

OCCURRENCE AND INCIDENCE OF PAPAYA RINGSPOT VIRUS IN PUERTO RICO

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ABSTRACT

Papaya ringspot virus (PRSV) is the most serious and destructive virus that affects papaya worldwide curtailing its production. PRSV has significant negative effects on the plant's development, fruit set and morphology. In an effort to contribute to the management of the disease caused by the virus in Puerto Rico, its incidence and occurrence was studied in papaya. The occurrence of PRSV associated with geographical elevation was determined with the generation of a predictive model, a Generalized Linear Model. The model's parameter estimation, discriminatory ability and significance of geographical elevation as a predictor variable were also assessed. The virus was found to be highly incident and most likely to occur at low elevations (0-200m), where most of papaya farms are located. The model created had good parameter estimation and was able to discriminate true PRSV positive and negative samples from all the samples. Geographical elevation proved to be a significant variable for predicting the occurrence of PRSV. The results from this study have implications for disease management in Puerto Rico.

Keywords: Papaya, papaya ring spot virus, elevation effects, PRSV

Introduction

Carica papaya (L.), commonly known as papaya, is a semi-woody shrub cultivated in tropical and sub-tropical regions. It is considered a cash crop because of its continuous production, successful seed germination and low maintenance requirements (Tennant et al., 2007). However, many diseases induced by etiological microorganisms affect papaya production and they range from fungi, bacteria, phytoplasms and viruses (Teixeira da Silva et al., 2007). There are many viruses that cause disease in papaya such as *Papaya ringspot virus* (PRSV), *Papaya leaf distortion mosaic virus* (PLDMV), *Papaya mosaic virus* (PapMV), *Papaya leaf curl virus*, *Tomato spotted wilt virus*, *Tobacco ringspot virus*, *Zucchini yellow mosaic virus*, among others (Tennant et al., 2007). PRSV, PapMV and PLDMV are the most economically important viruses that have a long history of affecting papaya production (Bauet al., 2008; Tennant et al.,

2007). Of these viruses, PRSV is the most damaging to papaya production in the world.(Gonsalves, 1998; Teixeira da Silva et al., 2007).

PRSV belongs to the Potyvirus genus in the Potyviridae family and is transmitted non-persistently by aphids. Hosts include members of *Caricaceae*, *Cucurbitaceae* and *Chenopodiaceae*. The virus causes ringspot disease and the symptoms it induces in papaya are leaf discolorations (mosaics), deformation and distortions (shoestring appearance and blisters). Fruit morphology is also affected by the development of bumps and ringspots in the outer layer, as well as changes in its shape (Tripathiet al., 2008). PRSV was first reported in Puerto Rico by Adsuar(1946) and initially described as a virus causing mosaic disease. It was restricted to the Southern coast of Puerto Rico and later spread to other regions in the island (Escuderoet al., 1994; Riollano, 1952; Rodríguez, 1978).Production of papaya has been affected since and continues to do so due to year-round production of papaya and practically completely infected fields within 9 months of planting (Zambrana-Echevarríaet al., 2016). The majority of commercial papaya fields are located in municipalities of the South coast region of Puerto Rico (USDA NASS, 2012). The Government of Puerto Rico designated this regionin the late 1970's for fruit and vegetable production in the island (Carro-Figueroa, 2002) causing agricultural intensification of papaya. Furthermore, PRSV had already been causing havoc in the island. Therefore, it is crucial to study the epidemiology of PRSV in Puerto Rico in order to have effective disease management programs, particularly for the south region of the island.

Plant disease is affected by the interactions between plant, pathogen and the environment, referred to as the disease triangle (Atiriet al., 2000). Quantitative measurements of disease or pathogen intensity influence the efficacy of management programs (Nutter Jr. et al., 2006). There are different measures of disease intensity and incidence is among them. It is measured as the proportion of plants or plant units (e.g. leaves) with disease (Madden and Hughes, 1995; Nutter Jr. et al., 2006). If the pathogen is being detected and measured with detection methods such as ELISA and PCR, then the term pathogen prevalence or incidence is used (Nutter Jr. et al., 2006). When assessing disease and/or pathogen incidence and occurrence, generalized linear models (GLM) are appropriate for modeling discrete data (Garret et al., 2002). A logistic regression is chosen in a GLM when analyzing proportions data where the predictor variable is continuous and the response variable is binary (Bewicket al., 2005; Garret et al., 2002). Modeling disease dynamics allows for management programs to best use resources, avoid or prevent disease cycles in order to increase the efficiency of agricultural production and provide tools for the study of dynamics between pathogen, host and vector (Jegeret al., 2004). A successful management of a disease depends on knowledge of the epidemiology of a disease (Sharma et al., 2008) and the etiological microorganism. Therefore, in the studythe incidence and occurrence of PRSV in

Puerto Rico was assessed using geographical elevation as a predictor variable. A GLM with a logistic regression was generated in order to associate the predictor variable and the occurrence of the virus.

MATERIAL AND METHODS

Sample collection and DAS-ELISA

A total of 116 papaya leaves with and without PRSV-like symptoms were collected in private homes of mainland Puerto Rico. Subsequently, the samples were tested for the presence of the virus with a DAS-ELISA PRSV kit from Agdia Inc. (Elkhart, Indiana). A sample was designated PRSV positive if the absorbance was $>2X$ than that of the negative control (approximately 0.3-0.4) and PRSV negative if the absorbance was $<2X$ than the negative control. Global positioning system (GPS) coordinates were recorded in each sample location. These coordinates were mapped using Google Maps Engine (<http://mymaps.google.com>) (Figure 1). The municipalities where collections took place were: Mayagüez, Maricao, Lares, Yauco, Sabana Grande, Coamo, Hormigueros, Villalba, Cayey, Aibonito, Barranquitas, Orocovi, Ponce, Utuado and Jayuya. Elevation was divided in three classes: low (0-299m), mid (300-599m) and high (600-899m). Table 1 shows the total samples collected at each elevation class and their result on the PRSV ELISA.

Statistical analyses

The exposure variable, geographical elevation, was divided in three classes as described above. PRSV incidence was calculated as the total of ELISA positives over the total samples collected (proportion of positives) at each elevation class and for all elevations (Table 2). A generalized linear model (GLM) was generated in R 3.2.2 (R Core team, 2015) in order to analyze the occurrence of PRSV at different elevations by associating the PRSV-ELISA result (positive/negative) with geographical elevation (in meters) as an explanatory variable. The GLM was constructed using the `glm()` function with a binomial error distribution and a logit link function. The model was fitted with the Maximum Likelihood method. The odds of obtaining a positive on the PRSV ELISA test, versus a negative, at 0m were calculated by taking the exponential of the coefficient from one of the unknown parameters ('Elevation') estimated by the model (Supplementary Figure 1). The Hosmer-Lemeshow (HL) statistic was calculated (Table 3) with the Resource Selection package to evaluate the extent to which the fitted model predicts the observed outcomes (goodness of fit). The data was separated into multiple groups (g): 5, 10 and

15. The observed χ^2 value and P-value were obtained in R with degrees of freedom of g-2. The significance of the explanatory variable in predicting the outcome of the response variable was evaluated with the Likelihood Ratio test (χ^2 statistic). In addition, the discriminatory ability of the model to differentiate between the proportion of true positives from all the positives (sensitivity; true positive rate), and true negatives from all the negatives (specificity; false positive rate) was assessed with a receiver operating characteristic (ROC) curve with the pROC package. The discriminatory ability of the model was further assessed quantitatively by finding the area under the curve (AUC). An AUC equal to 0.5 indicates no discrimination and equal to 1 indicates perfect discrimination (Cook, 2007) of the model. A P-value less than 0.05 was considered significant for all the statistical tests.

RESULTS AND DISCUSSION

Surveys of 116 papaya plants were conducted in Puerto Rico in order to determine the incidence of PRSV at different elevations (Figure 1) and the presence of the virus was detected with a PRSV DAS-ELISA. Papaya was found at all elevations sampled, however, it is most commonly found at lower elevations as opposed to higher elevations (Table 1). The proportion of PRSV positives was calculated per each elevation class to estimate virus incidence (Table 2). PRSV has higher incidence at lower elevations (0-200m) compared to mid and high elevations. A similar result was obtained for *Cucumber mosaic virus* in several crop hosts (Hordet al., 2001), for *Tomato spotted wilt virus* in lettuce (Cho et al., 1987) and several sweet potato viruses (Njeruet al., 2008) where their incidence was higher on location with lower elevations. In a study by Wan and Conover (1983), the incidence of papaya viruses, including PRSV, was assessed in papaya fields in Florida. It was found that PRSV had higher incidence in counties with commercial papaya fields. High incidence is demonstrative of high inoculum potential and efficiency of vector transmission (Maryset al., 2000; Wan and Conover 1983)

In order to associate geographical elevation with the occurrence of PRSV, a GLM was created with a logistic regression. At lower elevations, there is a higher probability of obtaining a positive result on the ELISA. Based on the parameter estimation of the Elevation Model (Supplementary Figure 1), at 0m the odds of obtaining a positive on the PRSV ELISA test is 2.1574 times more likely than a negative. The logistic regression of the GLM shows that as elevation increases, there is a lower probability of obtaining a PRSV positive in the ELISA (Figure 3). The quality of the model's parameter estimation was assessed with the Hosmer-Lemeshow Goodness of fit test (Table 3). For each group, the P-value was not statistically significant (>0.05) and the value of the observed chi-squared statistic was higher than the

expected value. Therefore, the model does a good fitting of the data and is able to make predictions based on the data obtained. A Likelihood Ratio test was carried out to assess the significance of elevation as a variable that predicts the result on the PRSV ELISA. This variable is significant at predicting the ELISA results ($\chi^2=28.2355$; d.f. =1, P -value=1.0742e-07) and it is statistically significant; therefore, geographical elevation is a significant variable for predicting the occurrence of the virus. Subsequently, the discriminatory ability of the GLM to differentiate between true positives and negatives was assessed with a ROC curve (Figure 3). The GLM generated in this study had higher sensitivity than specificity in the ROC curve with an AUC of 0.8344. This value is close to 1 (perfect discrimination); (Cook, 2007), which indicates that the model has good discrimination between true ELISA positives/negative samples from all the ELISA positives/negative samples (Figure 3).

The type of model constructed in this study, GLM, has been used previously in epidemiological studies of plant viruses. This is the case for Mora-Aguilera et al. (1992), where the temporal and spatial distribution of PRSV in fields of papaya was assessed, partly, with a GLM using number of aphids as a variable. Similarly, Leghtonen et al. (2006) used a GLM with a logistic regression to assess the occurrence and relationship of ryegrass infected with *Barley yellow dwarf virus* and a fungus. An extension of the GLM, the generalized linear mixed model, was used by Juarez et al. (2013) to analyze the distribution of cucurbit viruses related to zone and year in Spain.

Cultivation of papaya has historically – and to this day – taken place primarily in the South region of Puerto Rico, a coastal area of low elevations (0-200m) (Gobierno de Puerto Rico, Oficina de Gerencia y Presupuesto, n.d.; USDA NASS, 2012). In the late 1970's, after the decline of the sugar industry, the government of Puerto Rico changed their development model for the island, making agriculture one of the central aspects of the new economy. It acquired agricultural land previously used for sugar plantations, which were spread across the island. Different regions of Puerto Rico were made exclusively for growing a specific type of crop. For the South region, fruits were farmed intensively along with vegetables (Carro-Figueroa, 2002). Papaya was among the fruits that were cultivated in this region. Twenty years earlier to this change in agricultural practices, PRSV had been reported in the South region (Adsuar, 1948; Riollano, 1951) and was spreading to the other parts of the island (Rodríguez, 1978). The intensive farming of papaya in the South created an environment of high disease pressure and inoculum. Intensification in agriculture is a factor, among others, that drive virus emergence by facilitating encounters and host movements (Jones, 2009).

In this report, PRSV had higher incidence and occurrence in regions of lower elevations, where most papaya farms are located. This is the case for the municipality of Santa Isabel whom has 9 farms of papaya accounting for a total of approximately 180 acres (USDA NASS, 2012).

Although this municipality was not sampled in this study since sampling did not focus on fields, it belongs to the group of municipalities of low elevations (Gobierno de Puerto Rico, Oficina de Gerencia y Presupuesto, n.d.). The high incidence of PRSV on these areas demonstrates the need for better agricultural planning, practices and disease management. Some of the municipalities where collections took place have the highest values for minimum and maximum elevation, as well as for average elevation (Gobierno de Puerto Rico, Oficina de Gerencia y Presupuesto, n.d.). The municipalities in this group that were sampled in our study are Maricao, Lares, Villalba, Cayey, Aibonito, Barranquitas, Orocovi, Utuado and Jayuya. In these areas of mid to higher elevations, the incidence of PRSV is lower (Table 2) and the virus is less likely to occur as demonstrated by the GLM and logistic regression generated (Figure 2; Supplementary Figure 1). Commercial fields of papaya need to be more evenly distributed throughout the island and shift to higher elevations where PRSV is not as present and papaya can still grow (Table 1). A first step to a positive change to our agricultural practices and disease management would be to change the distribution and location of papaya farms in Puerto Rico. That would reduce the agricultural intensification occurring in the South.

In order to accomplish an effective management program, more epidemiological studies of PRSV and the disease it causes are needed, as well as research on the aphid vector's population dynamics and spatial distribution. A disease is the result of the interactions between hosts, pathogen (including its vector) and environment (Atiriet al., 2000). A vector population can influence disease and pathogen incidence, which depends on vector dynamics (Jegeret al., 2004). Previous to this study, Mora-Aguilera et al. (1992) reported in Mexico the temporal and spatial distribution of PRSV in relation to its vector. They found that the incidence of the virus was in fact related to the amount of vectors present. Before their report, there had not been quantitative studies on the epidemiology of PRSV. Furthermore, Kalleshwaraswamy and Kumar (2009) in a study of the effect of insecticides and oils on aphids that transmit PRSV determined that 9 months after planting, the incidence of the virus was higher in untreated plots. Our study cannot discard that the incidence and occurrence of PRSV in Puerto Rico is not influenced by the aphid's distribution in the island, which has not been reported to this date. A better understanding of the epidemiology of PRSV in Puerto Rico would require that different aspects of the disease triangle to be researched.

The study of epidemiology of PRSV in Puerto Rico is crucial in order to improve of our agricultural practices of papaya and influence effective disease management programs. In this study, it was found that PRSV is highly incident and occurs more at lower geographical elevations and that this is a variable that is good and significant for predicting the virus' occurrence. This reflects the agricultural intensification and high inoculum potential that is happening in municipalities of the south coast of Puerto Rico where there are numerous papaya fields.

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Figure 1. Topographical map with *Carica papaya* collection sites made during samplings at different elevations. Closed circles represent collection sites mapped with GPS coordinates.

Map was created in Google Maps with terrain layer.

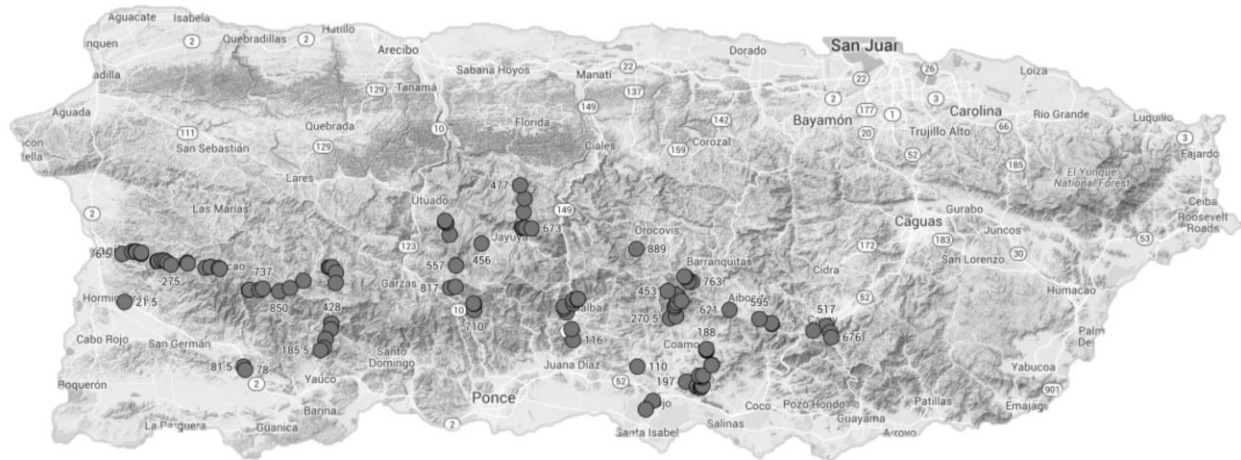


Figure 2. Logistic regression of the Elevation Model showing the probability of a positive result in the ELISA associated to elevation. The curve shows the predicted probabilities of success (ELISA positive) obtained from the estimation of the model's unknown parameters using the maximum likelihood method. Open circles show the observed values of the ELISA at each elevation sampled. McFadden's Pseudo R2= 0.2506

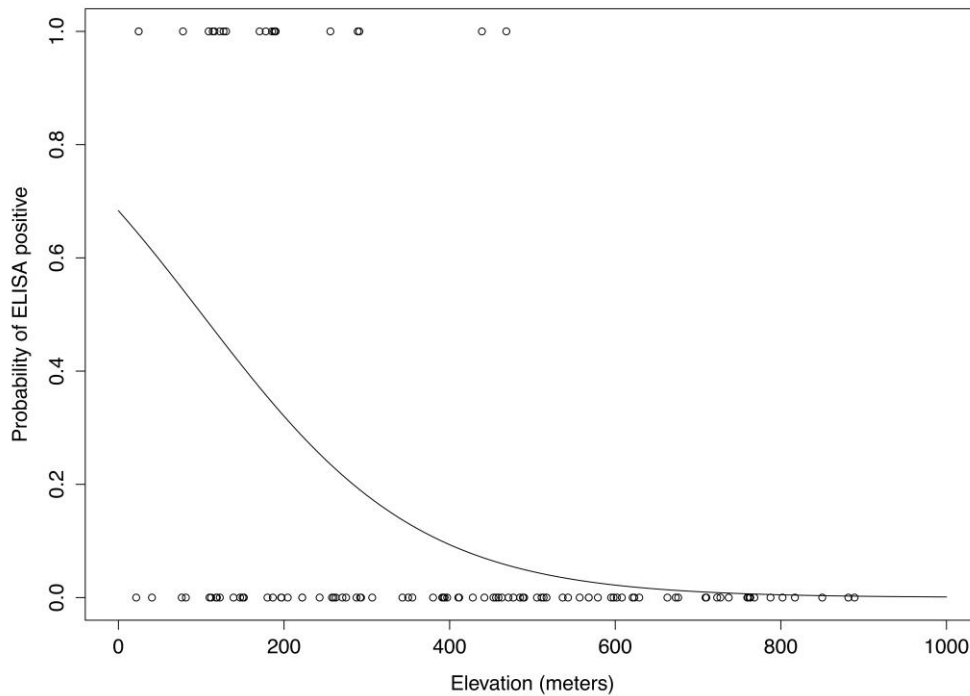
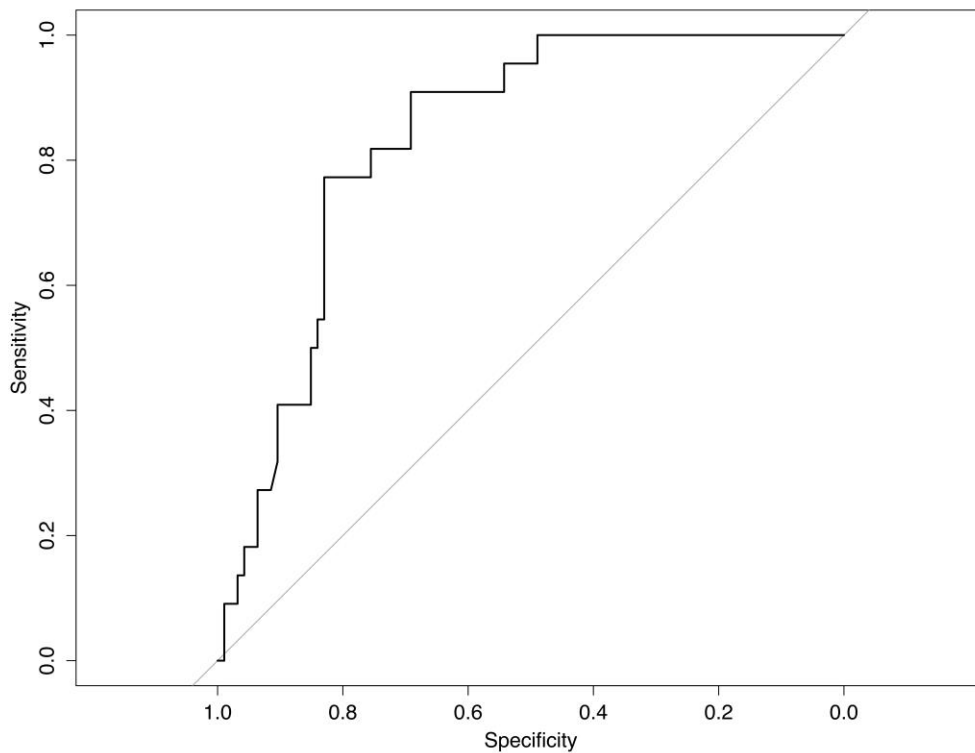


Figure 3.ROC curve for the assessment of the Elevation model’s ability to discriminate between ELISA positives and negatives. Sensitivity axis indicates the proportion of true positives (true positive rate) and the specificity axis indicates the proportion of true negatives (false positive rate). The area under the curve equals to 0.8344.



Supplementary Figure 1. Summary of the Generalized Linear Model created in R. The output shows the formula used in R to create the model with the glm() function. It also shows the model’s estimation of the parameters ‘Intercept’ and ‘Elevation’, their coefficients and standard error. Parameter estimation was performed with the Maximum Likelihood method within the glm() function

```
Call:
glm(formula = ELISA ~ Elevation, family = binomial(link = logit),
    data = data)

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.768900   0.500545   1.536   0.125
Elevation   -0.007604   0.001943  -3.914 9.06e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table 1. Number of papaya leaves collected at each elevation class and their result on the ELISA test for PRSV.

Elevation Classes (m)	ELISA Result		Total
	Negative	Positive	
low 0-299	31	20	51
mid 300-599	35	2	37
high 600-899	28	0	28
Total	94	22	116

Table 2. Incidence of PRSV at each elevation class. Incidence is measured as the proportion of positive samples over all samples. Proportions were calculated as the number of ELISA negative or positive samples over the total samples collected at each elevation class.

Elevation Classes (m)	Proportion of PRSV ELISA positives
low 0-299	0.3922
mid 300-599	0.0541
high 600-899	0.0000
Overall elevations	0.1897

Table 3. Goodnes of fit test of the Elevation Model. The test used was the Hosmer-Lemeshow Goodness of fit test with a chi-squared statistic (χ^2). The data was divided into multiple groups and the GOF was tested on each of them. P-value of 0.05 was considered as statistically significant.

Groups (g)	χ^2	Degrees of freedom	P-value
3	0.6602	1	0.4165
5	2.1316	3	0.5456
10	11.2730	8	0.1867
15	8.7880	13	0.7888