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Weather Parameters Influencing the Incidence of Citrus Canker Caused by AW Strain in the Rio Grande Valley

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WEATHER PARAMETERS INFLUENCING THE INCIDENCE OF CITRUS CANKER CAUSED BY A^W STRAIN IN THE RIO GRANDE VALLEY

A Thesis

by

AMIT SHARMA

Submitted in Partial Fulfillment of the

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December 2022

ABSTRACT

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Citrus canker caused by bacterium *Xanthomonas citri* subsp. *citri* (*Xcc*) seriously affects the citrus industry by making the fruit unmarketable due to unsightly lesions on the fruit. Canker caused by A^w strain of *Xcc* was reported in the citrus trees located in the residential areas of the Rio Grande Valley (RGV). Canker severity differs amongst cultivars/varieties, and it is influenced by prevailing environmental conditions. Multiple regression modeling of the disease incidence with the environmental variables such as temperature, humidity, windspeed, wind gust, and rainfall was performed to understand the environmental conditions that are favorable for spread of citrus canker caused by A^w strain in the RGV. Our results reveal two statistically sound models that are fit to the data. Model 1 predicts the significant effect of temperature, humidity, wind gust, and wind speed while Model 2 predicts the significant effect of temperature, humidity, wind gust, wind speed, and rainfall on citrus canker spread. However, Model 1 is preferred over Model 2 as Model 1 shows better model diagnostics than Model 2. Additionally, precipitation cannot affect the citrus canker negatively as in case of Model 2. Knowing the environmental conditions in the RGV that favors the spread of canker caused by A^w strain, one can design better management practices before disease becomes an epidemic.

DEDICATION

I want to dedicate my thesis not only to my mother Mrs. Neelam Sharma and father Mr. Mohan Lal Sharma for making me so much capable and desire to achieve great things but also my mother and father figure Mrs. Subash Kapila and late Mr. Satpal Kapila for providing me with immense support and confidence.

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CHAPTER I

INTRODUCTION

Citrus is widely cultivated in the tropical and subtropical climatic regions around the world (Nasreen et al., 2021; Srivastava, 2014). Citrus production is affected by a large range of pests and pathogens. Citrus canker is one of the major diseases of the citrus fruits that causes extensive damage. The disease severity varies with different citrus crop varieties/cultivars and prevailing climatic conditions (Nasreen et al., 2021). In the USA, canker disease was first reported in 1912 in Florida on imported seedlings from Japan (Berger, 1914). After various eradication campaigns, citrus canker is still endemic to Florida. The disease was observed in Gulf Coast states including Texas, Florida, and Louisiana in early 1900s (Stevens, 1914) caused by Asiatic strain but was eradicated by 1947 (da Graça et al., 2017; Dopson, 1964). However, it reappeared in 2015 in Cameron County, Texas on lime trees and one lemon tree infected with a different strain, A^w (da Graça et al., 2017). In 2016, the disease caused by A strain was reported in several citrus trees in the Upper Gulf Coast area (Gochez et al., 2020). The occurrence of the disease in residential citrus plants in Brazoria, Fort Bend, and Harris Counties (Gochez et al., 2020; Perez et al., 2021) raises a constant concern about the spread of the disease to other places in Texas.

The disease is caused by a gram-negative bacterium, *Xanthomonas citri* subsp. *citri* (*Xcc*) (Syn. *Xanthomonas axonopodis* pv. *citri*) (Das, 2003; Gottwald et al., 2002; Nasreen et al., 2021). The bacterium is categorized into several strains based on the host it affects, cultural and physiological characteristics, DNA-DNA homology, plasmid fingerprints, serology, and RFLP and PCR analysis (Gottwald et al., 2002). *Xcc* severely affects almost all citrus species causing Cancrosis A (Gottwald et al., 2002; Naqvi et al., 2022). *Xanthomonas citri* pv. *aurantifolii*, also known as Cancrosis B, affects a few species of citrus including lemons, Mexican limes, sour oranges, and pummelo in South America (Das, 2003; Patané et al., 2019). A variant of *Xanthomonas citri* pv. *aurantifolii* causes cancrosis C on Mexican lime and sour oranges in Brazil (Table 1.1). Another strain of bacteria known as A^* was reported from Oman, Saudi Arabia, Iran, and India affecting the Mexican limes (Das, 2003; Gottwald et al., 2002; Vernière et al., 1998). The most recent strain found in some parts of Florida and Texas is A^w strain affecting Mexican limes and alemow (da Graça et al., 2017; Perez et al., 2021; X. Sun et al., 2004). In Texas, Asiatic citrus canker is found in the Upper Gulf Coast areas including Harris County, Fort Bend County, and Brazoria Counties and the strain A^w isolates are found in South Texas including Cameron, Willacy, and Hidalgo counties (Perez et al., 2021).

Citrus canker on plant parts is the result of hyperplasia that renders the enlargement of the tissue of the plant due to the increased proliferation and excessive mitotic division rate of cells and can be seen as a conspicuous raised necrotic lesion on the plant part (Das, 2003). Typical symptoms of the disease include formation of 2-10 mm oily spots on leaf, twigs, and fruits which later turn corky tan brown and raised (Fig 1.1). Around the stem area the lesions may coalesce and girdle the stem. The old lesions on the leaves and fruits are often surrounded by yellow halo. The lesions do not penetrate deep into the fruit and thus not impairing internal fruit quality (Fig 1.2). In case of severe infection, there is die back of the twigs followed by dropping of the leaves (Das, 2003). The disease is very serious on grapefruits, sweet oranges, Mexican limes, lemons, and trifoliate orangs while kumquats, calamondin, mandarins are some of the resistant rootstocks (Das, 2003; Gottwald et al., 2002; Sharma & Sharma, 2009) (Table 1.2). The attack of citrus leaf miner favors the disease as it forms galleries by feeding on the epidermis cells and expose the mesophyll tissues to *Xcc* for direct infection (Naqvi et al., 2022).

In severe cases, citrus canker renders the premature fruit drop, unmarketable fruits, and loss of access to fresh fruit markets. If the preventive measures are not strictly followed the losses due to the disease can go up to 80% and hence it is important to manage the disease to reduce the fruit drop and other effects on fruit production and import (Gochez et al., 2020). It is generally very difficult to control the disease in the susceptible varieties when the environmental conditions are favorable. The successful management methods of disease constitute avoidance, exclusion, eradication, use of resistant varieties, protection, and therapy (Civerolo, 1982 and Gottwald et al., 2002). To avoid the pathogen in an area it is preferred to use moderate to high resistant varieties, new trees must be planted in an area with no history of the canker, the new planting material should never be taken from an area/country that had infection of the bacterium, the planting site chosen should not be climatically favorable for the development or progression of the disease. For exclusion of pathogen, the orchard workers must be made aware of the measures for managing the disease by disinfecting not only the orchard machinery but their clothing as well. The identification and then use of resistant plants are highly effective for long term management of the disease.

The disease propagates when there is free moisture on the surface of infected tissue and the bacterial exudates oozes out from the water-soaked lesions (Das, 2003; Stall et al., 1980). The bacterium inside the infected tissue produces extracellular polysaccharides that help in preventing the dilution and desiccation of the bacteria when it comes out on the surface (Goto, 1985). The bacteria along with the extracellular polysaccharides are dispersed to other uninfected plant parts by the rain splashes. After landing on new plant parts, the bacteria enter the systemics of the plants either by natural openings or by wounding. Under the favored conditions the symptoms start appearing within a weak as a lesion on the pant surface (Das, 2003 and Gottwald et al., 2002). It has been observed that 1 ml of the rainwater collected from the diseased foliage contains 105-108 colony forming units (cfu) of bacteria (Stall et al., 1980). Strong winds accompanied with rainfall aids in the infection process. Graham et al. (1992) observed wind speed of more than 8 m/s drives the bacterial penetration through natural openings or wounds. The bacterium dies when exposed to direct sunlight and drying. The bacterium survives in the plant tissue in the soil for a few months, however, it cannot survive without tissue in the soil for more than a few days (Goto, 1985). The bacterium present in the stem lesions can survive for a few years. The younger stages and the new growth of the plant is more susceptible than the older ones. Ideal temperature conditions for the infection process to take place is 20°-30°C (Koizumi; 1983). It may take up to 60 days to observe the symptoms after the infection process if the conditions are not ideal. The expansion of the symptoms on the plant surface is fast that is 1 mm/month for first 6-8 months and then it slows down (Graham et al., 2000). In contrast to wind driven rain which is the medium for the dispersal of the pathogen for short distances, the long-distance dispersal takes place by movement of the infected plant from one place to another, human intervention and using the contaminated equipment (Gottwald et al., 2002). Four foci of infection was developed at the point of infection in the orchard that varied from 230 m to 810 m from source of infection in a thunderstorm in 1989 in Florida (Gottwald et al., 1992). Severe rainstorms and tornados in 1996 helped the spread of citrus canker from 32 Km^2 to an area of 223 Km^2 (Gottwald et al., 2001). PCR based methods targeting the amplification the PthA, which is a virulent factor gene, can be used to detect the bacterium (Cubero & Graham, 2002). Also, strain A can be differentiated from B and C based on sequence variation of internal transcribed spacer (ITS) regions of 16s and 23s ribosomal DNA (Navarro et al., 1992). Rep-PCR uses enterobacterial repetitive intergenic consensus (ERIC) and BOX elements fingerprints to detect the difference in the strains within same pathotype (Cubero & Graham, 2002).

The cultural control to manage the disease includes the pruning of the infected parts followed by copper spray and using the windbreaks (Gottwald et al., 2002; Leite & Mohan, 1990). Biocontrol agents that have been reported against *Xanthomonas* includes *Erwinia herbicola, Bacillus subtilis* and *Pseudomonas fluorescens* (Nasreen et al., 2021). There are several chemical bactericides that are used as prophylactic measure and to minimize the building of inoculum on the infected plant. However, the success of the spraying depends upon many factors like resistant/susceptibility of the host, prevailing environmental conditions, and integration of other measures of managing disease. Eradication efforts and putting quarantine in the highly infested areas is another method to manage the spread of the disease as the movement of the citrus material is restricted within the quarantine boundary. There are 4 state regulated zones in Texas including the parts from Harris County, Fort Bend County, and Brazoria County, and Cameron County (Gochez et al., 2020). There should be an integrated use of all the above-mentioned measures to manage the disease from spreading within the endemic areas and to new areas. The environmental conditions including temperature, rainfall, moisture and wind speed were reported as the crucial factors for citrus canker caused by A strain to develop and spread (Hameed et al., 2022). This study focused on developing a mathematical model to check the influence of weather parameters on citrus canker disease incidence caused by A^W in RGV. We followed established models that include environmental variables such as temperature, wind speed, rainfall, and relative humidity along with other infection factors based on previous detections to develop regression models for citrus canker disease incidence in the RGV (Gottwald & Irey, 2007; Hameed et al., 2022; Irey et al., 2006; Neri et al., 2014; Raza et al., 2014). We considered additional components such as maximum temperature, minimum temperature, wind gust, sea level pressure, solar radiation, solar energy, and UV index that may affect disease incidence or spread. Overall, this study will help in better understanding of the citrus canker epidemiology under RGV weather conditions. Knowledge of the potential weather parameters that can promote citrus canker incidence caused by A^W strain in the RGV can help in designing better management strategies before the disease becomes an epidemic.

Figure 1.1: Canker symptoms caused by A^w strain on grapefruit leaves in the RGV. (Picture credit: Dr. M. Kunta, TAMU-K Citrus Center)

Figure 1.2: Canker symptoms caused by A^W strain fruit surface. The lesions do not penetrate deep in the fruit (Picture credit: Emma Perez, USDA APHIS PPQ).

Table 1.1: Distinct strains of the bacterium producing slightly different citrus canker symptoms on their respective hosts.

Bacterial strain	Canker type	Origin	Host range	Symptoms	References
Xanthomonas citri pv. *citri/ Xanthomonas axonopodis* pv. *citri* (Syn)	Cancrosis A or Asiatic citrus canker	Asia	Most Citrus fruits	Necrotic, raised, circular, 2-10mm, blister type, generally water-soaked lesions on leaves, twigs, and fruits. Older lesions on fruits and leaves are surrounded by yellow halo. Lesions do not penetrate fruit.	(Das, 2003; Gottwald and Graham, 2002; Naqvi et al., 2022)
Xanthomonas axonopodis pv. *aurantifolia*	Cancrosis B	South America	Lemons, Mexican limes, sour oranges, and pummelo	Symptoms of Cancrosis B is same as in Cancrosis A but the lesions are smaller in size.	(Das, 2003; Patané et al., 2019).

Table 1.1, cont.

Xanthomonas axonopodis pv. *aurantifolia*	Cancrosis C	Brazil	Mexican limes and sour oranges	Symptoms of Cancrosis C is same as in Cancrosis A.	(Das, 2003; Gottwald and Graham)
A^* strain		Oman, Saudi Arabia, Iran, and India	Mexican lime	Blister like lesions with more or less water-soaked margins and non-erumpent lesion on grapefruits unlike Cancrosis A	(Das, 2003; Gottwald et al., 2002; Vernière et al., 1998)
A^w Strain		Florida, United States of America	Mexican/key lime and Alemow	Typical canker symptoms appear on Mexican lime and alemow but not on grapefruit and sweet oranges.	(da Graça et al., 2017; Perez et al., 2021; X. Sun et al., 2004)

Table 1.2: Resistance/ susceptibility of different citrus cultivars to citrus canker

Category	Citrus cultivar	References
Highly resistant	Calamondin (*Citrus madurensis*) Kumquats (*Fortunella sps*.)	(Das, 2003; Goto, 1992; Gottwald et al., 1993; Graham, 2001; Leite Jr, 2002; Leite Jr & Mohan, 1984; Zubrzycki & Diamante de Zubrzycki, 1982)
Resistant	Mandarins (*C. reticulata*), Tahiti lime (*C. aurantifolia*), and Pummelo (*C. maxima*)	
Susceptible	Sweet oranges (*C. sinensis*), Sour oranges (*C. aurantium*), Lemons (*C. limon*), Tangelo (*C. tangelo*), Pummelo (*C. maxima*), Limes (*C. latifolia*), Trifoliate oranges (*Poncirus trifoliata*), and Citrumelos (*P. trifoliata* x *C. paradisi*)	
Highly susceptible	Grapefruit (*C. paradisi*), Mexican lime *(C. aurantiifolia*), and Lemons (*C. limon*)	

CHAPTER II

MATERIAL (DATA) AND METHODS

1. Exploratory data analysis (EDA)

1.1 Disease Incidence (DI) data

Citrus canker survey data for the period 2015-2022 was received from the United States Department of Agriculture for 13 species of *Citrus* including: Key lime (*C. aurantiifolia*), lemon (*C. limon*), sour orange (*C. aurantium*), orange (*C. sinensis*), grapefruit (*C. paradise)*, calamondin (*C. madurensis*), mandarin (*C. reticulata)* , tangerine (*C. reticulata)*, kumquats (*Fortunella species), Citrus species,* makrut lime (*C. hystrix*), pummelo (*C. maxima*) and trifoliate oranges (*Poncirus trifoliata)* (Fig 2.1). After performing exploratory data analysis (EDA), we found the total number of trees surveyed, the total number of positive trees, and the total number of negative trees for citrus canker for each month of 2021 and up to August 2022 (Fig 2.2). We focused on the geographical area with more positive canker trees than negative trees. Most of the trees which were found positive were key lime (*C. aurantiifolia*) while other positive trees for citrus canker including lemon (*C. limon*)*,* makrut lime (*C. hystrix*), pummelo (*C. maxima*), and trifoliate oranges (*Poncirus trifoliata)* were less in number, so we combined the data for the whole species surveyed to *Citrus*. Disease incidence for each month was then calculated by using formula (Cardoso et al., 2004; Hughes, 1999; and Seem, 1984).

$$\frac{Number\ of\ positive\ trees\ in\ a\ month}{Total\ number\ of\ trees\ surveyed\ in\ a\ month} * 100$$

In May 2021, the number of canker-positive trees were found to be zero and none of the trees were found to be negative, so we assume the number of negative trees as zero as well. Initially we had n=20 (corresponds to the months starting from January 2021 to august 2022). Since we had disease incidence zero (DI=0) in April and May 2021, we omit these two, rendering final data size n=18.

1.2 Weather data

Historical weather data (numerical) include the following variables for each day in the month starting from January 2021 to August 2022.

- Temperature (ºC)
- Maximum temperature (ºC)
- Minimum temperature (ºC)
- Humidity (%)
- Precipitation (mm)
- Wind gust (Km/h)
- Wind speed (Km/h)
- Sea level pressure (millibar)
- Solar radiation (W/m^2)
- Solar energy (W/m^2)
- UV index

The weather variable data were collected from online database of Visual Crossing Corporation (Ajaz et al., 2022; Bergero et al., 2022; Gharoie Ahangar et al., 2020; Gupta et al.

2020; Igobwa et al., 2022; R. Patil & Kumar, 2022; R. R. Patil & Kumar, 2021; Peter, 2021) which includes the data from multiple sources: Integrated Surface Database (ISD), Meteorological Assimilation Data Ingest System (MADIS), Global Historical Climatological Network daily (GHCNd) powered by National Oceanic and Atmospheric Administration (NOAA), and a German Meteorological Service: Deutscher Wetterdienst (DWD). The median value of each month for temperature (^oC), maximum temperature (^oC), minimum temperature (^oC), humidity (%), wind gust (Km/h), wind speed (Km/h), sea level pressure (millibar), solar radiation (W/m^2), solar energy (W/m^2), and UV index is calculated. For precipitation (mm) we utilize average for the statistical analysis.

2. Statistical modeling

In the study, the disease incidence is the response variable whereas the weather parameters are predictors/explanatory variables. The relationship between the citrus canker incidence and weather variables was modeled using correlation and multiple regression analysis. Even though we have time series data, it is safe to assume that there is independence between two observations in consecutive months. In the statistical analysis we use the software JMP Pro 16.2.0 software Copyright © 2020-2021 SAS Institute Inc.

The **correlation analysis** was performed between all the explanatory variables viz. median of temperature (^oC), maximum temperature (^oC), minimum temperature (^oC), humidity (%), wind gust (Km/h), wind speed (Km/h), sea level pressure (millibar), solar radiation (W/m^2), solar energy (W/m^2), UV index, and mean precipitation (mm).

We transform the response variable i.e., disease incidence (DI) by using **Box-Cox transformation** (Abraham & Ledolter, 2006). The best λ comes out to be -2 which refers to the transformation $(DI)^{-2}$. Since the least sum of squares of errors is also small for $\lambda = -1$.

Additionally, we also transformed the data to reciprocal of disease incidence i.e., $(DI)^{-1}$ (Fig 2.3). To model the relationship between the weather variables and the disease incidence, we implement **multiple regression** (Abraham & Ledolter, 2006; Gunst & Mason, 2019; Kumar & Kudada, 2018; Savary et al., 2000; G. Sun et al., 2018). The model is with response variable Y and $X_1, X_2, \ldots, X_n$ explanatory variables, the equation is as follows (Gunst & Mason, 2019)

$$Y = b + m_1X_1 + m_2X_2 + \ldots, mnXn$$

Where b is the intercept, $m_1, m_2, \ldots, m_n$ are coefficients corresponding to explanatory variables $X_1, X_2, \ldots, Xn$. Both $(DI)^{-2}$ and $(DI)^{-1}$ transformations of the response Y variable (Disease incidence) were used for regression analysis to select the best model.

We selected the best model using the following criteria (Abraham & Ledolter, 2006).

- Studentized residuals
- Parameter estimates
- Effects test
- Akaike information criterion (AIC)
- R^2 (Coefficient of determination) and adjusted R^2
- Durbin-Watson statistics
- PRESS residuals
- Root mean square error (RMSE)
- Mallow’s cp

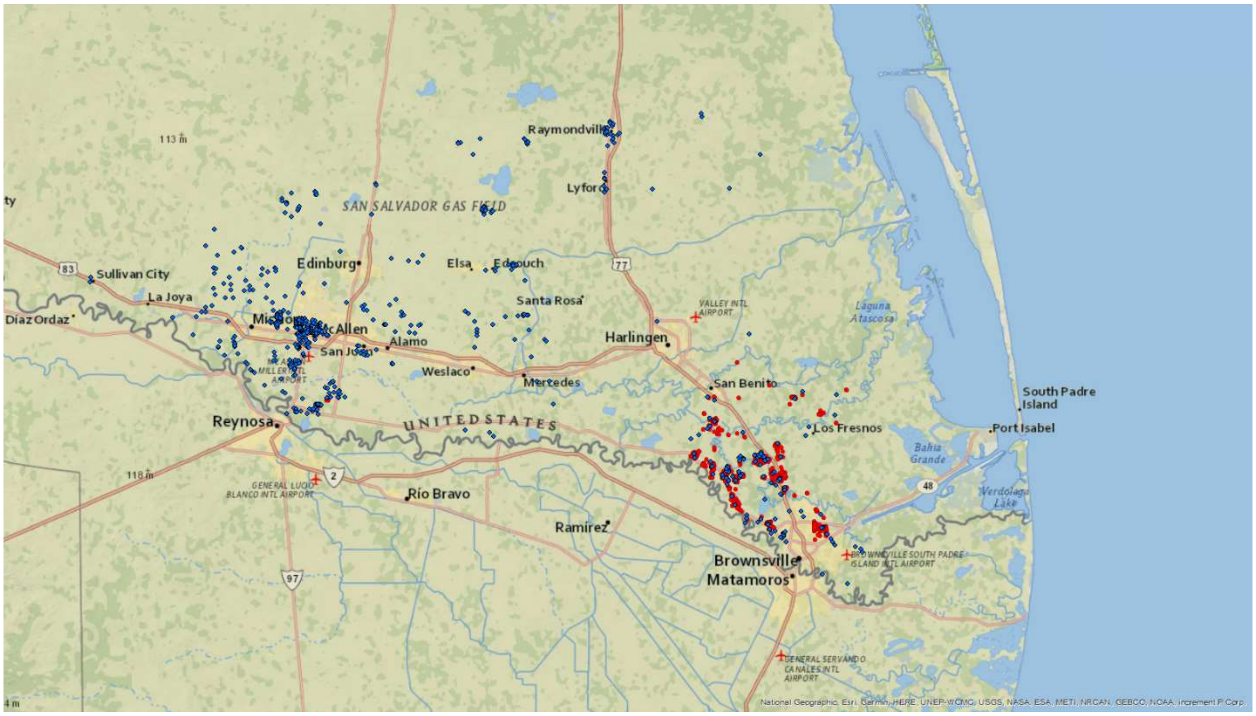


Figure 2.1: Citrus canker occurrence data for the period 2015-2022 obtained from United States Department of Agriculture. The red and blue points correspond to positive and negative citrus canker trees; respectively.

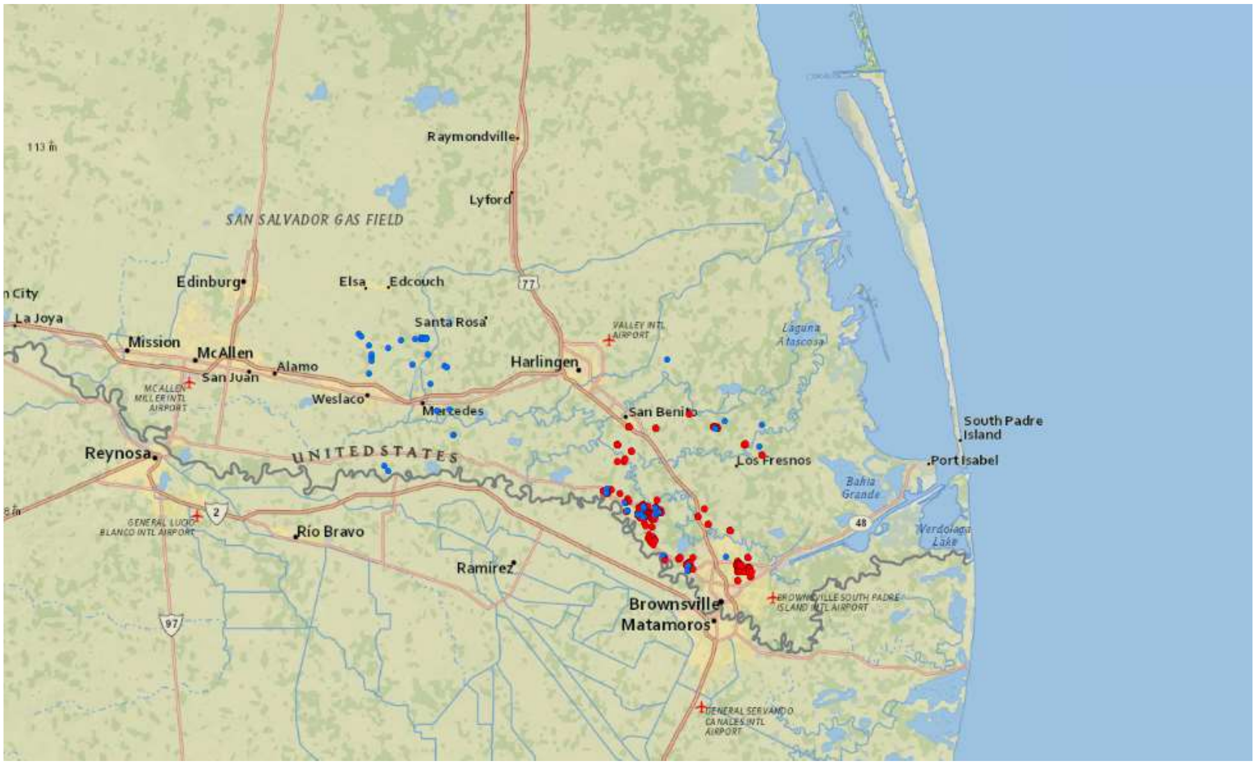


Figure 2.2: Citrus canker occurrence data for the period 2021-2022 with more focus on positive trees. The red and blue points correspond to positive and negative citrus canker trees; respectively.

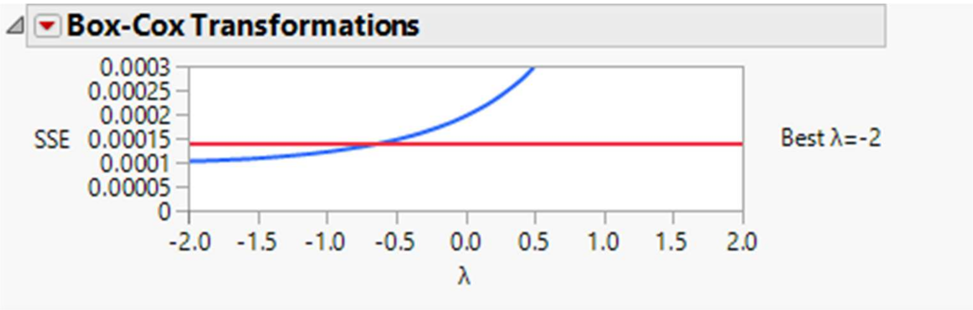


Figure 2.3: Box-Cox transformation of dependent Y variable (Disease incidence) representing best λ= -2.

CHAPTER III

RESULTS

1. Correlation analysis

High correlation either positive or negative was found between median temperature (ºC), maximum temperature (ºC), minimum temperature (ºC), and sea level pressure (millibar) (Table 3.1). As a result, we dropped the variables maximum temperature (ºC), and minimum temperature (ºC), and sea level pressure (milibar). Also, a high correlation was found between UV index, solar radiation (W/m^2), solar energy (W/m^2), and wind gust (Km/h). Thus, we dropped the explanatory variables UV index, solar radiation (W/m^2), and solar energy (W/m^2). The final set of parameters used for the citrus canker modeling is: median temperature (ºC), median humidity (%), median wind gust (Km/h), median windspeed (Km/h), and mean precipitation (mm).

2. Model diagnostics for selecting the best citrus canker model

Using multiple regression, two citrus canker predictive models were developed to determine the relationship between disease incidence and weather variables. Model 1 has the disease incidence data transformed to $(DI)^{-2}$ based on $\lambda = -2$ while model 2 has the disease incidence data transformed to $(DI)^{-1}$ based on $\lambda = -1$ (Box-Cox parameter transformation) (Chapter 2, Section 2). Both models were compared using various criteria as follows.

2.1 Studentized Residuals

Studentized residuals help in identifying the outliers that have an influence on the regression model. In both models, we have an outlier on time unit (row number 9) that exceeds the upper limiting red line. In Model 1 we have an outlier near value 9 (Fig. 3.1 A) and in Model 2, we have an outlier that exceeds 4 (Fig. 3.1 B).

2.2 Effects test

The effect test tells us which predictors in the model are or not significant. The effect test from Model 1 shows that temperature, humidity, wind gust, and wind speed are significant with a $p<0.05$ while mean precipitation is not significant with $p>0.05$ (Fig 3.2 A). Effect test for Model 2 shows that all the weather variables including temperature, humidity, wind gust, wind speed, and mean precipitation are significant.

2.3 Parameter estimates

Based on parameter estimates for Model 1 (Fig 3.3 A), the multiple regression equation is

$$Y = -0.01300 + 0.00007 X_1 + 0.00014 X_2 + 0.00012 X_3 - 0.00015 X_4,$$

where X_1 is median temperature, X_2 is median humidity, X_3 is median wind gust, X_4 is median wind speed. The mean precipitation comes out to be non-significant with $p>0.05$.

The multiple regression equation for Model 2 is:

$$Y = -0.207 + 0.001 X_1 + 0.002 X_2 + 0.002 X_3 - 0.002 X_4 - 0.002 X_5,$$

where X_1 is median temperature, X_2 is median humidity, X_3 is median wind gust, X_4 is median wind speed, and X_5 is mean precipitation (Fig 3.3 B).

2.4 Akaike information criterion (AIC)

Akaike information criterion (AIC) provides us with the estimate of predicted error thus helping in comparing and selecting the best quality statistical model. Among the candidate models, the one with less AIC value is preferred. The AIC value for Model 1 is –214.646 while the AIC value for Model 2 is –111.628.

2.5 R^2 (Coefficient of determination) and adjusted R^2

R^2 (Coefficient of determination) is one of the criteria used to select the statistical model. In a model, it determines the proportion of variability in the response variable that can be explained by the explanatory variables. The R^2 value for Model 1 is 0.681 while it is 0.661 for Model 2. Adjusted R^2 is a modified R^2 which has been adjusted for the number of predictors in the model. The addition of more predictors in the models leads to an increase in the value of R^2 which might be a result of overfitting. The adjusted R^2 value for Model 1 is 0.548 (Fig 3.4 A) and for Model 2 is 0.520 (Fig 3.4 B).

2.6 Durbin-Watson statistics

It is a statistical test to check the autocorrelation in the regression model output. The value for the test ranges from 0-4. Value 2 indicates no autocorrelation. The value below 2 indicates a positive while above 2 indicates a negative autocorrelation in the regression model output. The Durbin-Watson statistics for Model 1 is 2.108 while for Model 2 it is 2.008.

2.7 Predicted Residual Error Sum of Squares (PRESS) Residuals

Predicted Residual Error Sum of Squares (PRESS) Residuals are also used to compare the candidate regression models. It provides us with the predictive ability of the models. In general, a model with small value of PRESS is preferred. The PRESS for model 1 is 0.000007 while the PRESS for model 2 is 0.001907.

2.8 Root Mean Square Errors (RMSE)

Root Mean Square Errors (RMSE) is one of the ways to access the spread of data around the line of best fit. Mean square error is calculated by taking average of all the squared residuals which is the difference of observed and predicted value. Taking the square root of mean square error gives us RMSE. While comparing models, a model with lower value of RMSE is preferred. Model 1 has RMSE = 0.00038 (Fig 3.4 A) while Model 2 has a RMSE = 0.00662 (Fig. 3.4 B).

2.9 Mallow's Cp

Mallow's Cp is used to select the best model among the candidate models when numerous predictors are available for predicting an outcome. Its value should be around the number of predictors used in modeling +1. In our case, Model 1 has a Mallow's Cp value 5.98 whereas for Model 2, it is 5.96

Table 3.1: Correlation analysis of all the explanatory variables. High positive correlation shown in blue color while high negative correlation shown in red color.

	Temperature (max)	Temperature (min)	Temperature	Humidity	Wind gust	Wind speed
Temperature (max)	1.0000	0.9779	0.9916	-0.4475	-0.3123	0.1146
Temperature (min)	0.9779	1.0000	0.9912	-0.3411	-0.2077	0.1793
Temperature	0.9916	0.9912	1.0000	-0.3948	-0.2559	0.1676
Humidity	-0.4475	-0.3411	-0.3948	1.0000	0.3896	0.1722
Wind gust	-0.3123	-0.2077	-0.2559	0.3896	1.0000	0.7864
Wind speed	0.1146	0.1793	0.1676	0.1722	0.7864	1.0000
Sea level pressure	-0.7965	-0.8533	-0.8440	0.0912	-0.0774	-0.4895
Solar radiation	-0.0287	-0.0635	-0.0567	-0.1326	-0.6184	-0.6064
Solar energy	0.1806	0.1489	0.1676	-0.2142	-0.7084	-0.5753
UV index	0.1128	0.0764	0.1057	-0.1908	-0.7115	-0.5894
Mean precipitation	0.2254	0.2642	0.2271	0.1237	-0.3894	-0.3140

Table 3.1, cont.

	Sealevel pressure	Solar radiation	Solar energy	UV index	Mean precipitation
Temperature (max)	-0.7965	-0.0287	0.1806	0.1128	0.2254
Temperature(min)	-0.8533	-0.0635	0.1489	0.0764	0.2642
Temperature	-0.8440	-0.0567	0.1676	0.1057	0.2271
Humidity	0.0912	-0.1326	-0.2142	-0.1908	0.1237
Wind gust	-0.0774	-0.6184	-0.7084	-0.7115	-0.3894
Wind speed	-0.4895	-0.6064	-0.5753	-0.5894	-0.3140
Sea level pressure	1.0000	0.2621	0.0319	0.0945	-0.1506
Solar radiation	0.2621	1.0000	0.8945	0.8827	0.3200
Solar energy	0.0319	0.8945	1.0000	0.9747	0.3583
UV index	0.0945	0.8827	0.9747	1.0000	0.3712
Mean precipitation	-0.1506	0.3200	0.3583	0.3712	1.0000

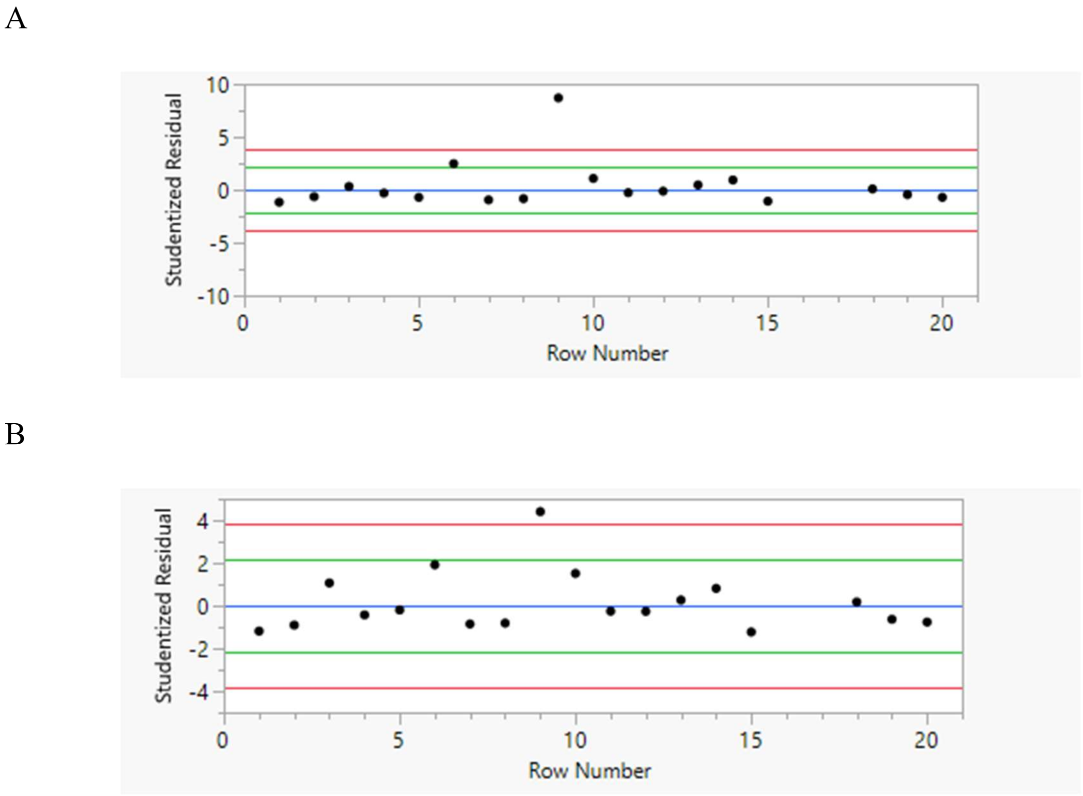
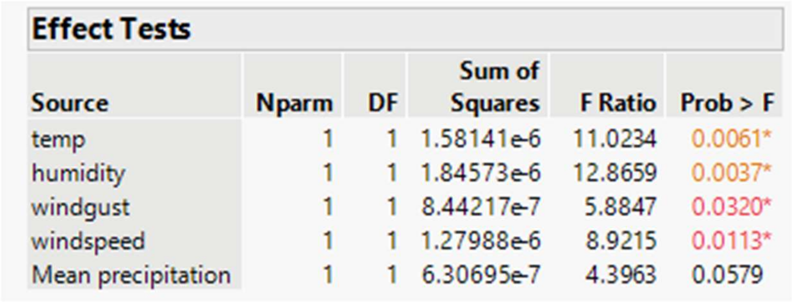


Figure 3.1: Studentized residuals with 95% simultaneous limits (Bonferroni) in red, individual limits in green A) for model 1 and B) for model 2.

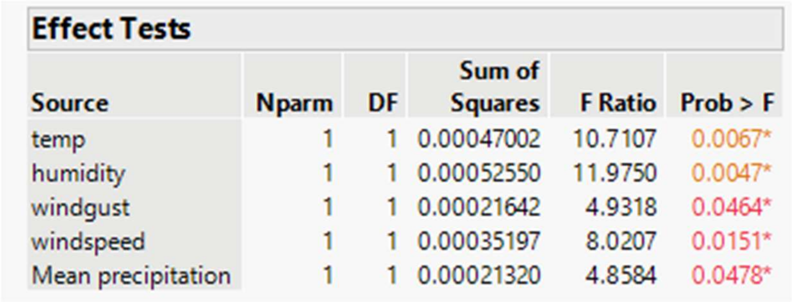
A



Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
temp	1	1	1.58141e-6	11.0234	0.0061*
humidity	1	1	1.84573e-6	12.8659	0.0037*
windgust	1	1	8.44217e-7	5.8847	0.0320*
windspeed	1	1	1.27988e-6	8.9215	0.0113*
Mean precipitation	1	1	6.30695e-7	4.3963	0.0579

B

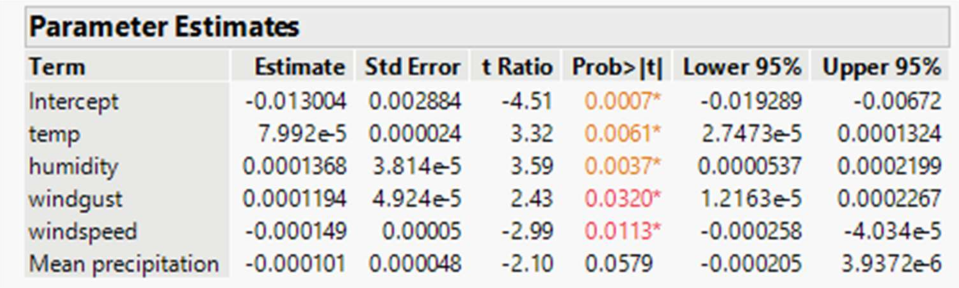


Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
temp	1	1	0.00047002	10.7107	0.0067*
humidity	1	1	0.00052550	11.9750	0.0047*
windgust	1	1	0.00021642	4.9318	0.0464*
windspeed	1	1	0.00035197	8.0207	0.0151*
Mean precipitation	1	1	0.00021320	4.8584	0.0478*

Figure 3.2: Effect test table A) for model 1 and B) for model 2 showing F and p value corresponding to temperature, humidity, wind gust, windspeed and mean precipitation.

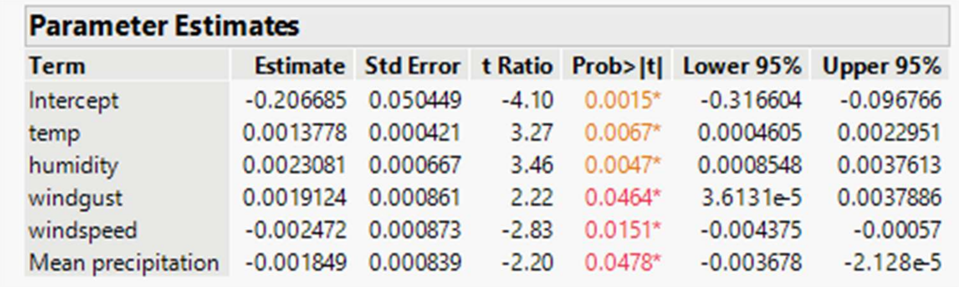
A



Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	-0.013004	0.002884	-4.51	0.0007*	-0.019289	-0.00672
temp	7.992e-5	0.000024	3.32	0.0061*	2.7473e-5	0.0001324
humidity	0.0001368	3.814e-5	3.59	0.0037*	0.0000537	0.0002199
windgust	0.0001194	4.924e-5	2.43	0.0320*	1.2163e-5	0.0002267
windspeed	-0.000149	0.00005	-2.99	0.0113*	-0.000258	-4.034e-5
Mean precipitation	-0.000101	0.000048	-2.10	0.0579	-0.000205	3.9372e-6

B

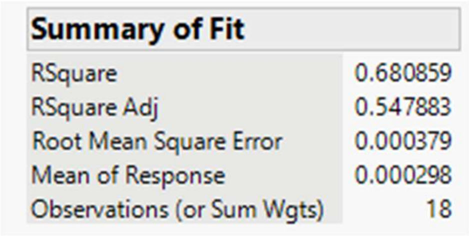


Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	-0.206685	0.050449	-4.10	0.0015*	-0.316604	-0.096766
temp	0.0013778	0.000421	3.27	0.0067*	0.0004605	0.0022951
humidity	0.0023081	0.000667	3.46	0.0047*	0.0008548	0.0037613
windgust	0.0019124	0.000861	2.22	0.0464*	3.6131e-5	0.0037886
windspeed	-0.002472	0.000873	-2.83	0.0151*	-0.004375	-0.00057
Mean precipitation	-0.001849	0.000839	-2.20	0.0478*	-0.003678	-2.128e-5

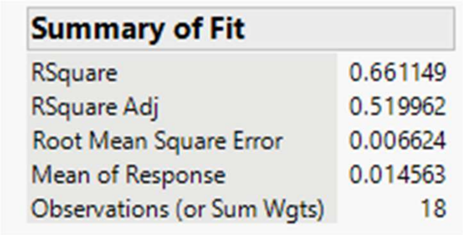
Figure 3.3: Parameter estimates A) for model 1 and B) for model 2 showing the intercept, and coefficients of temperature, humidity, wind gust, windspeed and mean precipitation.

A



Summary of Fit	
RSquare	0.680859
RSquare Adj	0.547883
Root Mean Square Error	0.000379
Mean of Response	0.000298
Observations (or Sum Wgts)	18

B



Summary of Fit	
RSquare	0.661149
RSquare Adj	0.519962
Root Mean Square Error	0.006624
Mean of Response	0.014563
Observations (or Sum Wgts)	18

Figure 3.4: Summary of fit A) for model 1 and B) for model 2 showing R^2, adjusted R^2, and root mean square error.

CHAPTER IV

DISCUSSION

To control of citrus canker in the areas where it is not present, we highly rely on quarantine and eradication to prevent at maximum, the introduction and establishment of citrus canker (Broadbent et al., 1992; Gottwald et al., 2002). In the region where the disease is prevalent with favorable environmental factors for disease spread, growing resistant varieties is recommended (Graham et al., 2004). Since we cannot control the weather of an area, we can try understating the environmental conditions of an area which affects the disease incidence and progression.

To understand and predict various factors in epidemics the concept of disease triangle was developed by George McNew in 1960s. It gave us insights to how the disease could be predicted, limited, or controlled. For a disease to occur, there is a need of susceptible host, virulent pathogen, and favorable environmental conditions that impacts the process of disease development. Six factors namely severity of the environment, time of the infection period, virulence potential of the pathogen, prevalence of the pathogen, susceptible/resistance level of host, and the age of the host plant interact with each other to determine the level of disease (G. L. McNew ; Scholthof, 2007). The variation in the environment is one of the factors that is being faced by plants throughout their lifetime. The fluctuations in the temperature, humidity, precipitation or drought, wind speed, micro-organisms, nutrients, and weeds could be beneficial or detrimental for the plants. These fluctuations also have a profound effect on disease incidence in plants (McNew, 1960).

In the development of citrus canker, it has been observed that temperature and moisture play an important role. Previous studies suggested that temperature in the range of 20ºC to 30ºC is crucial while relatively earlier studies suggested that citrus canker develops best at 30ºC to 35ºC (Christiano et al., 2009). The temperature can control the incubation of bacteria and symptoms expression in the host plant (Koizumi, 1976; Dalla Pria et al., 2006). In our study the disease incidence is slightly positively correlated with median temperature for both Model 1 and Model 2.

Moisture is very important for the disease as it governs the initial stages of the infection process, exudation of bacteria, and for bacterial dissemination (Arora et al., 2013; Pruvost et al., 2002). Our study also indicates that as the humidity increases the disease incidence for both the Models also increases.

It has been observed that more dispersal of the bacteria at greater wind speed than slower or no wind speed (Bock et al., 2010). Our study indicated that the disease incidence increased with increase in wind gust. We also found out that wind speed has a little negative or no effect on disease incidence.

The positive significant correlation of canker development has also been observed with increase humidity and rainfall (Arora et al., 2013; Khan & Abid, 2007). However, another report describes that there is a significantly decrease in the correlation of rainfall and disease incidence if the precipitation is less than threshold as precipitation won't have ample energy to spread the bacterium (Canteros et al., 2017). In Model 1 of our study, mean precipitation comes out to be non-significant while in Model 2, the disease incidence decreases with an increase in precipitation. The wind along with rain splashes rapidly increases the infection rate (Gottwald, 1989). Not only the weather but also the human movement from one place to another or working in orchards continues to expand the disease epidemic (Graham et al., 2004).

In our study on regression modeling of the citrus canker incidence in the RGV, despite the limitations of having less sample size, we have come up with 2 models. Model 1 predicts a significant effect of median temperature, median humidity, median wind gust and median wind speed except mean precipitation. Whereas Model 2 predicts that there is a significant effect of median temperature, median humidity, median wind gust, median wind speed, and mean rainfall on the disease incidence.

Since both Models provide statistically sound fit to the data, it is difficult to say which one is better. We give preference to Model 1 over Model 2 due to two main reasons:

- Scientifically, precipitation cannot affect the citrus canker disease negatively as in the Model 2
- Model 1 shows the better model diagnostics for regression analysis for the prediction of citrus canker in the Rio Grande Valley.

Studentized residuals for Model 1 and Model 2 satisfies the requirement that there is not a fixed pattern in both, and it is not fair to say if one is better than another. Note that in Chapter 3, Figure 3.1, the studentized residuals for both models falls within the control limits (red line).

Parameter estimation for Model 1 (Chapter 3, Figure 3.3 A) indicates that all the coefficients in front of median temperature, median humidity, median wind gust, and median wind speed except for mean precipitation are either positively or negatively significant while parameter estimates for Model 2 (Chapter3, Figure 3.3 B) shows all the parameters including mean precipitation is significant for disease incidence. Model 1 and Model 2 are statistically sound but scientifically we can argue that precipitation should not negatively affect the citrus canker incidence. So, in this case, we prefer Model 1 over Model 2.

AIC value also favors Model 1 over Model 2. As we prefer a model with lesser AIC value. The AIC value for Model 1 is –214.646 while the AIC value for Model 2 is –111.628 that leads us to prefer Model 1 over Model 2.

The R^2 value for Model 1 is 0.681 while it is 0.661 for Model 2. The adjusted R^2 value for Model 1 is 0.548 (Fig 3.4 A) and for Model 2 is 0.520 (Fig 3.4 B). Since the R^2 and adjusted R^2 value for Model 1 is higher than Model 2. Thus, we prefer Model 1 over Model 2 in this case as well.

Durbin-Watson statistics for Model 1 is 2.108 and 2.008 for Model 2. Since its value near 2 indicates no auto-correlation. So statistically, the value for Durbin-Watson statistics for both Model 1 and Model 2 are acceptable.

A model that has a small value for PRESS is preferred. In our case, Model 1 is favored since the PRESS for model 1 is 0.000007 while the PRESS for model 2 is 0.001907.

RMSE also favors Model 1 over Model 2 since a model with a lower value of RMSE is preferred. In our case, we have RMSE value 0.00038 and 0.00662 for Model 1 and Model 2 respectively.

Mallow’s Cp value are satisfactory for both models. In general, the value for Mallow’s Cp should be around number of predictors used in modeling +1. Model 1 has a Mallow’s Cp value 5.98 while Model 2 has a value of 5.96 which is around 6 in both cases and is statistically sound.

Many scientific studies have observed that rainfall act as one of the medium for dissemination of bacterium from one place to another (Bergamin & Hughes 2000; Canteros et al., 2017; Das, 2003; Goto 1962; Graham et al., 2004; Hameed et al., 2022; Nasreen et al., 2021; Stall et al., 1980). 5 out of 9 model diagnostics prefers Model 1 while the rest 4 provide us with information that both the Model 1 and Model 2 are significant. Thus, we conclude by suggesting Model 1 over Model 2.

Since the disease is highly contagious, under favorable environmental conditions just one infected fruit/tree can cause the epidemic. Unfortunately citrus canker is not curative but can be prevented by keeping a continual look on the environmental conditions and using copper chemicals intensively when the environmental conditions are conducive (Christiano et al., 2009). This study focused on developing a mathematical model to check the influence of weather parameters on citrus canker disease incidence caused by A^W strain in the RGV, avails the knowledge of which parameters including temperature, humidity, wind speed, wind gust, and precipitation and up to what extant governs the incidence of citrus canker in the RGV. The outcome of this study benefits Texas citrus industry in designing better management strategies before the disease becomes an epidemic in RGV.

To our knowledge, this is the first report that studied the influence of weather parameters on the incidence of canker caused by A^w strain. The work can be expanded further by considering citrus canker survey data for larger periods i.e., from 2015-2022, to come up with better mathematical model. Prediction models can also be generated by using some other statical analysis such as logistic regression.

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BIOGRAPHICAL SKETCH

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