabo en las palmas bajo condiciones de invernadero para confirmar su asociación con los síntomas observados.

Palabras Claves: Palmas, Amarillamiento Letal, Fungi, Tejido Vacular, Bacterias, Arecaceae

Objective

Isolate and identify the spectrum of pathogens found in the vascular tissue of tropical palms in the island.

Introduction

Vascular pathogens represent a wide but unique group of microorganisms that colonize the xylem and phloem of plants during the parasitic phase of their life cycle. Bacteria and fungi, infect through roots and enter the water and nutrient conducting vessels where they proliferate and obstruct the transportation of water, minerals and cell products. As a consequence, leaves, vascular tissue and roots die, which lead to impairment of the whole plant and eventually to death of the plant. Thus, there is a need to study vascular pathogens to be able to know their diversity and plant host range.

In Puerto Rico, palms are aesthetic icons and key components of the island culture, ecology, and tourism industry. As an agricultural commodity, there are about 255 palm tree farms with 1039 acres of production and annual revenues of 6 million dollars. The detection of *Haplaxius crudus,* crucial insect vector of Coconut Lethal Yellowing (CLY) and the discovery of a phytoplasma from the group 16SrIV in royal palm (*Roystonea borinquena),* fishtail palms (*Caryota mitis*) and Carpentaria palm *(Carpentaria acuminata),* have sparked interest in the potential impact of the presence of the disease in the island. Recent findings showed that most symptomatic palms sampled throughout the island are negative for phytoplasmas. Thus, this research seeks to find other potential pathogens associated with symptoms similar to those caused by CLY in palms. Identify the microflora of the vascular tissue of palms is needed in order to understand their role and management of palm disease outbreaks.

Unfortunately, there is almost a complete lack of knowledge about the biology and life history of palm pathogens and their symptoms. A better understanding of these relationships could lead to

the identification and documentation of complex associations between plant-pathogen, and that may also help explain the impacts of potential alternative sources of inoculum (Maixner, 2010).

Material and methods

Palm survey: Symptomatic palms were surveyed at five locations of the western and northern region of Puerto Rico (**Figs**. **1 and 2**).

Tissue Extraction: Palm vascular tissue was collected using a drill as described by Harrison et al., (2009). Each sample was grinded and passed thru a sieve, the smaller pieces were transferred to an erlenmeyer flask containing ultra pure water. All samples were placed in a shaker for 15 minutes at 25ºC temperature. **Microorganism Isolation:** From each sample, 100µl of palm vascular tissue suspended in sterile water were spread in potato dextrose agar (PDA), nutrient agar (NA) and trypticase soy agar (TSA). After 2 days at 29ºC temperature, microorganisms were purified in their corresponding media. **DNA Extraction:** Bacterial DNA was extracted using the Wizard® Genomic Purification Kit and for phytoplasmas DNA extraction was performed using a modified procedure of the DNeasy Plant Mini Kit (Qiagen®). Fungi DNA was extracted using the Plant DNA Extraction Kit (Qiagen®). DNA quality and concentration was determined using a nano-spectrophotometer (Nanopearl, IMPLEN, Germany). Microorganisms DNA amplification: Bacterial DNA was amplified using PCR and primers U519F/1392R (Marchesi, et al., 1997), for fungi primers ITS4/ITS5 for ITS rDNA region were used (White et al., 1990). Phytoplasma DNA was amplified by direct PCR using P1/P7 primers (Deng and Hiruki, 1991) for the 16SrDNA region. Nested PCR were conducted using FU5/RU3 primers (Seemüller et al., 1994). **DNA Sequencing and Analysis:** Amplified PCR products were purified using QIAquick Purification Kit, Qiagen® and sequenced at commercial facilities (Macrogen, MD USA). DNA sequence data was analyzed using NCBI data base.

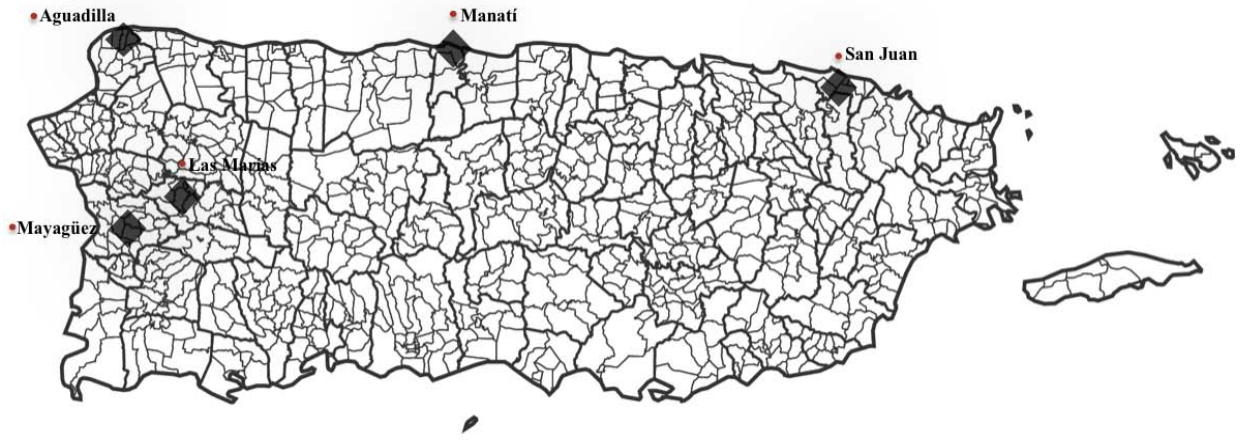

Fig. 1. Locations surveyed by collecting vascular tissue of palms at the western and northern region of Puerto Rico. Towns are highlighted by black diamonds.

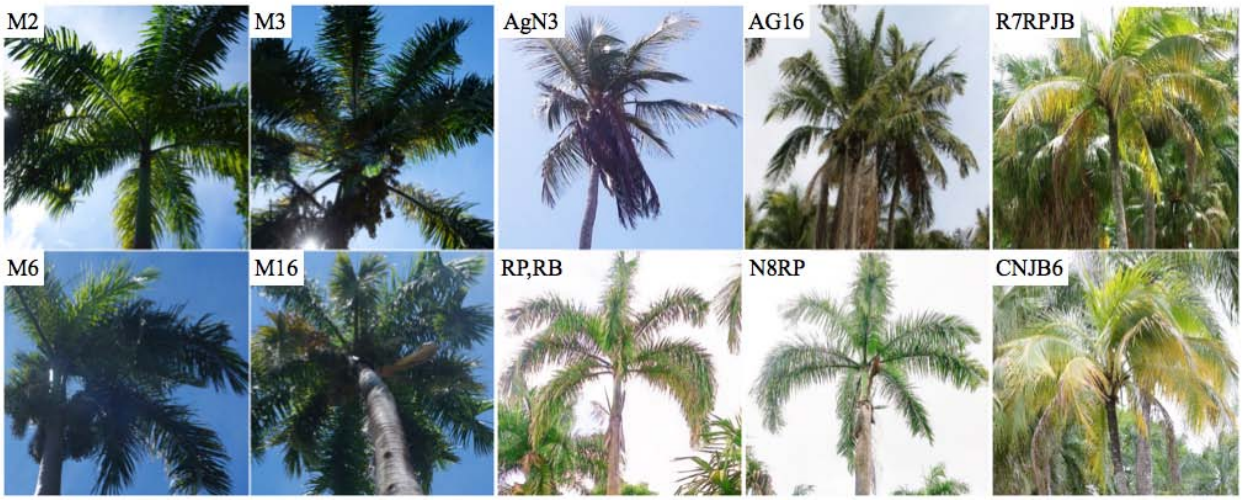

Fig. 2. Symptoms observed in palms. The following codes were used the towns: Ag = Aguadilla, M = Mayagüez and R, N or C = San Juan, and numbers to the palms.

Results

A wide diversity of bacteria (Fig. 3) and fungi (Fig. 4) were isolated from vascular tissue of symptomatic palms (n=15) (Fig. 2). Each organism was described based on morphology, structures, spores and biochemical tests (Figs. 8 and 9).

Bacteria: Forty eight percent (48%) of the isolated bacteria were Gram +. A PCR product of approximately 1500bp was observed in 1% agarose gels using primers U519F/1392R (**Fig**. **7**). Only 33% (9/25) of the isolated bacteria have homology with known species in databases. These were: *Bacillus subtilis*, *Myroides odoratus* and *Sphingomonas spp.*, the other 67% of the bacteria did not show homology with species in the databases, of these 22% are associated with non-culturable bacteria.

Fungi: Five fungal isolates were identified as *Pestalotiopsis microspora* and one as *Fusarium oxysporum* using morphological characters. Isolates identification were confirmed using DNA sequences data, showing 99% homology with species reported in databases. A PCR product of approximately 632bp was observed in agarose gels using primers ITS5F/ITS4R (**Fig**. **7**).

Phytoplasmas: A PCR product of approximately 750 bp was observed in 1% agarose gels using primers FU5/RU3 for nested PCR (**Fig.6**). PCR test was negative for phytoplasmas.

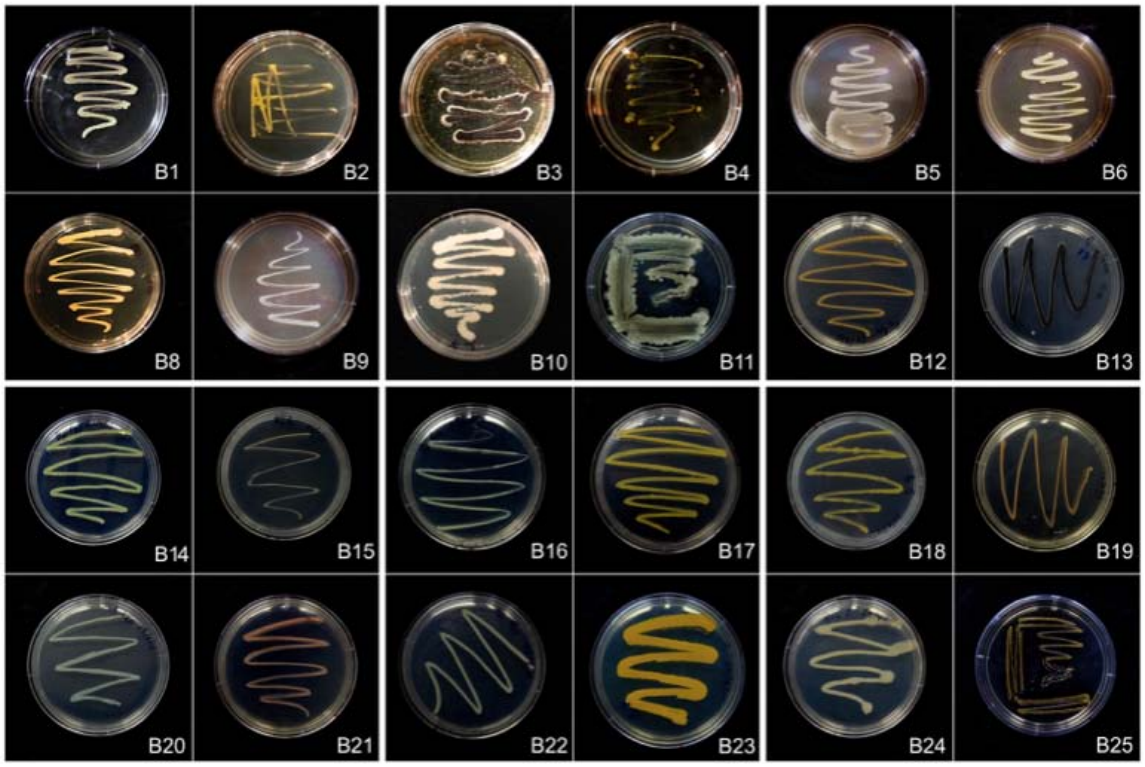

Fig. 3: Diversity of bacteria isolated from vascular tissue of the sampled palms on NA and TSA media.

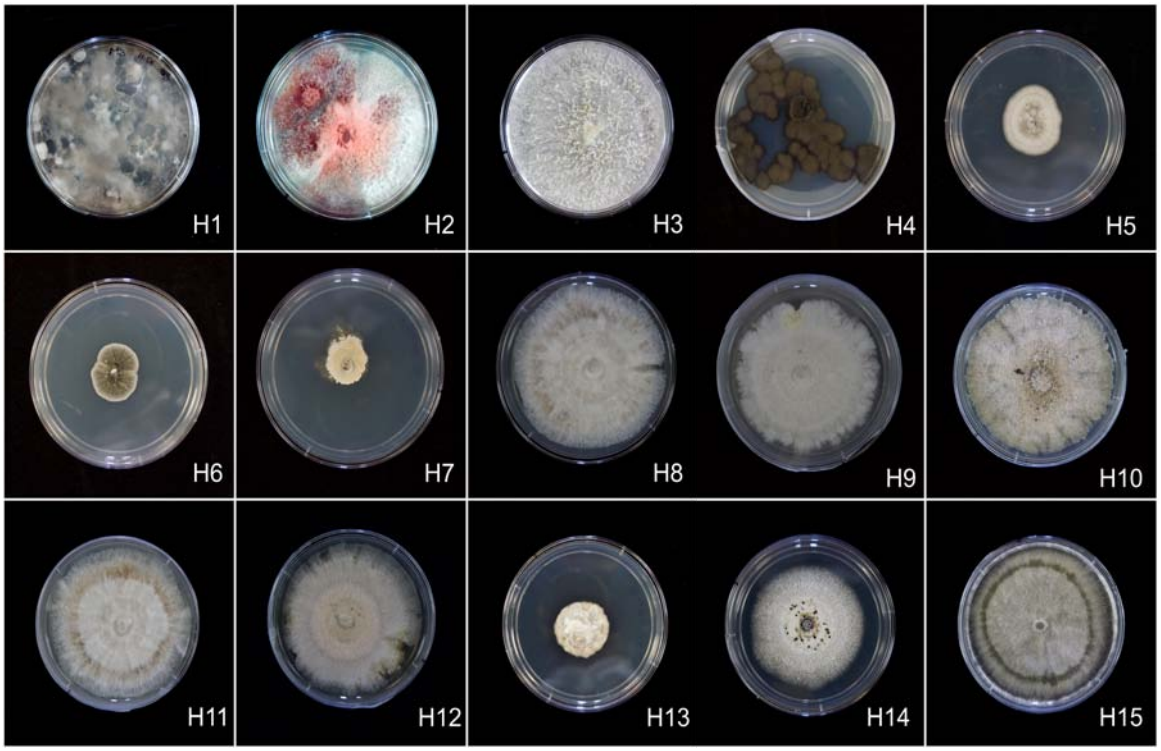

Fig. 4: Diversity of fungi isolated from vascular tissue of the sampled palms on PDA.

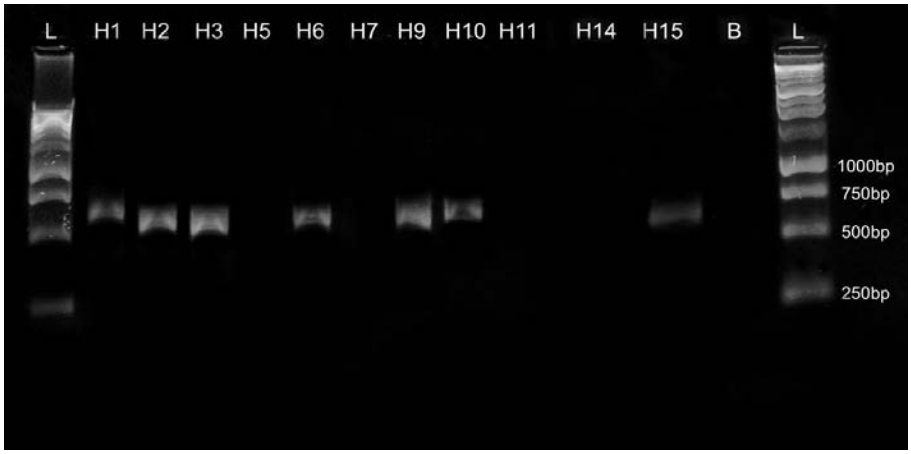

Fig. 5: Amplification of fungal DNA (samples H1 to H15) using primers ITS5F/ITS4R.

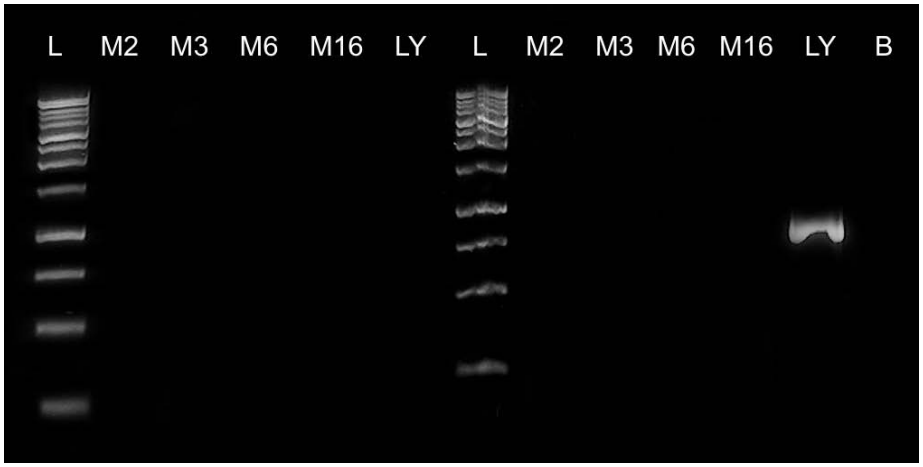

Fig. 6. Phytoplasma detection using primers FU5/RU3 for nested PCR. Samples were negative for phytoplasmas. Positive control was Lethal yellowing (LY) for approximately 750bp, B= Blank or negative control (DNA was replaced by ultra pure water). L= 1 kb ladder.

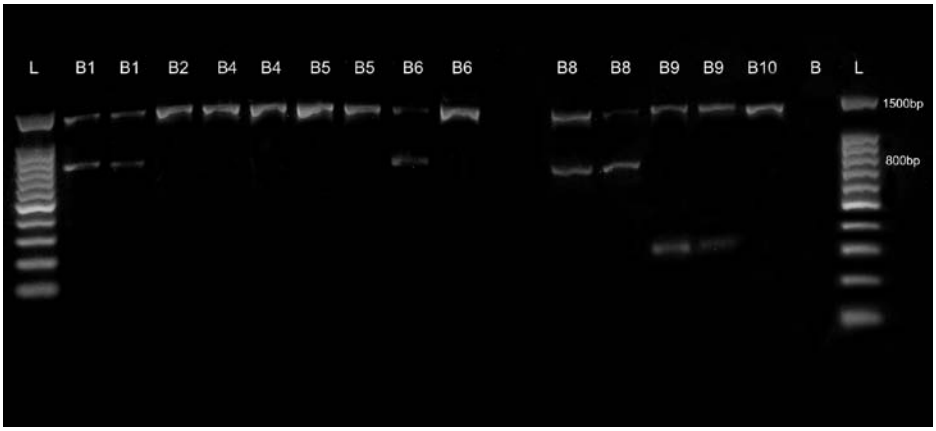

Fig. 7: Amplification of the16SrRNA of bacterial samples B1 to B10 using primers U519F/1392R.

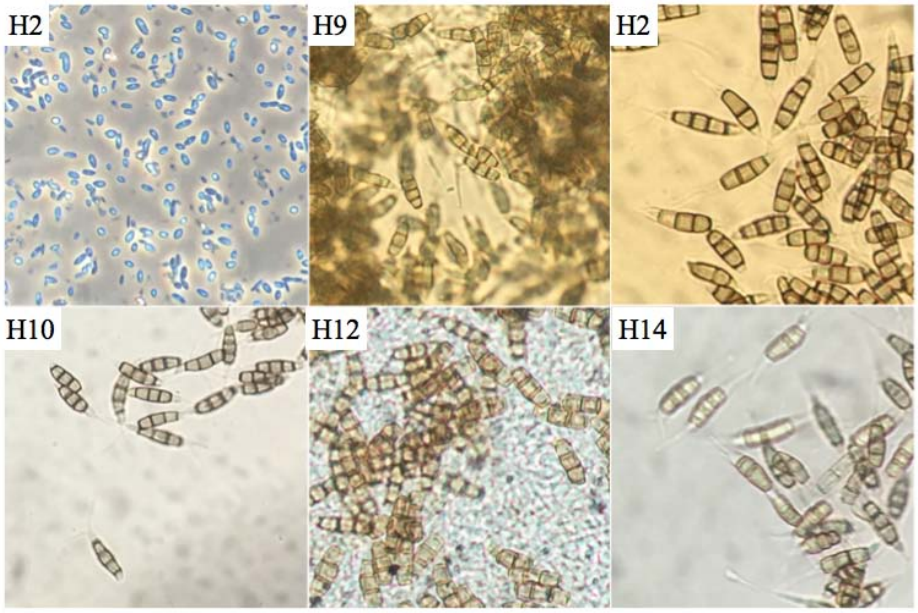

Fig. 8. Fungal reproductive structures: H2 are microconidias of Fusarium oxysporum, and H2, 9,10,12 and 14, conidias of Pestalotiopsis spp.

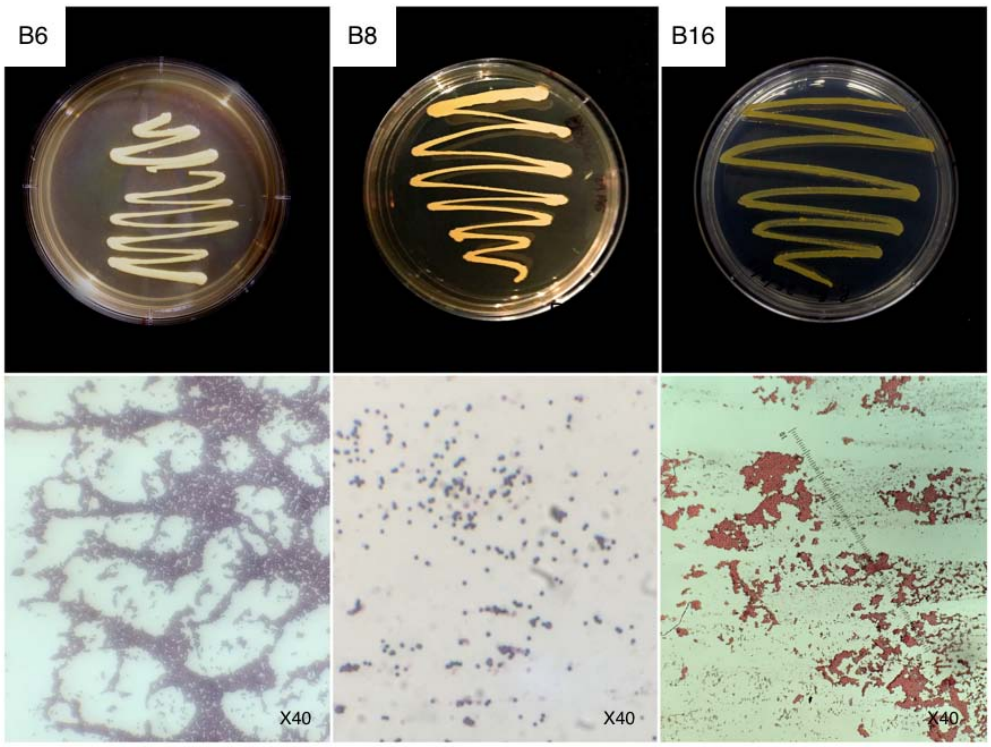

Fig. 9. Colony on NA and TSA and Gram stain of bacteria isolated from palm vascular tissue: B6 *Bacillus subtilis* (G+) NA; B8 *Myroides odoratus* (G+); and B16 *Sphingomonas* spp. (G-).

Conclusion

The severe disease known as Coconut Lethal Yellowing which is caused by a phytoplasma, was not detected by nested PCR assays in any of the palm sampled (n=15). A total of 25 bacteria and 15 fungal isolates were obtained from sampled tissues. Forty eight percent (48%) of the isolated

bacteria were Gram +. Only 33% (9/25) of the isolated bacteria have homology with known species in databases. These were: *Bacillus subtilis*, *Myroides odoratus* and *Sphingomonas spp.*, the other 67% of the bacteria did not show homology with species in the databases, of these 22% are associated with non-culturable bacteria. *Bacillus subtilis* is commonly used as a biological control agent in agriculture and is one of the gram-positive bacteria most studied in modern technology.

Five fungal isolates were identified as *Pestalotiopsis spp.* and one as *Fusarium oxysporum* using morphological characters. Isolates identification were confirmed using molecular data, DNA sequences have 99% homology with species reported in databases. Both are common plant pathogens. All species identified so far can be found in soils. Some microorganisms are or have the potential to be pathogenic to plants. Pathogenicity test are needed to have a better understanding of the patho-systems.

Upcoming experiments include continuing palms sampling to detect phytoplasma, continue DNA sequencing of isolated bacteria and fungi. Pathogenicity tests will be conducted with bacterial and fungal isolates in *Roystonea spp.* and *Cocos nucifera* under greenhouse conditions.

Acknowledgements

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Thanks are expressed to the Ing. Agro. Lorena Simbaña, graduate student for her help, dedication and mentoring and to Dr. Lydia Rivera Vargas for her mentorship and for providing me with opportunity to work in this project. To the Agro. Victor Gonzalez and Luis Collazo, lab technicians, for always being willing to help in everything, to my lab partners and the Department of Agro-Environmental Sciences.

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PHYLOGENETIC ANALYSIS OF *COLLETOTRICHUM* SPECIES ASSOCIATED WITH YAMS

Stephanie M. Plaza-Torres, Merari Feliciano-Rivera and Stephanie Fuentes-Aponte
University of Puerto Rico, Mayagüez Campus
Department of Agro-Environmental Sciences

Abstract

Colletotrichum is a genus of fungi that causes diseases and economic losses of numerous crops worldwide. Currently, there is a crucial dilemma with the taxonomy of these fungal species due to incomplete genetic data, high similarity and variability of morphological traits, and high similarity between species when using ITS (fungi barcode gene) sequences. Hence, this study evaluated the use of *in silico* RFLP analysis as a method of quick and accurate identification of *Colletotrichum* species associated with yam crops. Twenty-two isolates were obtained from a yam germplasm collection (and other private farms) and identified by morphological characters, pathogenicity tests, single gene analyses (GAPDH, beta-tubulin and ITS) and concatenated trees. *In silico* RFLP was conducted using Benchling and Ec-MLST with various combinations of three genes and six restriction enzymes to observe which pair produced more polymorphisms between different species and similarities between same species isolates. RFLP showed more polymorphisms when using the genes beta-tubulin and GAPDH in combination with the restriction enzyme HaeIII, but still little polymorphisms between the species *C. tropicale, C.ignotum, C. siamense* and *C. fructicola.* These species also had unclear phylogenetic relationships in the concatenated tree due to their evolutionary closeness and the similarity between the DNA regions used; the rest of the tree strongly supported the characterization of the isolates and presented a clearer phylogeny that was aligned with current literature. Evidently, they need to be sequenced for other gene regions with more specificity. Additionally, the simultaneous use of multiple enzymes and gene regions in RFLP may prove to be an excellent tool to work with the problem of species identification.

Keywords: *Colletotrichum*, phylogenetics, RFLP.

Resumen

Colletotrichum es un género de hongos patógenos que causa enfermedades y la pérdida económica de gran cantidad de cultivos alrededor del mundo. Actualmente, hay un dilema con la taxonomía de estas especies debido a falta de material genético, la alta variabilidad y similitud de rasgos morfológicos del hongo, y la similitud entre especies cuando se usa la región genética ITS (gen "barcode" para los hongos). Dado esto, se evaluó RFLP como un método rápido y certero para identificar especies de *Colletotrichum* asociadas a cultivos de ñame. Veintidós aislados fueron obtenidos de la colección de germoplasmas de ñame (y otras fincas privadas) e identificados por morfología, pruebas de patogenicidad, análisis molecular basado en un solo gen (GAPDH, beta-tubulina e ITS) y árboles concatenados. *In silico* RFLP fue hecho con los programas Benchling y Ec-MLST usando combinaciones de los tres genes mencionados y seis enzimas de restricción para observar que par producía más polimorfismos entre especies y similitudes entre aislados de la misma especie. RFLP mostró más polimorfismos al ser usado con los genes beta-tubulina y GAPDH en combinación con la enzima HaeIII, pero se vio poca variación entre los patrones de fragmentos de ADN de las especies *C. tropicale, C.ignotum, C.*

siamense and *C. fructicola.* Además, estas especies tuvieron relaciones filogenéticas poco claras en el árbol concatenado por su cercanía (evolutiva) y la similitud entre las regiones genéticas usadas; el resto del árbol apoyaba la caracterización de los aislados y mostraba una filogenia más clara y similar a las encontrada en otros estudios. Evidentemente, estas cuatro especies deben ser secuenciadas para otras regiones más específicas. Adicionalmente, el uso de múltiples enzimas y regiones genéticas en RFLP podría ser una excelente herramienta para trabajar con el problema de la identificación de especies de *Colletotrichum.*

Palabras Claves: *Colletotrichum,* filogenética, RFLP.

Introduction

Colletotrichum is an ascomycete that withholds numerous destructive pathogenic species that can infect an ample variety of plants and important crops worldwide (Gan *et al.*, 2012; Liu *et al.*, 2007). Puerto Rico is no exception to the scope of this pathogen; numerous *Colletotrichum* spp. have been reported around the island (Rivera-Vargas *et al.*, 2006). Unfortunately, *Colletotrichum* is a genus with great taxonomical confusion that requires extensive revision (Hyde *et al.*, 2009). The characterization of *Colletotrichum* spp. is withheld by the poor genetic data infrastructure available for the genus and the unreliability of morphological traits (Crouch *et al.*, 2009). Given this, we stress the importance of developing correct diagnosis tools for *Colletotrichum* in order to successfully control anthracnose disease. This study, motivated by the current identification dilemma, evaluate the use of RFLP, in combination with gene regions ITS, beta-tubulin and GAPDH, as a tool for the characterization of *Colletotrichum* isolates obtained from yams (*Dioscorea*) and employs concatenated trees to ensure the correct diagnosis of these isolates. Furthermore, the concatenated trees give insights of the phylogenetics of *Colletotrichum* spp. found in yams, which are currently unknown.

Materials and Methods

Genetic data retrieval

The ITS, beta-tubulin and GAPDH datasets were extracted from Stephanie E. Fuentes Aponte's master's thesis. Twenty-two isolates of *Colletotrichum* spp. were obtained from the foliar damage found in yam species *D. alata*, *D. esculenta,* and *D. rotundata* (these were acquired from the *Dioscorea* spp. germplasm collection in the University of Puerto Rico's Agricultural Research Station in Corozal, Puerto Rico and other private farms) after morphological, pathogenic and molecular characterization (using genes ITS, beta-tubulin and GAPDH). Eleven species of *Colletotrichum* were present among identified isolates: *C. siamense, C. ignotum, C. truncatum, C. alatae, C. gloeosporioides, C. cliviae, C. tropicale, C. fructicola, C. karstii, C. theobromicola, and C. aotearoa*. Table 1 shows each isolate's code and species; the beta-tubulin sequence was not obtained from isolates G122A, G62A, G153A, F52253A and F11244B.

Table 1. Results of morphological, pathogenic and molecular identification of isolates obtained from *Dioscorea* spp.

Isolate Code	Identified Species
G13A	*C. tropicale*
G143A	*C. tropicale*
G73D	*C. tropicale*
G153A	*C. tropicale*
G12B	*C. gloeosporioides*
F52252D	*C. gloeosporioides*
G12D	*C. siamense*
F11244A	*C. siamense*
G42B	*C. siamense*
G14B	*C. cliviae*
G61B	*C. cliviae*
F85241C	*C. cliviae*
G23B	*C. fructicola*
G62A	*C. fructicola*
G121B	*C. theobromicola*
G81	*C. aotearoa*
G122A	*C. karstii*
F133251A	*C. alatae*
F52253A	*C. alatae*
F52251A	*C. alatae*
F21182A	*C. truncatum*
F11244B	*C. ignotum*

In silico RFLP

The accuracy of different genes was tested to comprehend which gene region, Internal Transcribed Spacer (ITS), beta-tubulin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), provided a better method for classifying different species within *Colletotrichum* utilizing the fragment patterns produced by RFLP. *In silico* (computer based) RFLP was first conducted with EcMLST In Silico RFLP (Version 1.2; Whittam, 2006) to produce the enzyme digestions for the twenty-two *Colletotrichum* isolates (sequenced for ITS, beta-tubulin and GAPDH) using restriction endonucleases AauI, MseI, RsaI, HinfI, HaeIII, and EcoRI individually. Afterwards, successful gene-enzyme prospects were additionally digested using software Benchling (Wickramasekara and Singhal) to further illustrate how fragments would show in a gel electrophoresis. Using the fragment patterns generated, an ideal gene-enzyme pair was selected as a potential prospect for future applications of RFLP in vivo.

Concatenated trees

Data was prepared to run the phylogenetic analyses by joining the three gene regions, ITS, beta-tubulin and GAPDH (with sequence available), of each isolate into a larger sequence. The same was done with the three reference sequences (NCBI sequences that matched each genetic region of the isolates) found for each isolate. A MUSCLE (Multiple Sequence Comparison by Log-Expectation) alignment was performed with these concatenated sequences using the program MEGA 6 (Tamura *et al.*, 2013) to later build the concatenated tree. The phylogenetic analysis (to produce the concatenated tree) was done in MEGA 6 using a maximum likelihood analysis, with the Tamura-Nei model, and bootstrapping the tree 1000 times. Afterwards the clades within the tree were observed and compared to literature containing the same or a similar set of species.

Results and Discussion

In silico RFLP

After putting all the sequences in EcMLST In Silico RFLP, AauI was unable to cut ITS genes, EcoRI was unable to cut beta-tubulin genes, and, AauI and EcoRI were unable to cut GAPDH genes. The viability of ITS genes in combination with restriction enzymes for the classification of *Colletotrichum* species was discarded due to high incidence of misidentification when using this gene (Crouch *et al.*, 2009) and for similarity of ITS sequences within different species. As for GAPDH and beta-tubulin genes cut with different restriction enzymes, the results were promising. The fragment patterns generated by RsaI and HaeIII showed a great quantity of polymorphisms, different species showed unique fragment patterns more frequently, and also the enzymes produced many fragments, adding variability. Due to the lack of beta-tubulin sequences for isolates G122A, G62A, G153A, F52253A and F11244B, focus was shifted to the relationship of the GAPDH gene with RsaI and HaeIII. GAPDH sequences combined with the use of restriction enzyme HaeIII showed the most polymorphisms in digestion and the greatest quantity variation of fragments, from 2 fragments to 7 fragments (Figure 1). Three different *C. cliviae* isolates, F85241C, G14B, and G61B, had similar length fragments: 160 bp, 60 bp and 30 bp; since results were consistent between different isolates of the same species, there is more reassurance of the effectivity of the use of RFLP for fungi identification (Pongpisutta *et al.*, 2013). Also, the same similarity in fragment length and frequency was seen in *C. siamense, C. tropicale, C. fructicola* and *C. ignotum;* these species are closely related and difficult to differentiate with GAPDH (Weir *et al.*, 2012). As an alternative, RFLP could be done using genes that better differentiate these taxa: GS or SOD2 (Weir *et al.*, 2012). Additionally, the fragment patterns of *C. alatae* and *C. gloeosporioides* were similar as a result of their close phylogenetic relationships (Weir *et al.*, 2012).

Colletotrichum phylogenetics

The maximum likelihood analysis yielded a tree really similar to ones in literature (Weir *et al.*, 2012) and strongly supports that species were identified correctly. Even though most of the species' isolates matched up in clades, with high bootstrap values, with their respective NCBI reference sequences, there were occasions with species mismatch between isolate and reference, like in the case of *C. ignotum,* which was closely found with *C. fructicola* (Figure 2). Additionally, some isolates of *C. siamense* did not match correctly either (with other respective isolates from its same species), since they were in no definite clade. The top *C. tropicale* clade is well supported, but the neighboring species *C. siamense*, *C. fructicola* and *C. ignotum* don't

show clear relationships, due to the mismatch of reference and isolate and the high bootstrap value clades found between different species, and the phylogeny is not yet resolved for them. This uncertainty is due to the close phylogenetic relationships between these species which has been reported in literature (Weir *et al.*, 2012). To make these clearer, genes GS and SOD2 could be added to the analysis, since they have shown to distinguish well between these species (Weir *et al.*, 2012).

Conclusions

The GAPDH-HaeIII gene-enzyme pair could be proper for *Colletotrichum* species classification due to the consistency seen in fragment patterns within species, but still the gene regions used bear too much similarity along species. Even though, the GAPDH gene region has proven to be effective in species identification and phylogenetic analyses (Guerber *et al.*, 2003), there are still other gene regions, like GS or SOD2, to be used that could be useful to this application of the RFLP method. Furthermore, multiple enzyme digestion could be sought to generate even more distinct patterns, nonetheless the single gene-enzyme pair approach could be more useful. On the other side, the concatenated tree obtained strongly supports that these *Colletotrichum* isolates are correctly identified, but regarding the phylogeny, there are still some troublesome areas with the closely-related species *C. siamense*, *C. tropicale*, *C. fructicola* and *C. ignotum* (Weir *et al.*, 2012). Just like RFLP, the addition of genes GS and SOD2 could largely resolve this problem and help yield a clearer phylogeny of the *Colletotrichum* spp. found in yams.

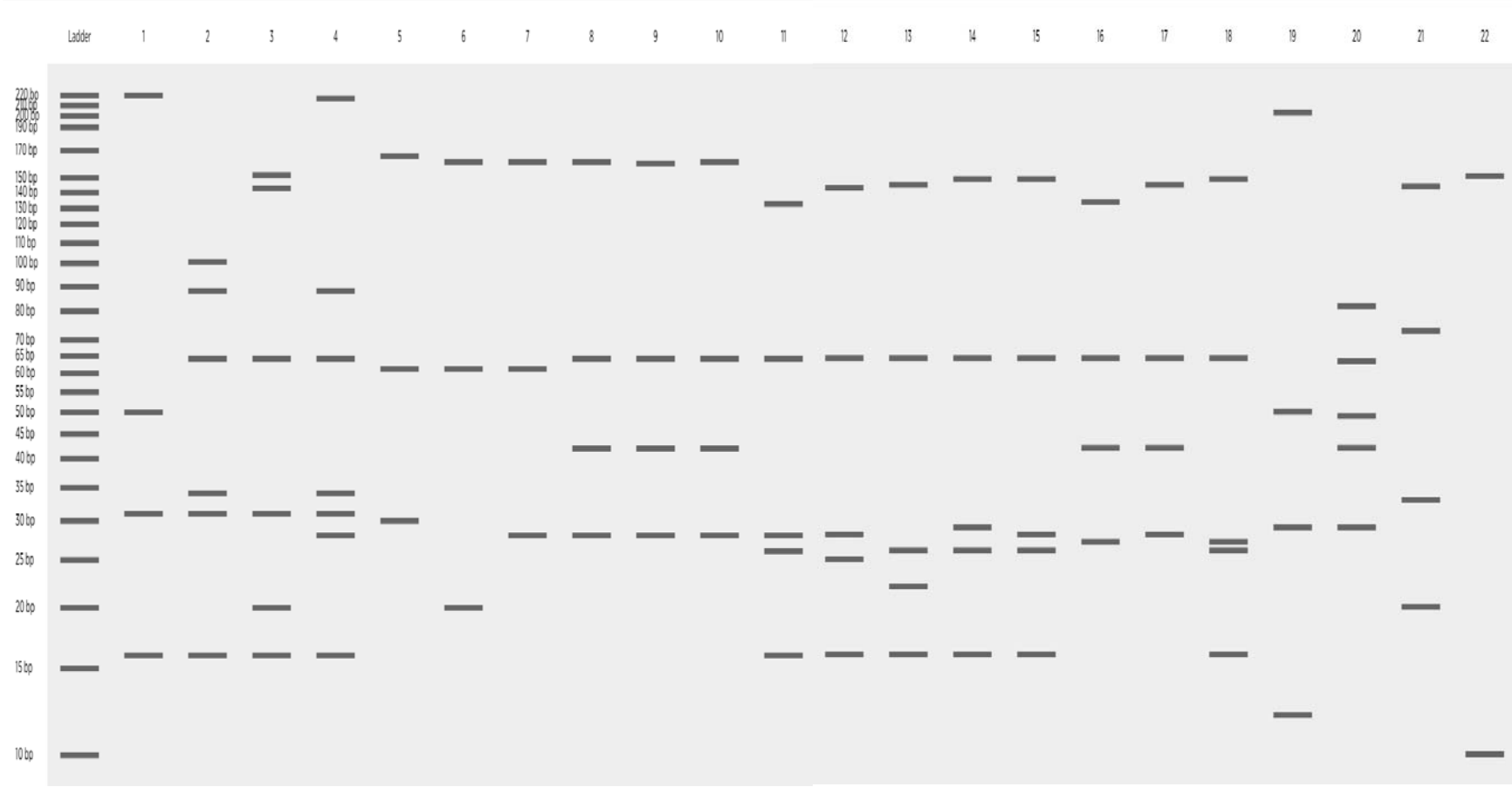

Figure 1. Virtual enzyme digestion of the twenty-two *Colletotrichum* spp. isolates using enzyme HaeIII (Wickramasekara and Singhal).

Ladder, Custom Ladder; 1, G73D (*C. tropicale*); 2. G153A (*C. tropicale*); 3, G143A (*C. tropicale*); 4. G13A (*C. tropicale)*; 5, G61B (*C. cliviae*); 6, G14B (*C. cliviae*); 7, F85241C (*C. cliviae*); 8. F133251A (*C. alatae)*; 9, F52251A (*C. alatae*); 10, F52253A (*C. alatae*); 11, F11244A (*C. siamense); 12*, G12D (*C. siamense*); 13, G42B (*C. siamense*); 14, G62A (*C. fructicola*; 15, G23B (*C. fructicola*); 16, F52252D (*C. gloeosporioides*); 17, G12B (*C. gloeosporioides);* 18, F11244B (*C. ignotum*); 19, F21182A (*C. truncatum;* 20, G121B (*C. theobromicola*); 21, G81 (*C. aotearoa*); 22, G122A (*C. karstii*).

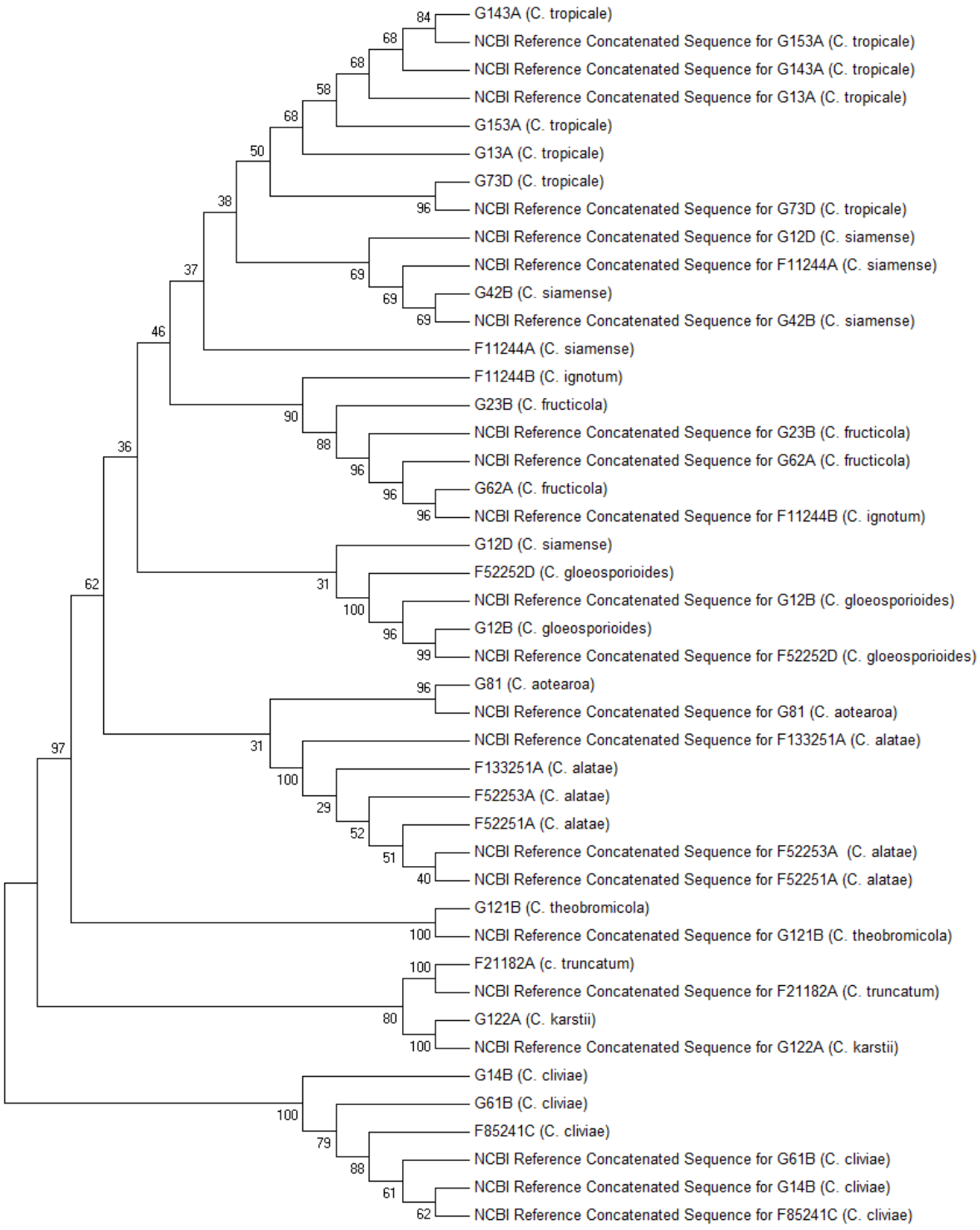

Figure 2. Concatenated tree (ITS, beta-tubulin, GAPDH) for the twenty-two *Colletotrichum* spp. found in yam.

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EFFECTS OF PHYTOSTIMULANTS AND NITROGEN RATES ON LEMONGRASS (*CYMBOPOGON CITRATUS*)

Llelenys Sanoguet Crespo and J. Pablo Morales-Payan,
Department of Agro-Environmental Sciences
University of Puerto Rico, Mayaguez Campus.

Abstract

Lemongrass (*Cymbogon citratus*) contains essentials oils which has aromatics and medicinal properties. Commercially those essentials oils can be extracted to produce vitamin A and other synthetic compounds. There are countries that lemongrass is grown for commercial purposes, but in Puerto Rico there is no commercial farm and is mainly on gardens. This research shows the effect of different phytostimulants and nitrogen fertilization on the productivity of lemongrass plants. Some phytostimulants have a positive effect on the productivity of biomass at low concentration of nitrogen, while if the concentration is increased the productivity of the plant decreases. However, other phytostimulants increases lemongrass biomass production by increasing the concentration of nitrogen fertilization. Finally, it was found that in some phytostimulants, including control group, the change in nitrogen concentration is not significant on lemongrass productivity.

Introducción

Lemongrass (*Cymbopogon citratus*) is an herbaceous perennial plant originally from India, the south of Asia. The lemongrass contains terpene compounds with aromatics and medicinal properties. Those essentials oils can be extracted to synthesize compounds like vitamin A, perfumes, detergents and others. Among the most used essentials oils form lemongrass are citral and limonene, because they have antibiotics, fungicide and bugs repellent properties (Ordóñez, et. al, 2004).

In countries like Brasil, India, Indonesia, Argentina, Guatemala and Spain lemongrass is grown for commercial purposes; while in Puerto Rico it can be found growing mostly in gardens, where it is used for medicinal purposes or as an insect repellent in homes (Soto Ortiz, et. al, 2002).

Lemongrass productivity is measured by biomass production and essential oil. There are few studies of the effect of fertilizers on lemongrass productivity. Although there are several reports on the effect of traditional phytoregulators such as gibberellins and cytokinins, there are no reports of phytostimulants. The objective of this research was to determine the effects of application of nitrogen fertilization and phytostimulants on the lemongrass growth and biomass production.

Materials and methods

The research was conducted in Mayaguez, Puerto Rico, in a greenhouse at the Alzamora Farm of the University of Puerto Rico-Mayaguez Campus. Plant set (basal shoots) 15-cm long and with roots were obtained from the Alzamora Farm. One basal shoot with roots was planted onto plastic containers 30 x 30 cm filled with a substrate. The substrate was prepared mixing a

commercially available growing medium (Promix®) (20%) and alluvial soil from Mayaguez (80%). Mentioning commercial names is done solely to specify the materials used in the research and does not imply an endorsement of the researchers or the UPR to particular brands, models or companies.

The treatments were combinations of N rates and phytostimulants applied to the susbtrate. At planting, the equivalent of 50 kg/ha of nitrogen, phosphorus and potassium was applied to the substrate of each container. Four weeks after planting, additional nitrogen (urea) was applied at the rates of 70 or 130 kg/ha, for a total N rate of 120 kg/ha ("regular rate") or 180 kg/ha ("high rate"). The phytostimulants were commercially available formulations of (1) an extract of the marine alga *Ascophyllum nodosum* (2 ml/L water) (Stimplex®), (2) free amino acids (5 ml/L) (Terra-Sorb Radicular®), (3) peptides (5 ml/L)(Inicium®), (4) fulvic acid (5 ml/L) (Fulvex®), (5) vitamins, enzymes and brassinosteroids (2 ml/L), (6) silicon and humic acid (2.5 ml/L) (Quick-Sol®). Phytostimulants were applied as soil drench every 14 days, with 200 ml of solution per plant. As control, plants without phytostimulants were used.

The 14 treatments were set in a complete block design with 9 replications (one plant per replication), in a split plot where the N rates were the large plot and phytostimulants were the smaller plots. Plants were watered as needed with drip irrigation. No fungicides or insecticides were used in the experiment. Plant height was measured every 14 days, chlorophyll concentration was measured with a Minolta SPAD meter® every 4 weeks after planting, and nitrogen in nitrate in the leaf sap was measured 9 weeks after planting with a Cardy® N sensor. Leaf biomass was harvested 90 d after planting, cutting the leaves at 15 cm above the substrate. Leaves were weighted fresh and after drying in an oven at 60 C for 48 hours. Results were submitted to statistical analyses.

Results and discussion

Plant height was not significantly affected by the treatments. There was a sharp increase in plant height from 15 to 50 cm in the first 17 days after plating the lemongrass (Figure 1). Thereafter, there was little additional increase in height, reaching approximately 60 cm until harvesting.

The treatments did not significantly affect the concentration of nitrogen in nitrate in the leaf sap and the chlorophyll concentration in the lemongrass leaves (data not shown). There were interactive effects of N and phytostimulants on the harvested leaf biomass of lemongrass. Plants fertilized with the low N rate produced significantly more leaf biomass when treated with the alga extract (22%), the amino acid formulation (46%), the peptide formulation (41%), and the fulvic acid formulation (35%) than plants treated with the other phytostimulants or than control plants (Figure 2).

At the high N rate, application of the alga extract, the peptide formulation, the vitamin complex and the silicon and humic acid formulation resulted in significantly more biomass than in the control plants or in plants that received the other phytostimulants (Figure 3).

The difference between leaf biomass production with low and high N rates depended on the phytostimulant used. Leaf biomass was greater in plants treated with the high N rate (as compared to the low N rate) when the alga extract, the vitamin complex and the silicon and humic acid phytostimulants were applied. With the other phytostimulants, there was no difference in biomass production regardless of N rate (Figure 4).

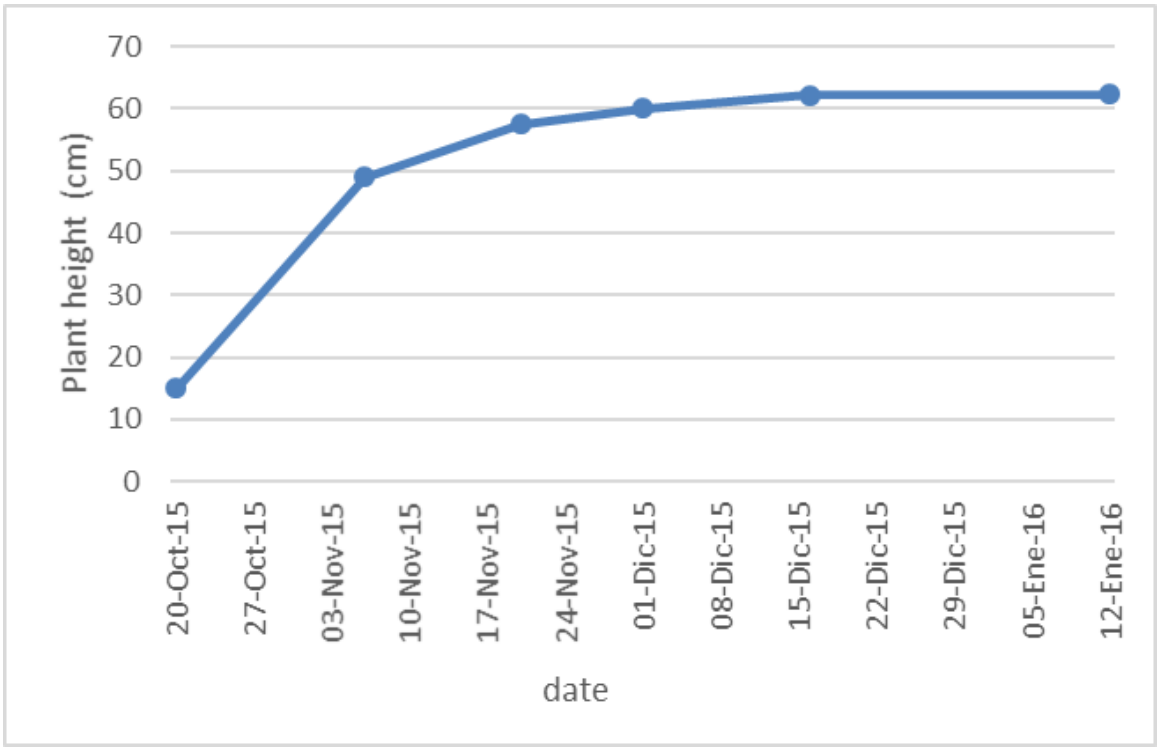

Figure 1. Plant height of lemongrass plants treated with phytostimulants and nitrogen. Mayaguez, Puerto Rico, 2015.

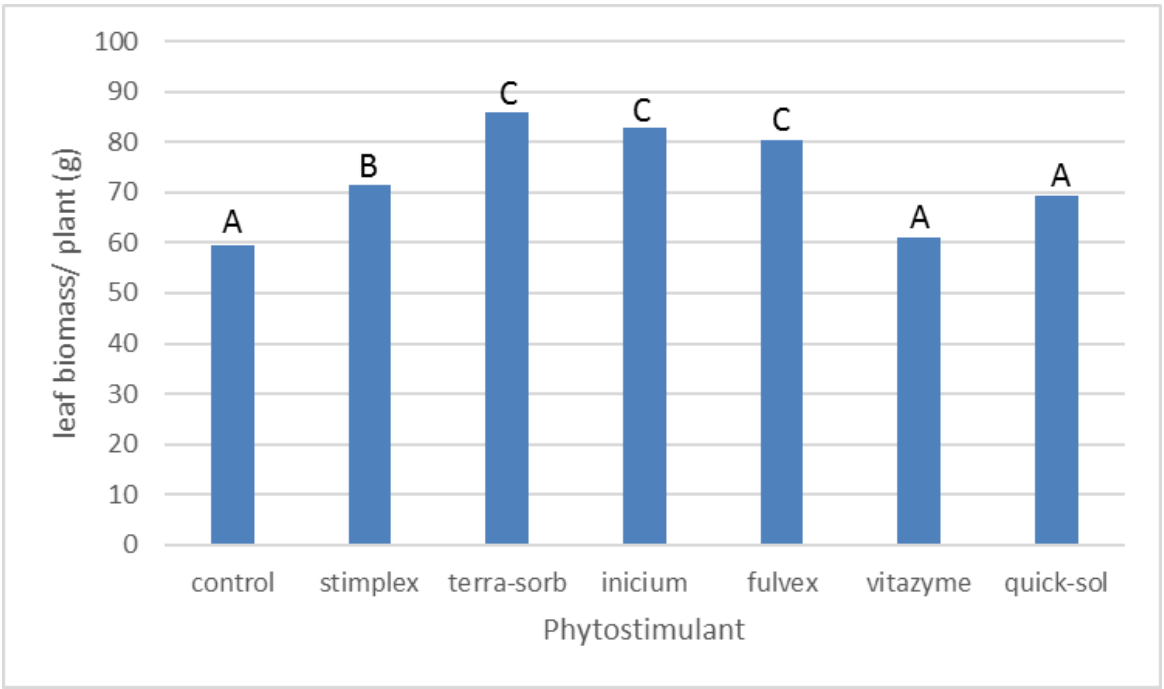

Figure 2. Leaf biomass from lemongrass plants treated with phytostimulants and 120 kg N/ha, Mayaguez, Puerto Rico, 2015. Bars with the same letter are not significantly different.

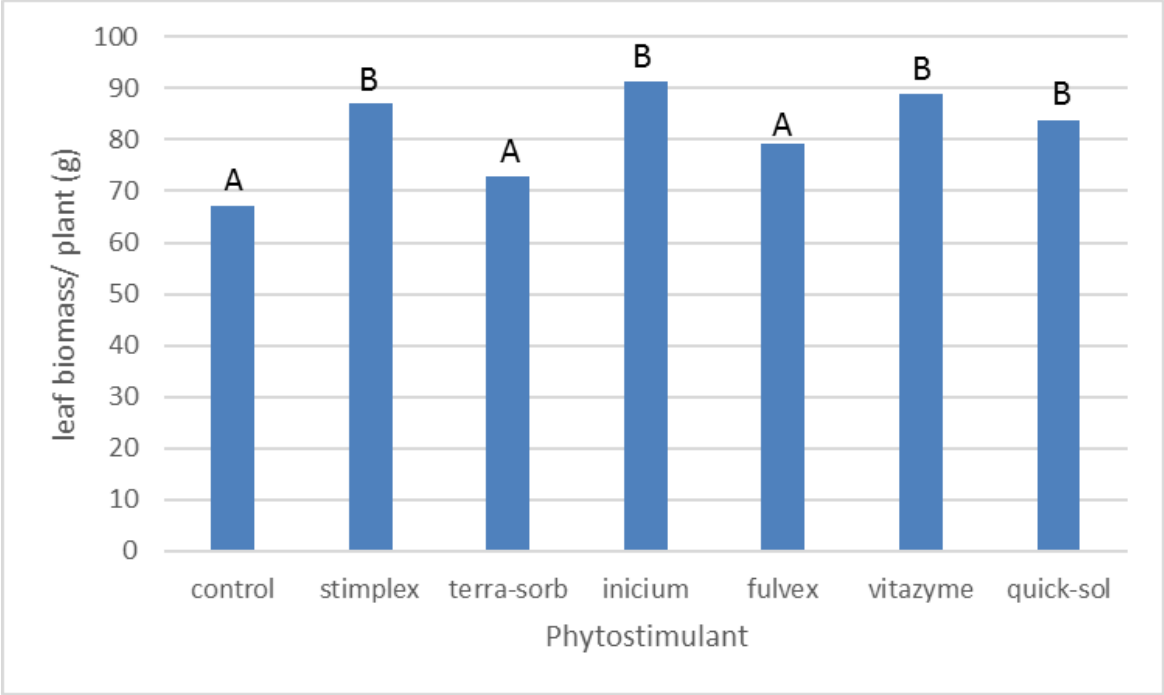

Figure 3. Leaf biomass from lemongrass plants treated with phytostimulants and 180 kg N/ha, Mayaguez, Puerto Rico, 2015. Bars with the same letter are not significantly different.

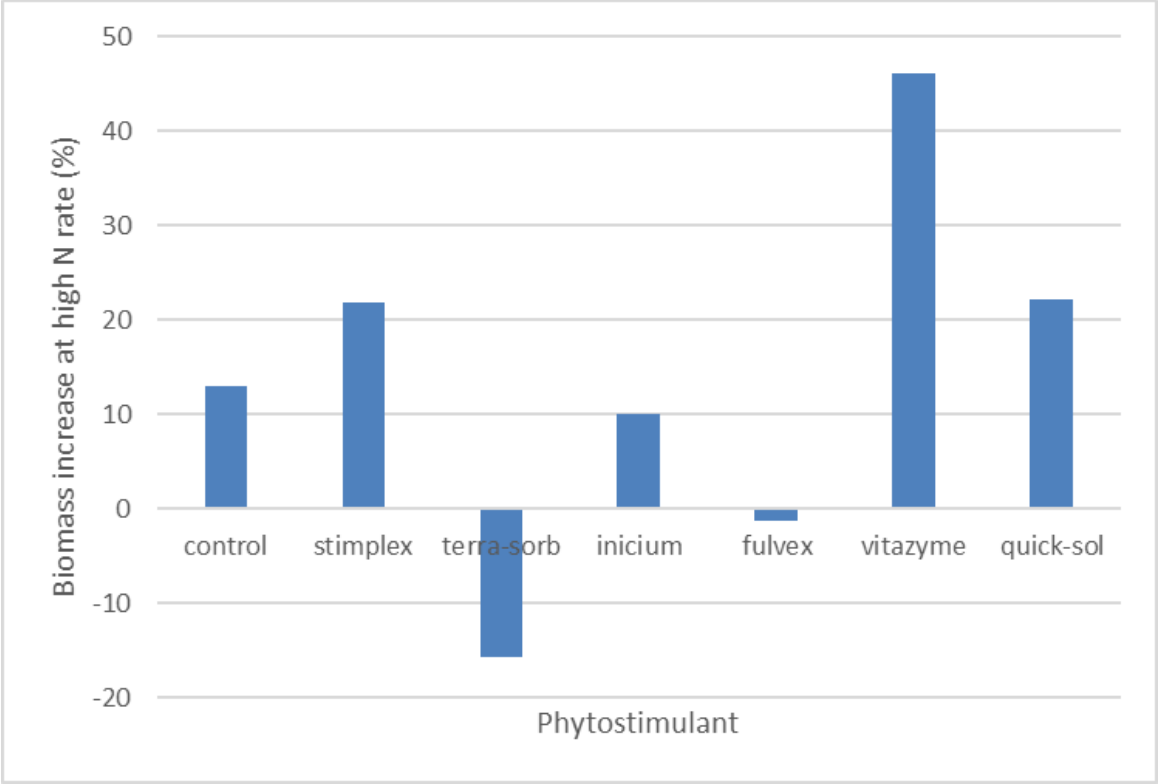

Figure 4. Difference in lemongrass leaf biomass from plants treated with phytostimulants when N rates increased from 120 kg/ha to 180 kg/ha, Mayaguez, Puerto Rico, 2015. Bars with the same letter are not significantly different.

In this research, two formulations increased biomass yield significantly regardless of N rate, the alga extract and the peptide mixture. The other formulations increased lemongrass biomass at one N rate or another, showing that results depend on N availability for the plant.

The potential effects of the phytostimulants tested in this research on plant growth have been shown in other studies in Puerto Rico and other places. Positive effects on growth can be attributed to amino acids and other organic acids, sugars, betain, cytokinins, gibberellins and other substances in each phytostimulants, which promote growth and/or increase tolerance to environmental stress. In the case of the silicon/humic acid formulation, silicon itself has been recognized as an essential and stimulant element for plants in the Poaceae family as is lemongrass.

Conclusion

In summary, these results show that at one N rate or the other, all the phytostimulants resulted in enhanced leaf biomass accumulation in lemongrass, and that the amount of the increase in biomass production depended on the phytostimulant and on the N rate. Therefore, while phytostimulants may be useful in increasing lemongrass yield, more research is necessary to determine the best N rates to obtain higher biomass yields when using specific phytostimulants.

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MANAGEMENT STRATEGIES TO REMEDIATE SODIC SOILS

Noelymar González, and David Sotomayor
Department of Crops and Agro-Environmental Sciences
Agronomy and Soil Science
University of Puerto Rico at Mayagüez

Abstract

High levels of exchangeable sodium (Na^+) occupying soil cation exchange sites (ESP) affect soil physical properties impacting machinery traffic, soil hydraulic infiltration rates, and soil water retention capacity. In addition, high levels of Na, Cl and other cations may affect crop growth via direct toxicity or by influencing soil water potential. These conditions predominate when there are sources of Na or other salts in the soil profile in arid and semi-arid soils. A seed company in south-eastern Puerto Rico (Guayama municipality) has classified 9% of their land area as being sodic with mean ESP values of 40%. Inbred maize grown for seed has been in general terms un-successful. A gypsum product Phusion™ was tested for its efficacy in remediating sodic soils. The product was applied to soil followed by water at calculated rates to reach ESP values of 7%. A second treatment was left unamended. Soil solution access tubes were used to measure soil pore-water at 30 cm. Soil water infiltration was measured as a function of the treatment application using a double ring infiltrometer in the field and a constant head soil core method in the laboratory. In plots amended with Phusion™ (#4 and #5), infiltration rates were 0.1 cm/hr in plot 4 and 3.15cm/hr in plot 5 with the double ring infiltrometer. With the constant head soil core method infiltration rates were 0.09 cm/hr for plot 4 and 3.11 cm/hr for plot 5. The unamended control plots (#1 and #6) had infiltration rates of 0.1 cm/hr in plot 1 with the double ring infiltrometer and 0.019 cm/hr in plot 6 with the constant head soil core method. Final results will be evaluated and analyzed after soil chemical analysis after application of the gypsum.

Keywords: Puerto Rico, Guayama, Sodic Soils

Resumen

Altos niveles de sodio (Na^+) intercambiable ocupando los lugares de intercambio catiónico del suelo afectan las propiedades físicas del suelo impactando el tráfico de maquinaria, las tasas de infiltración hidráulica y la capacidad de retención de agua del suelo. Adicionalmente, altos niveles de sodio, cloro y otros cationes pueden afectar el crecimiento de cultivos a causa de toxicidad directa o influenciando el potencial hídrico del suelo. Estas condiciones predominan cuando hay grandes cantidades de sodio y otras sales en el perfil de suelos de clima árido y semi-árido. Una compañía de semillas en el sureste de Puerto Rico (municipalidad de Guayama) ha clasificado 9% de sus tierras como suelos sódicos con un porcentaje de sodio intercambiable (ESP) aproximado de 40%. El producto de yeso "Phusion™" fue evaluado para su eficiencia en la remediación de suelos sódicos. El producto fue aplicado al suelo seguido por agua para alcanzar un ESP de 7%. Un segundo tratamiento se evaluó sin la enmienda de yeso. Se instalaron lisímetros en el suelo para medir el agua de los poros del suelo a 30cm de profundidad. Se midió la infiltración del agua en el suelo como evaluación de la efectividad de la enmienda utilizando un infiltrómetro de dos anillos en condiciones de campo y el método de agua constante en cilindros de suelo en el laboratorio. En las parcelas enmendadas con el yeso Phusion™ (#4 y #5) las tasas de infiltración de agua con el infiltrómetro de dos anillos fueron de 0.1cm/hr en la

parcela 4 y 3.15 cm/hr en la parcela 5. Con el método de agua constante en cilindros de suelo las parcelas enmendadas tuvieron tasas de infiltración de agua de 3.11 cm/hr en la parcela 5 y 0.09 cm/hr en la parcela 4. Las parcelas sin la enmienda (#1 y #6) tuvieron una tasa de infiltración de agua de 0.1 cm/hr en la parcela 1 con el infiltrómetro de dos anillos y 0.019 cm/hr en la parcela 6 con el método de agua constante en cilindro de suelo. Los resultados finales serán evaluados luego de hacer análisis químico del suelo después de la aplicación de la enmienda de yeso.

Palabras Claves: Puerto Rico, Guayama, Suelos Sódicos

Introduction

Sodic soils are those that have an exchangeable sodium percentage greater than 15% measured in the saturated paste extract, pH higher than 8.5 and an electrical conductivity (EC) lower than 4 dS/m. Sodic soils present partial or complete loss of its structure which develops a poor water infiltration. These characteristics of a sodic soil creates sodium (Na^+) and chlorine (Cl^-) toxicity for plants, consequently creating a negative impact in crop production and agricultural economy. Sodicity in soils can occur as a result of pedogenic or anthropogenic processes in arid or semi-arid climates. The southern coast of Puerto Rico is characterized by having a semiarid climate and a high agricultural activity. In order to develop agriculture in this region it is highly necessary to have supplemental irrigation systems. Some of these soils are classified as saline-sodic, saline or sodic and therefore present difficulties in soil management and consequently, a decrease in agricultural activity.

The amendment that has been utilized for more tan 250 years in the U.S and Europe for agriculture to manage sodicity in soils is gypsum ($CaSO_4 \cdot 2H_2O$) (Rasouli et al., 2013; Chen and Dick, 2011). As Juan A. Bonnet (1960) exposed, the application of gypsum followed by water is an effective chemical amendment to lixiviate the excess of exchangeable Na^+ of Sodic soils. Recent studies (D. Sotomayor, R. Barnes, 2014, not published) demonstrate the identification of Sodic soil in large extensions of land (9%) in the farm Dow AgroScience (Mycogen) in Guayama, PR. Therefore, the objective of this research was to evaluate the effect of gypsum as an amendment on the physical and chemical properties of a sodic soil.

Methods

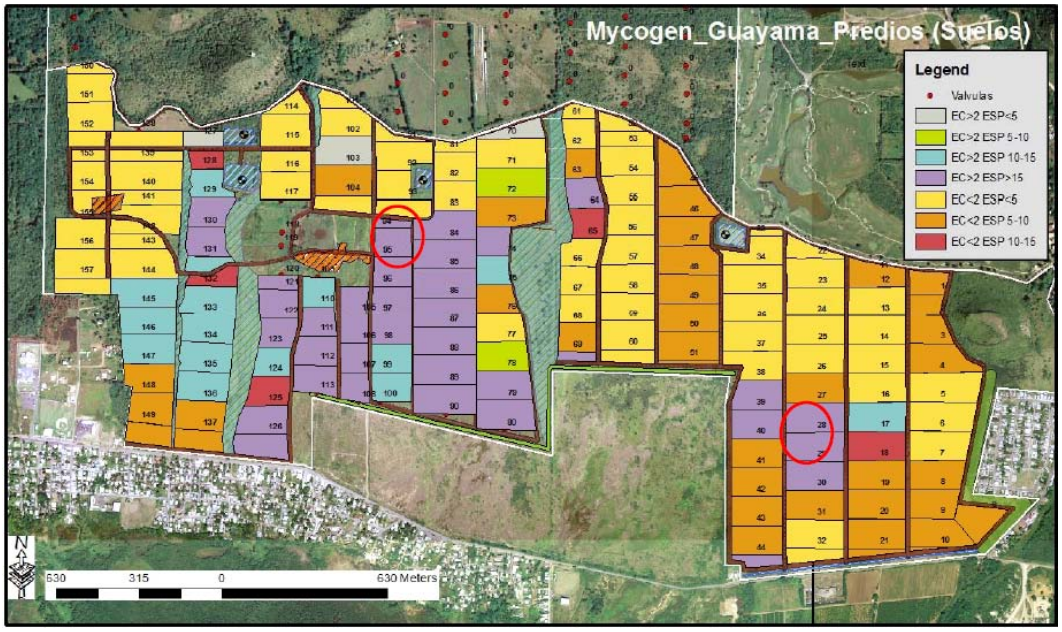
Setting

The soil of interest is located at Dow Agrosciences Research Farm in Guayama, Puerto Rico. The climate is semiarid and 16 out of 175 plots (9%) of the farm has soils classified as Sodic (D.Sotomayor y R.Barnes, 2014, not published). The plots of this farm are organized by valves; therefore, the area selected is within valve 94. Figure 1 shows a map of the farm identifying all the valves, the plot of interest is circled in red. The predominant soil (valve 94) is classified as: Vives clay series (Fine-loamy, mixed, superactive, isohyperthermic Fluventic Haplustepts) (US Taxonomy, Soil Web Survey). Other soil groups predominant in the farm are: Guaminí silty clay (Haplustepts), Paso Seco clay (Haplusterts), and Ponceña clay (Calciusterts).

Measurements of Plot

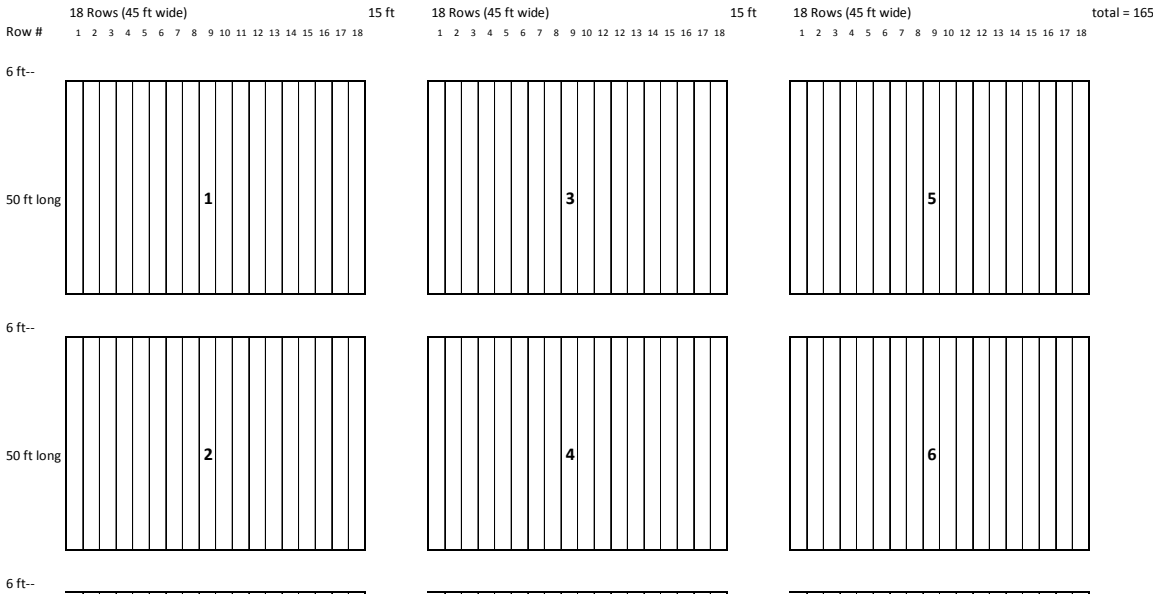
The selected area was 118 ft long and 165 feet width (total area of 19,470 ft^2). This area was divided in 6 plots that measured 50 ft long and 45 ft width (total area of 2,250 ft^2). The plots were separated vertically with 15 feet width aisles and horizontally with a 6 feet width aisle. The measurements of the area are described in Figure 2.

Figure 1. Map of the plots of the Dow AgroScience (Mycogen) Research farm at Guayama, PR.



The colors identifying the plots **Figure 1** correspond to the levels of the exchangeable sodium percentage (ESP) and the electrical conductivity (EC) in each plot (see leyend). Valve 94 was the setting of this research.

Figure 2. Diagram of Plot



This diagram was obtained from protocol "Experimental assay to remediate sodic and saline-sodic affected soils; Mycogen farm, Guayama" (D. Soyomayor and R. Barnes, November 5, 2014)

Gypsum Amendment

The gypsum selected for this research was Phusion Gypsum Calcium Sulfate. This product has a pH of 6.48, an electrical conductivity of 6720 μs/cm and demonstrated effervescence when tested with hydrochloric acid (HCl).

Gypsum Requisite:

The gypsum ($CaSO_4 \cdot 2H_2O$) requirement was calculated utilizing the exchangeable sodium percentage (ESP) which was calculated based on the sodium adsorption ratio (SAR) of the soil. The calculations were as follows:

Model:

(ESP to remove)(CIC meq/100g soil) = X meq Na/g soil

X meq Na = X meq Ca

X meq Ca = X meq $CaSO_4 \cdot 2H_2O$

(X meq $CaSO_4 \cdot 2H_2O$)(86 mg $CaSO_4 \cdot 2H_2O$/meq $CaSO_4 \cdot 2H_2O$) = 86X mg $CaSO_4 \cdot 2H_2O$/g soil

$$\left(\frac{86X mg\ CaSO4 \cdot 2H2O}{g\ soil}\right)\left(\frac{1kg/}{10^6 mg}\right)\left(\frac{10^3 g}{kg}\right)\left(\frac{1350 kg}{m^3}\right)(0.15\text{m})\left(\frac{10{,}000 m^2}{ha}\right) =$$

$$\frac{quantity\ of\ gypsum\ to\ be\ applied\ (\boldsymbol{kg})}{\boldsymbol{ha}}$$

Application:

ESP by SAR was 37%. To reach an ESP of 7% we must remove 30% ESP.

The calculation is as follows:

(0.3)(38 meq/100g soil) = 0.10703 meq Na/g soil

0.10703 meq Na = 0.10703 meq Ca

0.10703 meq Ca = 0.10703 meq $CaSO_4 \cdot 2H_2O$

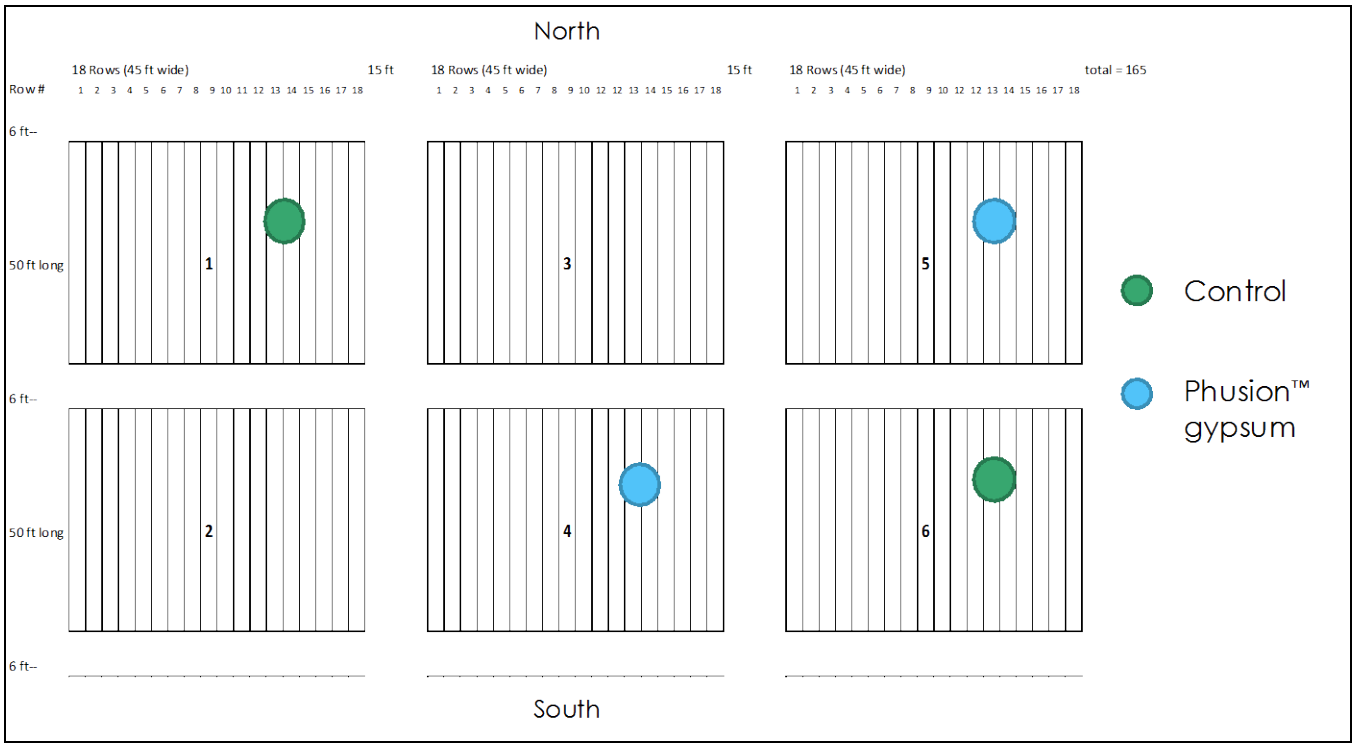
(0.10703 meq $CaSO_4 \cdot 2H_2O$)(86 mg $CaSO_4 \cdot 2H_2O$/meq $CaSO_4 \cdot 2H_2O$) = 9.2mg $CaSO_4 \cdot 2H_2O$/g soil

$$\left(\frac{9.2 mg\ CaSO4 \cdot 2H2O}{g\ soil}\right)\left(\frac{1kg/}{10^6 mg}\right)\left(\frac{10^3 g}{kg}\right)\left(\frac{1350 kg}{m^3}\right)(0.15\text{m})\left(\frac{10{,}000 m^2}{ha}\right) = \frac{\boldsymbol{19{,}853\ kg\ CaSO4 \cdot 2H2O}}{\boldsymbol{ha}}$$

Application of the treatment

The computed requirement to lixiviate the high levels of ESP in this soil was 19,853 kg/ha of calcium sulfate. Plots 4 and 5 were treated with the gypsum amendment Phusion™ Gypsum Calcium Sulfate (see Figure 3). Plots 1 and 6 were treated as control group (see Figure 3). Each plot was divided in 16 equal parts when gypsum was applied manually. We applied 363.2 kg/plot or 17,375 kg/ha of calcium sulfate due to availability of the product. After one week 3 inches of water were applied by irrigation and annual precipitation was measured. Annual precipitation for year 2015 was 21.97 inches, and for 2016 until march 18 was 4.46 inches. Plots 2 and 3 will be utilized for future research.

Figure 3. Experimental Design



Experimental Measurements:

Soil Chemical Properties

Soil sampling was performed in all 6 plots for chemical analysis before and after application of gypsum. Also, soil solution Access tubes were installed (after the application of the gypsum) to obtain solution from the soil pores for chemical analysis.

Soil Physical Properties

Saturated hydraulic conductivity was measured after the application of gypsum with 2 methods: the double ring infiltrometer method and the constant head soil core method; they were conducted under field and laboratory conditions respectively. The double ring infiltrometer method consisted of installing both rings 3 inches of depth where the outer ring was saturated

constantly and the inner ring was saturated until the floating metric ruler reached the value of zero, where the test began. Five sets of 20 minutes each were developed in each plot for water infiltration measurement with the double ring infiltrometer method.
The constant head soil core test was developed using cylinders with a volume of 72.38 cm^2 (4 inches long, 4 inches of diameter). These cylinders were installed completely in the soil and they were taken out carefully in order to not disturb the soil. Then, these samples of each plot were taken to the laboratory and the water infiltration test was developed using sets of 20 or 30 minutes until the soil reached equilibrium (approximately from 4 to 5 hours).

Results

Soil Chemical Properties

In average, the chemical analysis (Agsource Laboratories at Lincoln, Nebraska) before the application of the gypsum indicates that the soil has a pH of 9.1, salinity EC of 5.9 dS/m, and ESP of 33%. Other results were: CEC of 38.8 meq/100g soil, 3% of organic matter, 60 ppm NO_3, 21.74 ppm de P (Olsen Test), 18.1 meq Na^+/100g soil, 0.7 meq K/100g soil. In the saturated paste test results were: bulk density of 1.4 g/cm^3, 411 ppm salinity Cl^-, salinity SAR (sodium adsorption ratio) of 37.3. The soil had an average humidity of 25.4%. The soil samples after application of gypsum have not been analyzed yet. When analyzed each plot individually, the variable that demonstrated significant variability in values among plots was the electrical conductivity (EC); plots 1, 4, 5, and 6 have an EC of 4 ds/m, 7.7 ds/m, 15 ds/m, and 5.6 ds/m respectively. These data can be observed for each individual plot and all soil samples taken in Table 1 and Table 2.

The solution extracted from the soil solution access tubes has been from 0 to 5 mL in all plots. However, this quantity is not enough for chemical analysis.

Soil Physical Properties

Saturated hydraulic conductivity (Ks): Double ring infiltrometer method

These tests were developed in plots 1,4 and 5. Plots 1 (unamended) and 4 (amended) resulted in an infiltration of 0.1cm/hour and plot 5 (amended) resulted in an infiltration of 3.15cm/hour (see Figure 4).

Saturated hydraulic conductivity (Ks): Constant head soil core method

These tests were developed in all plots except for 1 and 3. The Ks values for the plots analyzed were: 0.09 cm/hr for plot 4 (amended), 3.11 cm/hr for plot 5 (amended), and 0.019 cm/hr for plot 6 (unamended) (see Figure 5).

Table 1. Preliminary Result - soil chemical analysis										
Plot	Gypsum Treatment	pH	CEC meq/100g soil	Bulk Density (g/cm3)	OM %	Na meq/100g	Ca meq/100g	NO3 ppm	Olsen P ppm	K meq/100g
1	no	8.9	44.8	1.44	2.9	21.40	18.43	89	11.2	0.52
1	no	8.1	38.2	1.36	3.7	5.54	24.62	74	13.4	0.40
2	no	9.6	45.5	1.46	2.1	34.88	8.75	110	22.6	0.72
2	no	9.6	32.7	1.39	4.1	5.07	21.03	75	20.7	0.49
3	no	9.5	41.3	1.44	2.8	21.53	16.1	46	9.48	0.51
3	no	9.4	37.3	1.31	3.5	6.80	24.38	42	8.96	0.52
4	yes	9.3	38.3	1.44	2.5	22.97	12.84	39	10.7	0.55
4	yes	9.7	39.1	1.43	2.3	25.23	11.56	39	13.4	0.61
5	yes	8.9	41.8	1.45	2.8	23.84	14.87	55	17.8	0.59
5	yes	9.1	40.7	1.41	2.6	25.05	12.67	48	31.1	0.82
6	no	8.8	32.2	1.38	3.4	10.97	15.68	41	54	1.30
6	no	8.9	33.7	1.41	3	14.52	14.36	62	47.5	1.21
		9.15	38.8	1.41	2.975	18.15	16.27	60	21.74	0.69

Table 2 Saturated Paste Extract - Salinity Test							
Plot	Gypsum Treatment	Salinity Cl ppm	Salinity SAR	ESR	ESP by SAR	ESP by CEC	Salinity EC (dS/m)
1	no	430.4	35.84	0.54	34.96	47.77	5.86
1	no	103.5	12.39	0.19	15.67	14.50	2.28
1	no	451.7	42.79	0.64	39.09	.	6.18
1	no	128.3	14.04	0.21	17.40	.	2.21
2	no	694.9	63.47	0.95	48.77	76.67	10.3
2	no	57.4	14.33	0.21	17.69	15.50	2.37
3	no	425.4	39.27	0.59	37.07	52.13	5.57
3	no	116.3	19.03	0.29	22.21	18.23	2.76
4	yes	625.8	47.43	0.71	41.57	59.96	7.72
4	yes	.	.	.	.	64.52	.
5	yes	1384.8	89.88	1.35	57.41	57.03	14.76
5	yes	.	.	.	.	61.56	.
6	no	388.6	27.76	0.42	29.40	34.07	4.75
6	no	133.3	41.83	0.63	38.55	43.08	6.64
		411.7	37.34	0.56	33.32	45.42	5.95

Piezometer

From 10 attempts, the piezometer has not detected any water in the subsoil.

Figure 4. Ks curve for double ring infiltrometer in plot 5.

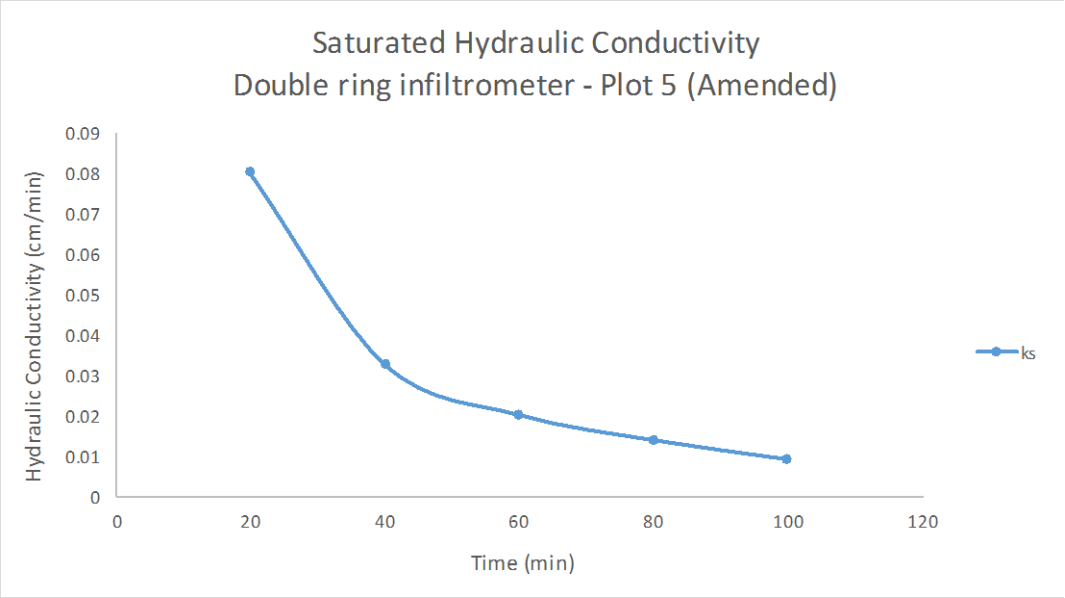
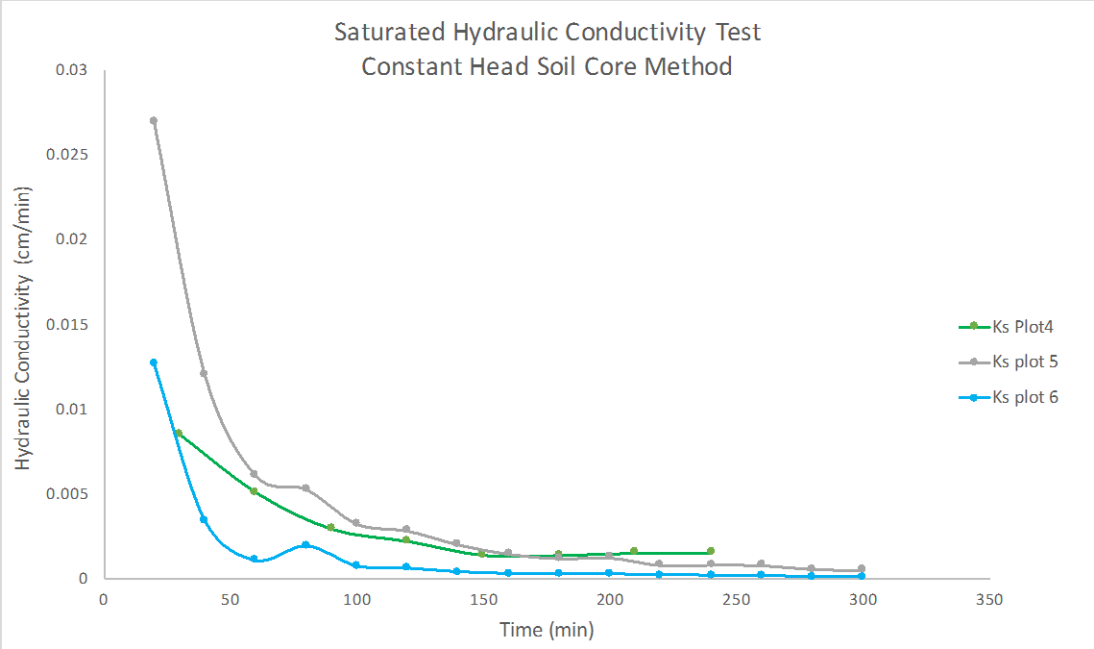


Figure 5. Ks curve for constant head soil core method for plots 4, 5 (amended) and 6 (unamended).



Discussion

Soil Chemical Analysis

The results obtained from the soil chemical analysis confirms the description of this soil as Sodic with some saline-sodic spots. The value of ESP chosen was the one computed from SAR (Seilsepour, et.al, 2009). Final results will be evaluated after we obtain data from the soil chemical analysis after the application of gypsum.

Solution from the soil solution access tubes ranged from 0 to 5 mL for all plots, due to this reason chemical analysis could not be performed because a higher volume is needed. A possible reason why this method had faced difficulties may be due to the lack of pores in this soil, making it hard for water to be available in the soil for extraction.

Soil Physical Properties

Saturated hydraulic conductivity test: Double ring infiltrometer

Infiltration tests performed with the double ring infiltrometer were performed in Plots 1, 4 and 5. Plots 1 and 4 resulted in an infiltration rate of 0.1cm/hr, and after 3 hours no change in infiltration was observed and for this reason the data was collected with only one set of one hour. The infiltration rate for these plots was extremely slow. However, the infiltration test performed in plot 5 (amended) resulted in 3.15 cm/hour and the Ks curve has a behavior expected for this type of test (greater infiltration at the beginning and a stabilized infiltration at the middle and end of the trial). Comparing the amended plots (4 and 5) with the unamended plots (1 and 6), the plot 5 demonstrated a higher infiltration rate when compared to the plot 1. This may be an indicator of gypsums effectivity as an amendment in the soil physical property of water infiltration. Also, this infiltration rate for plot 5 may be due to a high electrical conductivity of 15ds/m compared to the electrical conductivity of plot 1 of 4 ds/m. On the other hand, when plot 4 (amended) with an electrical conductivity of 7.7 ds/m is compared to plot 1 (unamended) they both resulted in the same infiltration rate.

Saturated hydraulic conductivity (Ks): Constant head soil core method

This infiltration method was easier to develop compared to the double ring infiltrometer method because it was conducted under laboratory conditions, the cylinders were smaller and because infiltration occurred faster the test was conducted more extensive (from 4 to 5 hours) (more data was collected compared to the double ring infiltrometer method). In this test, the splot 5 (amended) continued to show the fastest infiltration rate compared to the other plots (4 – amended and 6 - unamended) with a Ks of 3.11 cm/hr. Plot 4 (amended) demonstrated a Ks of 0.09 cm/hr and plot 6 (unamended) demonstrated a Ks of 0.019 cm/hr. When comparing infiltration rates of the amended sub-plots 4 and 5 with the unamended sub-plot 6, we can observe in the curve of **Figure 5** that the amended sub-plots have higher infiltration rates than the unamended plot. These results may be an indication of a positive effectiveness of the gypsum as an amendment for sodic soil management. Also it is important to mention that these plots have different electric conductivity; plot 5 has an EC of 15ds/m, plot 4 has an EC of 7.7 ds/m, and sub-plot 6 has an EC of 5.6 ds/m. Plot 5, although amended with gypsum, has the highest EC compared with the other plots; therefore, the high infiltration rate may be due to the high level of EC other than the application of the amendment. However, when comparing plots 4 (amended) and 6 (unamended) that have a similar EC, we can determine by the Ks values and the curve in

Figure 5 that plot 4 has a higher infiltration rate than the unamended plot 6. These results may be due to the effectiveness of gypsum as an amendment to lower ESP in sodic soils.
Plots 1 and 3 could not be tested in this method because they did not saturate by capillarity after 10 days of exposure to water and they started to show growth of fungus. Therefore, these core samples should be retaken from a different spot in the sub-plots to see if the sample saturation and Ks test can be conducted successfully.

Piezometer

No data was collected from the piezometer because it has not detected water in all attempts (10) although the soil literally appears to be humid. More attempts should be performed to obtain better data.

Conclusions

The tests that demonstrated trustable data were the soil chemical analysis and the saturated hydraulic conductivity constant head soil core method. The other tests, saturated hydraulic conductivity with double ring infiltrometer, the soil solution access tubes and the piezometer need an improvement in methodology in order to be utilized to obtain trustable data. In the Ks test with the constant head method, amended plots with gypsum had higher infiltration rates (Ks values) than the control group. However, further analysis must be done individually to determine if factors like the difference of EC between plots could have an impact in the difference of infiltration tests. In addition, chemical analysis after the application of gypsum must be conducted for better evaluation of the calcium sulfate as an amendment for sodic soil remediation.

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