Geographical distribution and detection of two potyviruses occurring in *Momordica charantia* (Cucurbitaceae) in Puerto Rico[1,2]

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ABSTRACT

Transmission dynamics of viruses within and among cultivated plant species are often well known. Much less studied, especially in the tropics, is the dynamic of virus exchange between cultivated and nearby wild or weedy species, even though such exchanges are known to occur. To develop the best strategies for crop protection and general disease control, spatial distribution of viruses in non-cultivated plants needs to be understood. This research focuses on the potyviruses that infect *Momordica charantia* (Cucurbitaceae), an alien naturalized invasive vine in Puerto Rico. A total of 390 symptomatic and asymptomatic plants were sampled throughout Puerto Rico, including adjacent islands of Culebra and Vieques. Samples were subjected to an enzyme linked immunosorbent assay (ELISA) for general potyvirus screening, and to ELISAs specific for *Papaya ringspot virus* (PRSV) and *Zucchini yellow mosaic virus* (ZYMV). The species distribution model algorithm MaxEnt was used to predict suitable environments for the potential presence of potyvirus symptoms, potyvirus, PRSV or ZYMV in *M. charantia*. Almost half of the samples of *M. charantia* tested positive for ZYMV, PRSV or both viruses. Twice as many samples were positive for PRSV (39%) than for ZYMV (21%). About 14% of samples were positive for both potyviruses. Plants that tested positive for PRSV were three times more likely to be positive for ZYMV than were plants that were negative for PRSV. Plants that tested positive using the general potyvirus ELISA

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were much more likely to exhibit symptoms than plants testing negative for potyvirus. In comparison, PRSV and ZYMV samples testing positive or negative were equally likely to exhibit virus-like symptoms. When we classified samples according to habitat (agricultural, rural-nonagricultural, or urban), the presence/absence of symptoms and test results for potyvirus, PRSV and ZYMV were not dependent on habitat classification. By contrast, a MaxEnt model using 20 environmental variables was able to predict areas of Puerto Rico where environmental conditions are favorable for the potential presence of virus symptoms, potyvirus, PRSV or ZYMV in *M. charantia*. Conditions predicted by our model to be moderately to strongly suitable for the presence of PRSV in *M. charantia* covered a much larger area of Puerto Rico than they did for ZYMV. The vegetable growing region in the central to eastern south coast was predicted to have highly suitable environmental conditions for the presence of both potyviruses in *M. charantia*.

Key words: *Potyviridae*, papaya ringspot virus, zucchini yellow mosaic virus, Caribbean, MaxEnt, DIVA-GIS, landscape ecology

RESUMEN

Distribución geográfica y detección de dos Potyvirus que ocurren en *Momordica charantia* (Cucurbitaceae) en Puerto Rico

La dinámica de transmisión de los virus dentro y entre las especies de plantas cultivadas suele ser bien conocida. Mucho menos estudiada, especialmente en los trópicos, es la dinámica de los intercambios de virus entre especies cultivadas y especies silvestres o malezas, aunque se sabe que tales intercambios ocurren. Para desarrollar las mejores estrategias para la protección de cultivos y el control general de enfermedades, es necesario comprender la distribución espacial de los virus en las plantas no cultivadas. Esta investigación se enfoca en los Potyvirus que infectan *Momordica charantia* (Cucurbitaceae), un bejuco exótico invasor naturalizado en Puerto Rico. Se tomaron muestras de 390 plantas, tanto sintomáticas como asintomáticas, en todo Puerto Rico, incluidas las islas adyacentes de Culebra y Vieques. Las muestras se sometieron a un ensayo inmunoabsorbente ligado a enzimas (ELISA) para la detección general de Potyvirus y a ELISAs específicos para el virus de la mancha anular de la papaya (PRSV) y el virus del mosaico amarillo del calabacín (ZYMV). Se utilizó el algoritmo del modelo de distribución de especies MaxEnt para predecir las localidades de ambientes adecuados para la posible presencia de síntomas o detección de Potyvirus, PRSV o ZYMV en *M. charantia.* Casi la mitad de las muestras de *M. charantia* fueron positivas para ZYMV, PRSV o ambos virus. El doble de muestras fue positivo para PRSV (39%) que para ZYMV (21%). Alrededor del 14% de las muestras fueron positivas para ambos Potyvirus. Las plantas que resultaron positivas para PRSV tenían tres veces más probabilidad de ser positivas para ZYMV que las plantas que resultaron negativas para PRSV. Las plantas que resultaron positivas en el ELISA general para Potyvirus tenían muchas más probabilidad de presentar síntomas que las plantas negativas para Potyvirus. Por el contrario, para PRSV y ZYMV, las muestras con resultados positivos o negativos tenían la misma probabilidad de presentar síntomas similares a los causados por el virus. Cuando clasificamos las muestras según el hábitat (agrícola, rural no agrícola, o urbano) la presencia/ausencia de síntomas y los resultados de las pruebas de Potyvirus, PRSV y ZYMV (positivo o negativo), estas no dependieron de la clasificación del hábitat. Por el contrario, el utilizar 20 variables ambientales pudo predecir áreas de Puerto Rico donde las condiciones ambientales son favorables para la posible presencia de síntomas o detección de virus, Potyvirus, PRSV o ZYMV

en *M. charantia.* Las condiciones que se pronosticaron como moderadamente hasta muy adecuadas para la presencia de PRSV en *M. charantia* cubrieron un área mucho más grande de Puerto Rico que para ZYMV. Se predijo que la región de cultivo de hortalizas en la costa sur central y oriental tendría condiciones ambientales muy adecuadas para Potyvirus en *M. charantia*.

Palabras clave: *Potyviridae*, Caribe, mosaico amarillo del calabacín, mancha anular de la papaya, MaxEnt, DIVA-GIS, ecología de paisaje

INTRODUCTION

Viral diseases, especially potyviruses (family *Potyviridae,* genus *Potyvirus*), limit production of cucurbits worldwide (Lecoq et al*.,* 1998; Provvidenti,1996). *Potyviruses* are transmitted by aphids in a non-persistent manner (Brunt, 1992; Gadhave et al., 2020). Transmission takes place rapidly in seconds to minutes. In tropical and subtropical regions of the world, the watermelon strain of the *Papaya ringspot virus* (PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV) are two potyviruses that cause severe disease, significantly reducing the yield and quality of most cucurbits (Lecoq et al*.,* 1998; Ling et al*.*, 2009; Purcifull et al*.,* 1984).

In Puerto Rico, where melons and squashes are often cultivated, PRSV and ZYMV are common and not only occur in a wide range of crops, but also in native and naturalized wild species (Chen et al*.,* 2008; Paz-Carrasco and Wessel-Beaver, 2002; Rodrigues et al., 2012). Despite the importance of these crops in Puerto Rican agriculture, very little is known about the infection dynamics between crops and native or naturalized wild plants. Two aphids known to be the most common vectors of potyviruses, the green peach aphid, *Myzus persicae*, and the melon aphid, *Aphis gossypii* (Gadhave et al., 2020)*,* are distributed throughout Puerto Rico and feed on many types of plants. If wild cucurbits are affected in the same manner as cultivated plants, then they may serve as viral reservoirs as occurs in other systems (reviewed in Cooper and Jones, 2006) and should be considered when designing strategies for weed management, disease prevention and the development of plant resistance to viruses.

PRSV isolates are divided into two groups, the W and P biotypes, both of which can infect cucurbits (Purcifull et al*.,* 1984; Romay et al., 2014). In addition to infecting cucurbits, the P biotype also infects papaya and can devastate commercial production (Gonsalves et al., 2010). PRSV is a highly variable virus with Asiatic origins and may have been introduced to the Americas some 300 years ago (Bateson et al*.,* 2002; Gibbs et al., 2008; Olarte Castillo et al*.,* 2011). ZYMV is one of the most destructive and widespread viral pathogens affecting cucurbits worldwide (Lecoq et al., 1998). It causes severe symptoms on leaves, such as yellowing, leaf deformation, and stunting. In the later stages of infection, leaves develop a yellow mosaic and often show dark green blisters (Zellnig et al., 2014).

As an island, Puerto Rico is an ideal place to conduct studies on the frequency and distribution of these viruses under natural conditions (Prendeville et al., 2012). The large number of farms producing cucurbits offers the potential for a sizable amount of virus inoculum; wild cucurbits are present and there is an ornamental industry that frequently imports plants providing a source of viruses and vector introductions. Focusing on a widespread weedy cucurbit, *Momordica charantia*, we addressed the following questions: Do potyviruses affect wild plants in the same way as those grown in cultivation? Are these potyviruses associated with one another? Are asymptotic plants virus-free? And finally, is the distribution of potyvirus-infected *M. charantia* environment dependent?

MATERIALS AND METHODS

Study area and collection of samples. Puerto Rico, the smallest of the main islands of the Greater Antilles, is located between N18°00’ and N18°30’ and W66°15’ and W68°00’ and covers approximately 8,940 km^2 (Del Mar López and Villanueva Colón, 2006). The island has six different ecological life zones and is biologically diverse (Ewel and Whitmore, 1973). The maritime tropical climate is characterized by moderate temperatures throughout the year (mean = 26.7° C) without the extremes seen in continental regions of similar latitudes. Precipitation is moderately seasonal and highly variable geographically as topography produces rain shadows, orographic precipitation and convectional daytime storms (Del Mar López and Villanueva Colón, 2006).

From November 2008 to July 2009, *M. charantia* was sampled in areas that varied by ecological zone, elevation, geomorphology, hydrography and proximity to cucurbit farms (which occur primarily in the southern part of the island). Sample habitats were classified as urban, agricultural or rural-nonagricultural. Young plant tissue from both asymptomatic plants and plants showing virus-like symptoms such as leaf mottling, deformation, or chlorosis were collected. Samples were placed in plastic bags (Ziploc®, S.C. Johnson, Racine, WI)[7] containing wet paper towels and held in a cooler until brought to the laboratory. Until further testing could be done, samples were either lyophilized and stored at -20° C or stored at -80° C without being lyophilized.

[7]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

Processing of samples. A commercial enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Inc., Elkhart, IN) was initially used to carry out a general test for potyviruses (POTY) on 390 samples of *M. charantia*. Samples showing a positive POTY reaction were then subjected to a double antibody ELISA (DAS-ELISA) (Clark and Adams, 1977) for either PRSV or ZYMV using commercial kits (Agdia, Inc., Elkhart, IN). Some samples were not tested for PRSV and ZYMV, explaining the small differences in the total number of samples. When a sample tested negative for POTY but positive for PRSV and/or ZYMV, the sample was counted as a positive for potyviruses. Only if a sample tested negative for POTY, PRSV, and ZYMV was a sample considered negative for potyviruses. The presence of potyviruses in samples that tested positive for POTY with ELISA was confirmed with reverse transcription polymerase chain reaction (RT-PCR) using universal potyvirus primers (Grisoni et al., 2006).

Statistical tests of association. Each sample was classified as positive or negative (present or absent) for PRSV, ZYMV, POTY, and virus-like symptoms. In addition, the sample's collection site (habitat) was classified as urban, agricultural or rural-nonagricultural. Chi-square tests were used to test the null hypothesis of independence (non-association) between PRSV and ZYMV, between sample habitat and virus presence, and between virus-like symptoms and virus presence using InfoStat version 2014 (Di Rienzo et al., 2014).

Predicated geographic distribution of potyviruses. We used the species modeling software MaxEnt [Maximum Entropy Species Distribution Modeling, version: 3.3.3k (Phillips et al., 2006); current version available at http:// biodiversityinformatics.amnh.org/open_source/maxent/, accessed on 1 Oct 2022] to develop four models to predict the potential distribution of potyviruses symptoms, and detection of potyviruses, PRSV and ZYMV in *M. charantia* on the island. The geo-referenced location of each positive *M. charantia* sample was matched with 20 environmental variables [elevation, plus 19 environmental layers gleaned from BioClim (www.worldclim.org/bioclim); Table 1] to produce the prediction models. For each model, 80% of the samples were used for training (model development) and 20% for testing (model validation). A total of 10 replicates (runs) were conducted for each model. The area under the receiver operator curve (AUC) was used to judge the predictive capability of the models. Models with AUC values ≥ 0.70 were considered reliable while models with AUC values ≥ 0.90 were considered to be highly reliable (Elith, 2000; Phillips and Dudík, 2008). A jackknife test of variable importance was conducted for each of the four models to identify the variable with the greatest predictive effect when used alone and to identify the variable result-

TABLE 1.—*Environmental variables used in MaxEnt (version 3.3.3e) to build prediction maps for the occurrence of viruses and symptoms in* Momordica charantia *in Puerto Rico.*

Layer[1]	Variable
BIO1	Annual mean temperature
BIO2	Mean diurnal range [mean of monthly (maximum temperature - minimum temperature)]
BIO3	Isothermality (BIO2/BIO7) (x 100)
BIO4	Temperature seasonality (standard deviation x 100)
BIO5	Maximum temperature of warmest month
BIO6	Minimum temperature of coldest month
BIO7	Temperature annual range (BIO5-BIO6)
BIO8	Mean temperature of wettest quarter
BIO9	Mean temperature of driest quarter
BIO10	Mean temperature of warmest quarter
BIO11	Mean temperature of coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter
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[1]Standard codes from http://www.worldclim.org.

ing in the greatest decrease in gain when omitted. The threshold between suitable and unsuitable environments was estimated using the MaxEnt option "maximum training sensitivity plus specificity" as recommended by Liu et al. (2016). MaxEnt results were exported to the program DIVA-GIS (https://www.diva-gis.org), from which we projected the models on maps of Puerto Rico. Red, orange, yellow and green on maps indicate areas where environmental conditions were highly, moderately, marginally, or not suitable, respectively, for the presence of virus symptoms, potyviruses, PRSV or ZYMV.

RESULTS

Only about a third of the samples (39.2%) exhibited virus-like symptoms (Table 2). Considerably more samples (57.7%) tested positive for POTY indicating that many samples were asymptomatic yet carrying potyviruses. Virus-like symptoms in *M. charantia* included mottling, chlorosis, stunting and leaf deformation (Figure 1). Among samples with single infections (one virus present), plants with PRSV were much

TABLE 2.—*Frequency and percentages of presence (positive) or absence (negative) of virus-like symptoms, and positive or negative enzyme linked immunosorbent assay (ELISA) test results for general potyvirus (POTY),* Papaya ringspot virus *(PRSV) and* Zucchini yellow mosaic virus *(ZYMV) in single and/or mixed infections in 390 samples of* Momordica charantia *collected from 2006 to 2010 in Puerto Rico.*

Evaluation or test	Evaluation or test result		
	Positive	Negative	Total[1]
Virus-like symptoms	153 (39.2%)	237 (60.8%)	390
POTY	225 (57.7%)	165 (42.3%)	390
PRSV – in single infections	86 (25.0%)	258 (75.0%)	344
PRSV – in single + mixed infections	134 (39.0%)[2]	210 (61.0%)	344
ZYMV – in single infections	24 (7.2%)	323 (92.8%)	348
ZYMV - in single + mixed infections	73 (21.0%)[3]	275 (79.0%)	348
PRSV and ZYMV – mixed infections	48 (14.0%)[4]	296 (86.0%)	344
PRSV and/or ZYMV – single + mixed infections	159 (46.2%)[5]	185 (53.8%)	344

[1] Totals vary slightly because not all samples were tested for PRSV and ZYMV.
[2] Samples with PRSV alone or in a mixed infection with ZYMV.
[3] Samples with ZYMV alone or in a mixed infection with PRSV.
[4] Samples that tested positive for PRSV and ZYMV.
[5] Samples that tested positive for (a) PRSV or (b) ZYMV or (c) PRSV and ZYMV.

more common (25.0%) than those with ZYMV (7.2%) (Table 2). Samples testing positive for PRSV infections (as either a single or mixed infection) were nearly twice as common as samples with ZYMV infections. Mixed infections of PRSV and ZYMV were found in only 14% of the 344 samples tested. However, among the 159 samples (46.2%) testing positive for at least one of these viruses (corresponding to the category "PRSV and/or ZYMV" in Table 2) mixed infections were common: 48 samples (30.2% of 159) tested positive for PRSV and ZYMV (Table 3).

Relationship between PRSV and ZYMV. The relative frequency of positive tests for ZYMV depended on whether the sample was positive or negative for PRSV. Samples were three times as likely to be positive for ZYMV when a sample was also positive for PRSV than when a sample was negative for PRSV (Table 3). This difference was highly significant (χ^2 test, P<0.0001).

Relationship between virus infection and virus-like symptoms. The relative frequency of samples showing virus-like symptoms was strongly dependent on whether a sample tested positive or negative for POTY. Symptoms were present in 46.7% of the samples testing positive for POTY while only 29.1% of samples testing negative exhibited symptoms, a highly significant difference (χ^2 test, P=0.0004) (Table 4). By contrast, the relative frequencies for both PRSV and ZYMV were independent of the test results (χ^2 test, P =0.5580 for PRSV and 0.1656

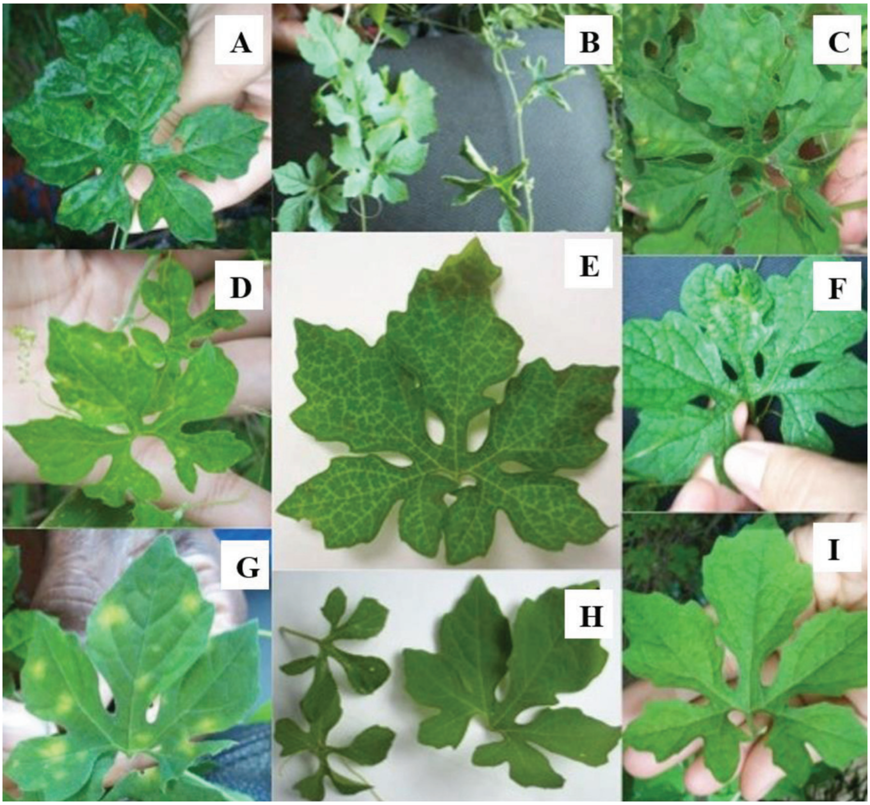

FIGURE 1. Symptoms in *Momordica charantia* associated with potyvirus infections. Leaf deformation (A, B, F, H); chlorotic spots (C, D, G); chlorotic veins (E); dwarfing (H); interveinal chlorosis (I). Symptoms associated with *Papaya ringspot virus* (PRSV) or *Zucchini yellow mosaic virus* (ZYMV) infections were undistinguishable in these field samples collected in Puerto Rico.

TABLE 3.—*Frequency of positive or negative results in enzyme linked immunosorbent assays (ELISA) for* Papaya ringspot virus *(PRSV) and* Zucchini yellow mosaic virus *(ZYMV) in 344 samples of* Momordica charantia *collected between December 2006 and July 2010 in Puerto Rico. The hypothesis that the proportion of positive to negative tests for ZYMV is independent of the test result for PRSV was tested with chi-squared.*

	ZYMV ELISA				
PRSV ELISA	Positive	Negative	Total	χ^2 value	Probability
Positive	48	86	134	27.99	<0.0001
Negative	25	185	210		
Total	73	271	344		

TABLE 4.—*Frequency of positive or negative results in enzyme linked immunosorbent assays (ELISA) for general potyvirus (POTY),* Zucchini yellow mosaic virus *(ZYMV) or* Papaya ringspot virus *(PRSV) and presence or absence of virus-like symptoms in* Momordica charantia *samples collected from 2006 to 2010 in Puerto Rico. The hypothesis that the proportion of symptomatic to asymptomatic plants was independent of ELISA test results was tested with chi-squared.*

	Virus-like symptoms						
	Present		Absent				
ELISA result[1]	Number	%	Number	%	Total	χ^2 value	Probability
POTY positive	105	46.7	120	53.3	225	12.33	0.0004
POTY negative	48	29.1	117	70.9	165		
PRSV positive	50	37.3	84	62.7	134	0.34	0.5580
PRSV negative	85	40.5	125	59.5	210		
ZYMV positive	24	32.9	49	67.1	73	1.92	0.1656
ZYMV negative	115	41.8	160	58.2	275		
PRSV positive/ ZYMV positive	11	22.9	37	77.1	48	8.43	0.0380
PRSV positive/ZYMV negative	39	45.3	47	54.7	86		
PRSV negative/ZYMV positive	13	52.2	12	47.8	25		
PRSV negative/ZYMV negative	72	38.9	113	61.1	185		

for ZYMV). However, when all possibilities of both single and mixed PRSV-ZYMV infections were considered, the relative frequencies of virus symptoms varied depending on the ELISA result (χ^2 test, P = 0.0380). Virus-like symptoms were infrequent in samples with mixed infections (23% with symptoms present) and quite common in samples with PRSV absent and ZYMV present (52.2% with symptoms present). About 38.9% of samples testing negative for both PRSV and ZYMV exhibited virus-like symptoms, and 45.3% of samples that tested positive for PRSV and negative for ZYMV showed symptoms.

Relationship between virus and virus symptoms, and habitat classification. The relative frequencies of viral symptoms and positive tests for POTY, PRSV and ZYMV were all independent of habitat classification (Table 5). Thus, viral symptoms and infections were equally likely to appear in agricultural, rural/nonagricultural and urban habitats.

Predicated geographical distribution of potyvirus symptoms and potyviruses in M. charantia. Although the samples of *M. charantia* were not collected in a strictly random manner, nearly all of the island's municipalities were visited for sampling (Figure 2). Maps of potential distribution based on suitable environmental conditions (Figures 3, 4, 5 and 6) were generated using all 20 environmental variables in MaxEnt. For each 20-variable model, the relative contributions of the best five environmental variables are shown in Table 6. AUC values among the four models varied from 0.749 to 0.839. By the criteria we described above, the models were considered reliable, although not strongly so. In general, the best variables made relatively small contributions (6.6% to 18.4%) to their respective models. There were two exceptions: the 25.8% contribution of mean temperature of driest quarter in the ZYMV model and the 25.2% contribution of precipitation in warmest quarter for the symptoms model. These latter two variables were consistently important in all four models. These two variables were also often the most important variable when used alone in a model (the letter "A" in Table 6).

There were both differences and commonalities among the four maps of the predicted suitability distributions for the presence of symptoms or virus in *M. charantia* (Figures 3, 4, 5 and 6). Particularly noticeable was a common area of moderately to highly suitable conditions (orange and red on maps) in the central to eastern south coast of Puerto Rico on all four maps. These maps also showed that large parts of the interior of Puerto Rico are predicted to be unsuitable (green on maps) for virus or virus symptoms. Maps for symptoms (Figure 3) and potyvirus (Figure 4) were very similar except for a region in the central east interior of Puerto Rico that had an area of high predicted

TABLE 5.—*Frequency of ELISA positive or negative results in enzyme linked immunosorbent assays (ELISA) results for general potyvirus (POTY),* Zucchini yellow mosaic virus *(ZYMV) or* Papaya ringspot virus *(PRSV), and of symptomatic and asymptomatic plants in each of three habitats where samples of* Momordica charantia *were collected from 2006 to 2010 in Puerto Rico. The hypothesis of independence of virus test result or plant symptoms versus habitat was tested with chi-squared.*

	Habitat				
Virus test result or sample symptoms	Agricultural	Rural, non-agricultural	Urban	χ^2 value	Probability
POTY positive	20	153	49	2.60	0.2720
POTY negative	23	104	38		
PRSV positive	13	89	32	0.37	0.8323
PRSV negative	24	137	46		
ZYMV positive	6	51	15	0.85	0.6451
ZYMV negative	31	179	63		
PRSV positive / ZYMV positive	6	35	7	9.04	0.1714
PRSV positive /ZYMV negative	7	54	25		
PRSV negative /ZYMV positive	0	16	8		
PRSV negative /ZYMV negative	24	121	38		
Symptomatic plant	16	100	36	0.25	0.8813
Asymptomatic plant	27	157	51		

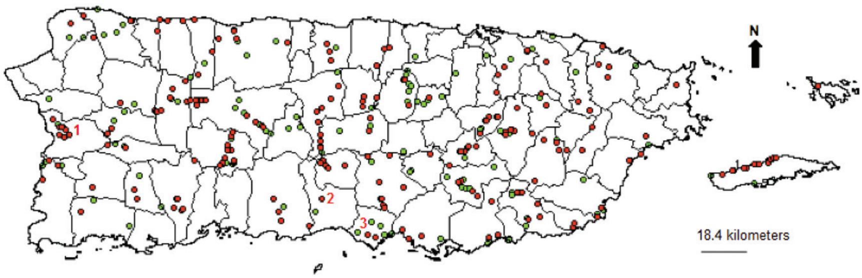

Figure 2. *Momordica charantia* collection sites and occurrence of *Potyvirus* on the islands of Puerto Rico (left), Culebra (top right) and Vieques (bottom right). Virus detection was obtained through potyvirus–ELISA serological tests. Map made with DIVA–GIS. Dark and light points (red and green when in color)[1] represent a cluster of samples that were positive or negative, respectively, for *Potyvirus* (either potyvirus in general, *Papaya ringspot virus* or *Zucchini yellow mosaic virus*).

[1] See digital version in http://revistas.upr.edu/index.php/jaupr/

suitability for the presence of symptoms in *M. charantia* compared to only moderate to marginal suitability for potyvirus. Both maps also showed similar suitable environmental conditions along the valleys of the Río Grande de Arecibo and Río Grande de Manatí (but with stronger suitability for symptoms than for potyvirus), the western half of the north coast, and coastal Vieques. For PRSV and ZYMV, the models predicted areas suitable for PRSV (Figure 5) to be rather different from that of ZYMV (Figure 6). Conditions predicted to be moderately

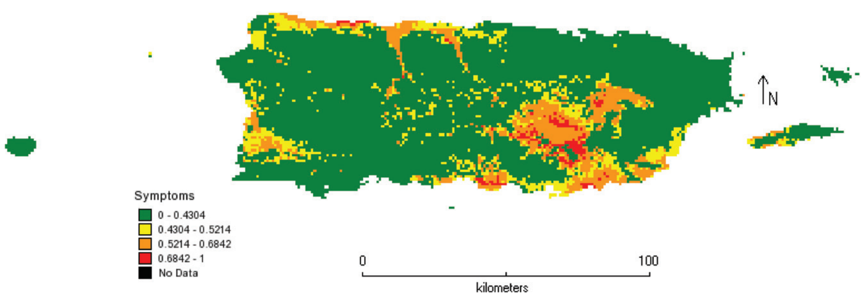

Figure 3. Potential distribution of *Momordica charantia* with viral disease symptoms in Puerto Rico, Culebra, and Vieques as predicted by MaxEnt (version 3.3.3k) using a model based on 20 environmental variables. Environmental suitability/unsuitability was determined using the "maximum training sensitivity plus specificity" threshold in MaxEnt. Biggest area (green, when in color)[1] corresponds to environments predicted to be unsuitable for the presence of viral disease symptoms (less than or equal to the threshold probability of 0.4304). The other areas correspond to environments predicted to be marginally suitable, moderately suitable and highly suitable, (yellow, orange and red, respectively)[1] for the presence of viral disease symptoms in *M. charantia*.

[1] See digital version in http://revistas.upr.edu/index.php/jaupr/

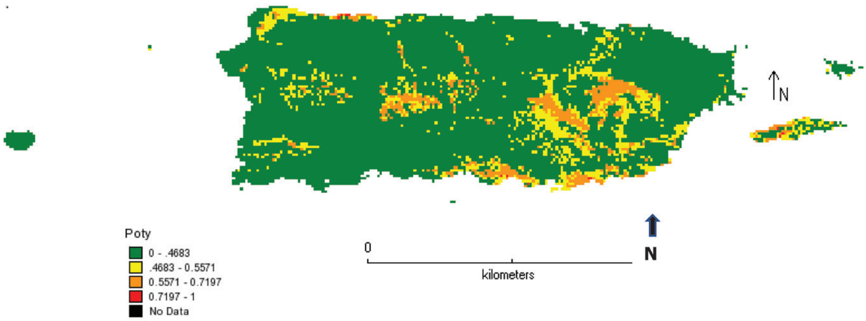

FIGURE 4. Potential distribution of *Momordica charantia* infected by potyviruses in Puerto Rico, Culebra, and Vieques as predicted by MaxEnt (version 3.3.3k) using a model based on 20 environmental variables and samples testing positive for potyvirus in ELISA. Environmental suitability/unsuitability was determined using the "maximum training sensitivity plus specificity" threshold in MaxEnt. Biggest area (green, when in color)[1] corresponds to environments predicted to be unsuitable for the presence of potyviruses (less than or equal to the threshold probability of 0.4683). The other areas correspond to environments predicted to be marginally suitable, moderately suitable and highly suitable (yellow, orange and red respectively)[1] for the presence of potyviruses.

[1] SEE DIGITAL VERSION IN HTTP://REVISTAS.upr.edu/index.php/jaupr/

to strongly suitable for the presence of PRSV in *M. charantia* covered a much larger area of Puerto Rico than they did for ZYMV. For PRSV, areas with suitable environmental conditions included the central west coast (where conditions were only marginally suitable for ZYMV) and

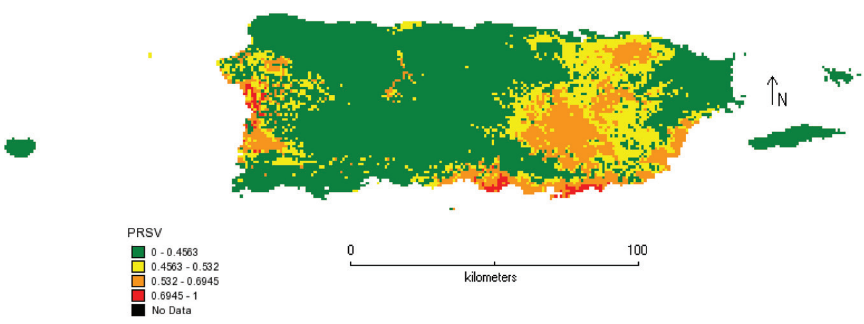

FIGURE 5. Potential distribution of *Momordica charantia* infected by *Papaya ringspot virus* (PRSV) in Puerto Rico, Culebra, and Vieques as predicted by MaxEnt (version 3.3.3k) using a model based on 20 environmental variables and samples testing positive for PRSV in DAS-ELISA. Environmental suitability/unsuitability was determined using the "maximum training sensitivity plus specificity" threshold in MaxEnt. Biggest area (green, when in color)[1] corresponds to environments predicted to be unsuitable for the presence of PRSV (less than or equal to the threshold probability of 0.4563). The other areas correspond to environments predicted to be marginally suitable, moderately suitable and highly suitable (yellow, orange and red, respectively)[1] for the presence of PRSV.

[1] SEE DIGITAL VERSION IN HTTP://REVISTAS.upr.edu/index.php/jaupr/

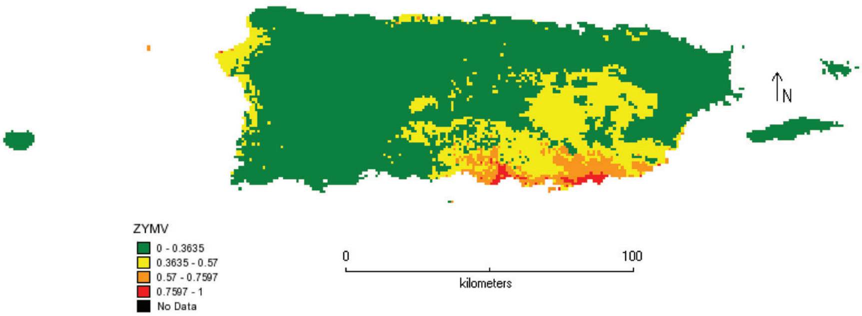

FIGURE 6. Potential distribution of *Momordica charantia* infected by *Zucchini yellow mosaic virus* (ZYMV) in Puerto Rico, Culebra, and Vieques as predicted by MaxEnt (version 3.3.3k) using a model based on 20 environmental variables and samples testing positive for ZYMV in DAS-ELISA. Environmental suitability/unsuitability was determined using the "maximum training sensitivity plus specificity" threshold in MaxEnt. Biggest area (green, when in color)[1] corresponds to environments predicted to be unsuitable for the presence of ZYMV (less than or equal to the threshold probability of 0.3635). The other areas correspond to environments predicted to be marginally suitable, moderately suitable and highly suitable (yellow, orange and red, respectively)[1] for the presence of ZYMV.

[1] SEE DIGITAL VERSION IN HTTP://REVISTAS.upr.edu/index.php/jaupr/

the eastern north coast and nearby interior (where conditions were unsuitable for ZYMV in *M. charantia*). Nevertheless, some areas of the island were predicted to have similar suitability conditions for PRSV and ZYMV in *M. charantia*, particularly the central to eastern south coast. For both PRSV and ZYMV, conditions on Vieques were unsuitable in contrast to the distribution for virus symptoms and potyvirus that predicted areas of suitability.

DISCUSSION

For thousands of years, humans have intentionally moved plants from their native regions to novel areas where they have become established as crops or weeds. These introductions often bring with them disease-causing organisms and their vectors, many of which have spread beyond the point of introduction with little or no constraints. In fact, accidental introduction of viruses and vectors to new geographic regions are a major cause of emerging invasive plant diseases (Anderson et al., 2004; Woolhouse et al., 2005), and weeds with fast growth and high nutrient value may be most likely to serve as effective virus reservoirs (Cronin et al., 2010). How these introductions interact with resident crops is largely unknown.

Our model system was the pantropical weed, *M. charantia,* a cucurbit related to cultivated crops such as watermelon, melon, pumpkin,

TABLE 6.—*Summary of MaxEnt analyses using 20 environmental variables for virus symptoms, potyviruses,* Papaya ringspot virus *(PRSV) and* Zucchini yellow mosaic virus *(ZYMV) in* Momordica charantia *sampled in Puerto Rico. For each analysis, the five variables with the highest percent contribution in the MaxEnt 20-variable model are shown. The letter "A" indicates the most predictive variable when used alone in a model. The letter "B" corresponds to the variable (of 20 possible variables) that results in the largest decrease in gain when it is omitted, and therefore likely has unique information not present in other variables.*

	Contribution to the 20-variable MaxEnt model							
Environmental Variables[1]	Symptoms		Potyviruses		PRSV		ZYMV	
BIO2 – Mean diurnal range							7.0%	
BIO3 - Isothermality							6.6%	
BIO4 – Temperature seasonality					9.5%			
BIO9 – Mean temperature of driest quarter	12.0%		18.4%	A	9.6%		25.8%	A
BIO11 – Mean temperature of coldest quarter					12.6%	A		
BIO12 – Annual precipitation	12.2%							
BIO13 - Precipitation of wettest month			12.4%			B		
BIO15 – Precipitation seasonality	14.1%							
BIO17 – Precipitation of driest quarter			15.4%		8.0%		9.5%	
BIO18 – Precipitation of warmest quarter	25.2%	A	9.6%		14.6%		12.9%	B
Elevation	13.6%	B	11.9%	B				
AUC[2]	0.783		0.749		0.785		0.839	

[1]Standard environmental codes from http://www.worldclim.org.
[2]Area under the receiver operator curve (AUC) in the MaxEnt analysis using all 20 environmental variables and all presence data.

and cucumber (Robinson and Decker-Walters, 1999). PRSV and ZYMV are commonly encountered in these crops in Puerto Rico (Paz-Carrasco and Wessel-Beaver, 2002). Aphids are transmitters of *Potyvirus* and are relatively and proportionately more available in agricultural systems than in wild plants. Thus, the probability of viruses moving from cultivated plants to wild plants should be high. PRSV and ZYMV in *M. charantia* should be expected to be more commonly encountered in proximity to cucurbit farms.

Momordica charantia often exhibited potyvirus symptoms (Figure 1) similar to those found in cultivated cucurbits. It is possible that the symptoms we observed in *M. charantia* were caused by infections of a virus or viruses outside the *Potyviridae* family since we only tested for potyvirus, PRSV and ZYMV. However, previous work (Paz-Carrasco and Wessel-Beaver, 2002) suggests that cucurbits in Puerto Rico are seldom infected by viruses other than potyviruses. On the other hand, infected *M. charantia* (based on ELISA) was often asymptomatic (Table 4). This may be a consequence of the type of genetic resistance (for example, resistance that limits movement of the virus in the plant) or a low viral load at the early stages of infection. Natural host populations typically have greater genetic diversity and more complex age structures than populations in agroecosystems, especially where crops are monocultures of similar genotypes. In wild communities, plant species are under natural selection that may favor the persistence of multiple alleles and the emergence of new ones that would offer some protection against virus infection or virulence (Desbiez et al., 2011).

We found that *M. charantia* in Puerto Rico frequently carried PRSV and ZYMV and that co-infection of these two viruses occurred more often than what one would expect by chance. In a survey of viruses in Puerto Rico, Paz-Carrasco and Wessel-Beaver (2002) found about 18% of their samples of cultivated cucurbits to have mixed infections of PRSV and ZYMV. We saw a similar percentage in our study of *M. charantia:* 14% of the samples had mixed infections of these two viruses. We also observed an interesting interaction between these two potyviruses in *M. charantia*: 35.8% of samples positive for PRSV, but only 11.9% of samples negative for PRSV, were infected with ZYMV (Table 3). In other words, the presence or absence of one potyvirus influenced whether or not the other potyvirus was present.

In Puerto Rico, there are four major localities where cucurbit crops are grown: the central to eastern south coast (particularly the municipalities of Guánica, Juana Díaz and Santa Isabela), and the western north coast (Isabela). We classified these sites as agricultural. Other *M. charantia* samples were categorized as coming from either a rural, primarily non-agricultural habitat, or an urban habitat. Using this classification approach, we were unable to detect a higher frequency of virus incidence in agricultural habitats (Table 5).

By contrast, the MaxEnt modeling approach predicted that favorable to highly favorable environmental conditions for the presence of virus symptoms, potyvirus, PRSV and ZYMV in *M. charantia* are found in the important vegetable growing region of the central to eastern south coast of Puerto Rico as well as on the western north coast (Figures 3, 4, 5 and 6). This finding suggests that the opportunity of some type of virus interaction between cultivated and wild cucurbits is highest in areas associated with cucurbit production on the island, and that environmental conditions favorable to potyvirus incidence in *M. charantia* might be similar to conditions favorable to potyvirus incidence in cultivated cucurbits. Potyviruses are known to be ubiquitous in cucurbit crops in the south coast vegetable region of Puerto Rico (Paz-Carrasco and Wessel-Beaver, 2002).

In our sampling we found PRSV to be much more prevalent than ZYMV in *M. charantia* (Table 2). Likewise, the MaxEnt maps predicted the areas of environmental suitability for PRSV to be much more widespread than that for ZYMV (Figures 5 and 6). By contrast, Paz-Carrasco and Wessel-Beaver (2002) found a higher incidence of ZYMV than of PRSV in their survey. But that survey concentrated on the south coast vegetable growing area while our survey was island wide.

There are several limitations to the use of MaxEnt for species distribution modeling. Predictions made with MaxEnt are thought to be more reliable when the variables used are not correlated (Merow et al., 2013). At least some of the environmental variables included in our MaxEnt model are likely to be correlated. Yackulic et al. (2013) point out that sampling bias can often affect the results of MaxEnt. However, this is more of an issue in studies that depend on museum or herbarium specimens. We acknowledge that our sample was not truly random since sampling was done primarily in localities with road access. However, the road network in Puerto Rico is very dense: it ranks in the top ten worldwide in roads per 100 square kilometers (NationMaster, no date). Given the large number of samples we collected across the entire island of Puerto Rico and adjacent islands of Vieques and Culebra, we consider our sample of *M. charantia* to be adequate. A MaxEnt analysis uses presence data even though our study included both presence and absence data. The inability of MaxEnt to use available absence data indicates that prediction information may have been lost.

Although in Table 6 we point out which are potentially important environmental variables in determining suitability for the presence of potyviruses in *M. charantia*, the nature of MaxEnt modelling does not allow us to conclude that these are, in fact, the most important variables. The percentage contribution of a variable to the model is highly dependent on which other variables are included in the model and the order in which the variables are entered into the model, among other things. But this list does provide insights into which environmental variables could be more closely studied in future research.

A central question in plant virus ecology is: Under what conditions do viruses have negative, neutral, or positive influences on host fitness? Historically, plant viruses in agriculture have been conceptualized as pathogens because those studies were usually initiated because of apparent adverse effects on crop yield or marketability, the effects of which were also observed in wild plants. However, sometimes viruses may be merely commensal and cause little or no disease, and at other times viruses may even benefit their hosts (Roossinck, 2005). The ubiquitous occurrence of plant viruses in tropical regions would undoubtedly suggest a plethora of interactions (Wren et al., 2006; Malmstrom et al., 2011). Indeed, while *M. charantia* may serve as a reservoir of potyviruses (Chin et al., 2007b), its viral load may have minimal effects on plant fitness. Furthermore, this load may be inconsequential to local crops without avenues for transmission. There is also evidence that PRSV can manipulate its aphid vectors to promote viral dispersion, for example, by increasing the fitness of vectors feeding on PRSV-infected plants (Gadhave et al., 2020).

Momordica charantia can be used as a model to monitor plant viruses (Chin et al., 2007a) in Puerto Rico and other parts of its native and invasive range. Future studies should focus on identifying other viruses that may be infecting *M. charantia*, determining whether the diversity in *M. charantia* may play a determining role in virus occurrence, and studying whether viral infections in wild plants have the same detrimental effect as in crops. The distributional relationships between these viruses and their vectors and the transmission relationships among wild plants and crops need to be better studied. Answers to these and other issues may contribute to additional crop protection strategies for cucurbits and other crops.

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