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Fluctuación poblacional de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) en el cultivo del sorgo del sur de Anzoátegui, Venezuela[1,2]

María Virginia Bertorelli[3] y Barlin Orlando Olivares[4]*

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RESUMEN

Este estudio tuvo como objetivo evaluar la fluctuación poblacional de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) en etapa larval, en las diferentes etapas fenológicas del sorgo y su relación con el tipo de labranza, las condiciones climáticas, y distintos sistemas de uso del sorgo en el Fundo Las Tunas, municipio Simón Rodríguez, estado Anzoátegui, Venezuela. Para la evaluación se utilizaron cuatro sistemas de uso: S1 (Sorgo-*Stylosanthes brasilianum-Brachiaria dictyoneura*); S2 (Sorgo-*Centrosema brasilianum-B. dyctioneura*); S3 (Sorgo-*B. dyctioneura*); y S4 (Sorgo monocultivo) bajo dos tipos de labranza (liviana y profunda). La determinación de la fecha de ocurrencia de las etapas fenológicas del cultivo se realizó dos veces por semana durante todo el ciclo del cultivo. Los resultados del estudio revelaron que la fluctuación poblacional de *S. frugiperda* no se vio influenciada por el sistema de uso,

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[2] Proyecto de transferencia e innovación de tecnologías agrometeorológicas para contribuir al desarrollo rural integral de la producción agrícola nacional con influencia en los polos agrarios socialistas. Sub-proyecto: desarrollar y transferir tecnologías e información con base en investigación agrometeorológica, apoyada en el fortalecimiento y ampliación de la red agrometeorológica del estado Anzoátegui. Institución responsable: Instituto Nacional de Investigaciones Agrícolas (Venezuela)/Gerencia de Investigación e Innovación Tecnológica.

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pero sí por el tipo de labranza, se presentaron mayores valores de fluctuación población del insecto (>0.80 larvas por planta) con la labranza profunda. Se evidencia una estrecha relación entre estos factores de fluctuación poblacional del insecto, condiciones climáticas y los días después de la siembra; un mayor ataque de la plaga ocurrió en el periodo vegetativo cuando se presentaron bajos montos de precipitación diaria y altas temperaturas.

Palabras clave: agricultura, sorgo, plaga, fitófago, precipitación

ABSTRACT

Population fluctuation of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in sorghum cultivation in Southern Anzoategui, Venezuela

The objective of this study was to evaluate the population fluctuation of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) at the larval stage of the different phenological stages of sorghum and its relationship with the type of tillage, climatic conditions and different systems of sorghum use in the Las Tunas, Simón Rodríguez municipality, Anzoátegui state, Venezuela. For this, four systems of use were used: S1 (Sorghum -*Stylosanthes* brasilianum- *Brachiaria dictyoneura*); S2 (Sorghum -*Centrosema brasilianum* - *B. dyctioneura*); S3 (Sorghum-*B. dyctioneura*); and S4 (monoculture sorghum) under two types of tillage (light and deep). The determination of the date of occurrence of the phenological stages of the crop was carried out twice a week during the entire crop cycle. The results of the study revealed that population fluctuation of *S. frugiperda* was not influenced by the system of use, but by the type of tillage, with deep tillage having the highest fluctuation values of the insect population (> 0.80 larvae per plant). There is a clear relationship between these factors of population fluctuation of insect, climatic conditions and the days after sowing; a greater attack of pests occurred in the vegetative period when there were low amounts of daily precipitation and high temperatures.

Key words: agriculture, sorghum, pest, phytophagous, precipitation

INTRODUCCIÓN

Una de las principales plagas de la producción de sorgo en los llanos venezolanos lo representa el insecto denominado cogollero, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Esta es una especie polífaga nativa del trópico con una amplia distribución geográfica en Suramérica; causa daños severos en brotes tiernos, especialmente en los cogollos de los cereales maíz (*Zea mays* L) y sorgo (*Sorghum bicolor* L. Moench) (Willink et al., 1993; Flores et al., 2009; Goergen et al., 2016; Sarmento et al., 2002; Olivares et al., 2018a).

Los sistemas de producción de sorgo y maíz en los llanos orientales venezolanos son los más afectados, requiriendo hasta tres o más aplicaciones de insecticidas para controlar este insecto. El alto número de aplicaciones de insecticidas se debe fundamentalmente al recorrido de las larvas sobre la superficie del suelo por sí mismas o ayudadas por el viento, y condiciones meteorológicas favorables, lo que ocasiona una infestación generalizada en lotes cuando eclosionan los huevos (Murillo, 1991; Olivares et al., 2018a).

Partiendo de este último aspecto, estudios relevan que el sistema de labranza en terrenos cultivados de sorgo se relaciona directamente con la incidencia del cogollero, es decir, la siembra directa pareciera generar una menor incidencia que el sistema de labranza convencional. Se atribuye este hecho a una mayor presencia de enemigos naturales en el suelo protegido de la siembra directa y a que las hembras adultas no pueden distinguir tan claramente las plantas como lo podrían hacer en suelo desnudo (Willink et al., 1994; Willink y Osores, 1994).

Así mismo, se establece el rol determinante de las condiciones climáticas en la aparición de plagas y enfermedades (Contreras, 2009; Olivares et al., 2016a; Paredes-Trejo y Olivares, 2018), especialmente en la influencia de la temperatura del aire y la precipitación como factores claves para el óptimo desarrollo de los insectos plaga y enfermedades en plantas (Olivares et al., 2016b; Chirinos y Olivares, 2011). En este sentido el uso de información climática disponible en zonas de interés agrícola del sorgo puede asegurar el éxito en la realización de las labores del cultivo tales como la preparación del suelo, aplicación de fertilizantes, insecticidas o herbicidas (OMM, 1990; Marco, 2001; Martínez, 2007).

Para el caso del cultivo de sorgo en el estado Anzoátegui, este se adapta a una amplia gama de condiciones agroecológicas, produciendo granos incluso en condiciones desfavorables como las sequías meteorológicas (Olivares y Zingaretti, 2018; Cortez et al., 2018; Parra et al., 2018). Comúnmente se observan asociaciones entre el sorgo y especies forrajeras multipropósito como *Stylosanthes brasilianum*, *Brachiaria dictyoneura* y *Centrosema brasilianum* donde los pequeños y medianos productores integran tecnologías novedosas basadas en forrajes multipropósito en sus sistemas de producción para mejorar su seguridad alimentaria, la generación de ingreso, la eficiencia en el uso de la tierra, y la utilización y conservación de los recursos naturales (Heulveldop et al., 1986; Villapaldo y Ruiz, 1993; Hernández et al., 2017; Olivares et al., 2018b).

Recientemente, en la zona de estudio se ha manifestado el interés de estudiar los fenómenos biológicos que se observan periódicamente adaptados a ritmos estacionales y que están estrechamente relacionados con el clima y el curso anual del tiempo atmosférico (Mejía, 2005; Contreras, 2009, Olivares et al., 2012b; Rodríguez et al., 2013; Cortez et al., 2016). Se estable que el uso de la tierra, aunado a las prácticas de labranza y el ambiente físico se relacionan en la aparición de *S. frugiperda* en terrenos cultivados de sorgo.

El objetivo de este trabajo es evaluar la dinámica poblacional de *S. frugiperda* en las etapas fenológicas del cultivo del sorgo y su relación con las condiciones climáticas en cuatro sistemas de uso agrícola en el estado Anzoátegui, Venezuela. Esta investigación proporcionará un nuevo enfoque en la zona de estudio, incrementando el conocimiento existente, y apoyado en fundamentos teóricos para el uso potencial de un adecuado control, basado en un método de control físico que forme parte de las estrategias de un Manejo Integrado de Plagas.

MATERIALES Y MÉTODOS

Área de estudio

Las evaluaciones se realizaron en una parcela agrícola llamada Las Tunas cuyas coordenadas son 8° 45' 42" N y 64° 3' 45" W con una altura de 285 m.s.n.m; ubicada en la Parroquia el municipio Simón Rodríguez al sur del estado Anzoátegui, Venezuela. El clima en el sur de Anzoátegui es de tipo *Awi*, definido como marcadamente tropical isotérmico, con una estación seca bien definida de diciembre a abril. Según Olivares et al. (2013, 2017b), el promedio de precipitación anual en el sur de Anzoátegui es 1,119 mm.

La región presenta un bioclima seco tropical según la clasificación de Holdridge (1947), con evaporación media anual de 2,626 mm; la temperatura media anual de 26.9° C y la humedad relativa media anual de 78%. En la vegetación nativa predominan las sabanas, ocasionalmente interrumpidas por bosques de galería y morichales asociados a los cursos de agua (Vegas-Villarrubia y Herrera, 1993, Olivares, 2014; Olivares et al., 2015). En el Cuadro 1 se presentan los valores promedios de los elementos climáticos de la zona.

Los índices climáticos indican que la zona posee un régimen de humedad subhúmedo (cinco meses secos en los cuales la precipitación es menor que 0.5 ETo), con un déficit hídrico anual de 711 mm y un exceso de 245 mm (Olivares et al., 2012a). Presenta además una alta agresividad climática, definida como el efecto erosivo de las precipitaciones según Lobo et al. (2009), dado que el Índice de Fournier Modificado (IFM) tiene un valor de 153.3, de acuerdo con los criterios de Arnoldus (1980).

Los suelos representativos de la zona en estudio presentan un pH moderadamente ácido de 5.8; con contenidos de fósforo cercanos a los 6 µg/g, calcio: 35 µg/g, magnesio: 37 µg/g, potasio: 10-30 µg/g, hierro: 0.5-30 µg/g, manganeso: 0.5-12 µg/g, zinc: 0.1-1 µg/g, bajo contenido de materia orgánica (0.51 %), aluminio intercambiable de de 0.44 cmol/kg de suelo y conductividad eléctrica de 0.28 dS/m (Olivares et al., 2015).

Evaluación fenológica y datos climáticos

La evaluación fenológica se realizó dos veces por semana durante todo el ciclo del sorgo (*S. bicolor*) (Cuadro 2). Inicialmente se marcó un metro lineal en cada tratamiento y se procedió a determinar el evento, la fecha de

Cuadro 1.—*Comportamiento promedio (1970-2010) de los elementos climáticos en el área de estudio (Fuente: Olivares et al., 2015).*

Elemento	Ene	Feb	Mar	Abr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dic	Anual
Precipitación (mm)	10.0	5.9	9.5	37.3	107.2	174.0	194.1	217.0	164.0	109.5	59.7	30.6	1119
Evaporación (mm)	188.7	200.8	259.4	244.2	223.4	161.2	157.1	165.7	171.8	177.6	164.1	163.7	2277.7
T.max (°C)	31.5	32.2	33.4	33.8	33.2	31.4	31.0	31.8	32.4	32.5	32.1	31.3	32.2
T. Media (°C)	25.8	26.4	27.3	27.9	27.6	26.6	26.3	26.7	27.1	27.0	27.0	26.0	26.8
T. Min (°C)	20.2	20.7	21.1	21.9	22.3	21.9	21.6	21.7	21.9	22.0	22.0	20.9	21.5
HR (%)	69.7	67.0	63.8	64.6	69.8	76.9	77.3	75.5	73.3	71.6	72.1	71.4	71.1
Rg (Cal/cm^2/día)	390.8	483.3	481.7	489.9	447.7	424.0	450.5	477.7	456.7	461.5	396.9	377.7	444.9
Vel. del viento -0.65 m (km/h)	6.0	6.8	7.2	6.9	6.2	5.1	3.9	3.4	3.4	3.8	4.6	5.4	5.2
Eto (mm)	124.0	137.2	161.2	156.0	142.6	120.0	127.1	136.4	129.0	136.4	117.0	117.8	1604.7

Eto: evapotranspiración de referencia (Penman – Monteith – FAO; calculada usando el CIRH (Santibañez, 2005)

Cuadro 2.—*Etapas de crecimiento de sorgo (Fuente: Vanderlip, 1979)*

Etapa	Definición	Características
E0	Emergencia	50% de plantas con coleóptilo visible en la superficie del suelo
E1	tres hojas	50% de plantas con el nudo de la tercera hoja visible
E2	cinco hojas	50% de plantas con cinco hojas completamente extendidas
E3	Diferenciación del punto de crecimiento	50% de plantas con 7 a 10 hojas completamente extendidas (según el material genético)
E4	Hoja bandera visible	50% de las plantas con la hoja bandera completamente extendidas
E5	Embolsamiento	50% de las plantas en las cuales se siente la panoja dentro de la hoja bandera
E6	Media floración	50% de las plantas mostrando la panoja
E7	Masa suave	La mitad del peso seco de los granos ha sido acumulado. Estado lechoso
E8	Masa dura	75% del peso seco de los granos ha sido acumulado
E9	Madurez fisiológica	100% del peso seco. El grano adquiere su color.

ocurrencia de este, el número de plantas totales y el número de plantas en el evento. Se utilizaron datos de precipitación total (mm) y temperatura media del aire (°C) durante el año 2012, proporcionados por la red agrometeorológica del Instituto Nacional de Investigaciones Agrícolas (INIA) El Tigre, Anzoátegui.

Selección de tratamientos

Para las evaluaciones en campo se consideró el material vegetal híbrido Chaguaramas VII, sembrado el 14 de septiembre de 2012 en cuatro tratamientos denominados: sistemas de uso del suelo (superficies de cultivos intercalados) y dos tipos de labranza: profunda (un pase de arado de cincel y tres pases de rastra, TLP) y liviana (cuatro pases de rastra, TLL). Los sistemas de uso del suelo fueron los siguientes: S1 (Sorgo-*Stylosanthes capitata - Brachiaria dictyoneura*), S2 (Sorgo-*Centrosema brasilianum-Brachiaria dictyoneura*), S3 (Sorgo- *Brachiaria dictyoneura*) y S4 (Sorgo monocultivo). La fertilización en el sorgo monocultivo fue en bandas con fórmula completa mientras que en los demás sistemas de uso del suelo se fertilizó al voleo con fertilizante residual complementado con urea.

Evaluación de niveles poblacionales de larvas de S. frugiperda

El diseño utilizado fue en bloques al azar con dos repeticiones, en un arreglo de tratamientos en parcelas divididas. Las parcelas principales se adjudicaron a los sistemas de labranza (TLP y TLL) y las parcelas secundarias estuvieron representadas por las épocas de muestreo. Los muestreos se efectuaron a los 26, 36, 44, 51 y 58 días después de la siembra (DDS). Las variables medidas fueron: número de plantas por m^2, plantas dañadas, plantas infestadas (plantas con larvas vivas) y larvas vivas por planta. Los contajes de las larvas vivas por planta y las demás variables se realizaron en el campo utilizando el método de observación directa semanalmente una vez detectada la plaga. Para ello, se muestrearon 10 plantas consecutivas de las dos hileras centrales de los bloques evaluados.

El área experimental abarcó una superficie de 5,500 m^2. Las dimensiones de las parcelas fueron 15 m de largo por 30 m de ancho (450 m^2). Para los fines de muestreo, cada parcela se dividió en cuatro subparcelas de 7.5 m x 15 m y una superficie de 108 m^2. Se escogieron tres puntos al azar, delimitándose en cada punto un metro cuadrado. Posteriormente se hizo el análisis de la varianza (ANOVA) del programa Infostat v2018 (Di Rienzo et al., 2011).

RESULTADOS Y DISCUSIÓN

Dinámica poblacional de S. frugiperda

En el Cuadro 3 se puede observar que hubo diferencias altamente significativas entre los días después de la siembra (DDS) (fechas), así como en la interacción entre la fecha y tipo de labranza en la dinámica poblacional de *S. frugiperda* en etapa larval. En la primera evaluación para determinar la fluctuación poblacional no se encontraron larvas. A partir de la segunda (29 DDS), alcanzó el máximo valor (0.90 larvas por planta) a los 51 DDS. En esta fecha, se puedo apreciar el cambio en la densidad poblacional del cogollero que pasó de 0.20 a 0.92 larvas por planta. La población luego descendió a 0.05 larvas por planta a los 58 DDS. Al respecto, Piñango et al. (2001) señala que este aumento en los niveles de la densidad podría estar determinado por el desarrollo de las mazorcas como principal alimento en sustitución del follaje.

Al comparar los sistemas de uso, la Figura 1 muestra un comportamiento similar en las poblaciones de larvas de *S. frugiperda* para los dos tipos de labranza. Sin embargo, se puede notar que, en la cuarta evaluación, correspondiente a los 51 (DDS) para ambas labranzas (Figura 1a), el sistema de uso (S4) compuesto únicamente por el Sorgo monocultivo mostró un n**ú**mero de larvas superior al resto de los sistemas de uso (>0.80 larvas/plantas), posiblemente asociado a una menor presencia de enemigos naturales en el suelo con labranza profunda. A los 51 DDS se observó un incremento significativo en la incidencia de *S. frugiperda*, relacionado con las marcadas deficiencias hídricas en la zona, lo que acentúa el daño causado por la plaga y que obliga a tener más precauciones para lograr un control eficaz con los insecticidas en esas condiciones ambientales.

Por su parte, en la Figura 1b se observa que los tratamientos en función a los tipos de labranza se comportaron de forma similar, no

CUADRO 3.—*Análisis de varianza de la dinámica poblacional de S.* frugiperda *(etapa larval) para los cuatro tratamientos y dos tipos de labranza.*

Fuente de variación	GL	Suma de cuadrados	Cuadrado medio	F	P
Fecha	4	67.692	16.923	105.28	0.0003**
Tipo labranza (TL)	1	0.490	0.490	416.32	0.0312*
Rep (Rep)	1	0.063	0.063	0.40	0.5628 ns
Tratamiento (Trat)	3	0.557	0.185	0.99	0.4071 ns
Fecha x Rep	4	0.642	0.160		
TL x Rep	1	0.001	0.001		
Fecha x TL	4	6.301	1.575	8.44	0.0001 **
Fecha x Trat.	12	2.892	0.241	1.29	0.2680 ns
TL x Trat.	3	1.696	0.565	3.03	0.0426 *
Fecha x TL x Trat	12	3.522	0.293	1.57	0.1460 ns
Fecha x TL x Rep x Trat	34	6.345	0.186		
Total	79	90.205			

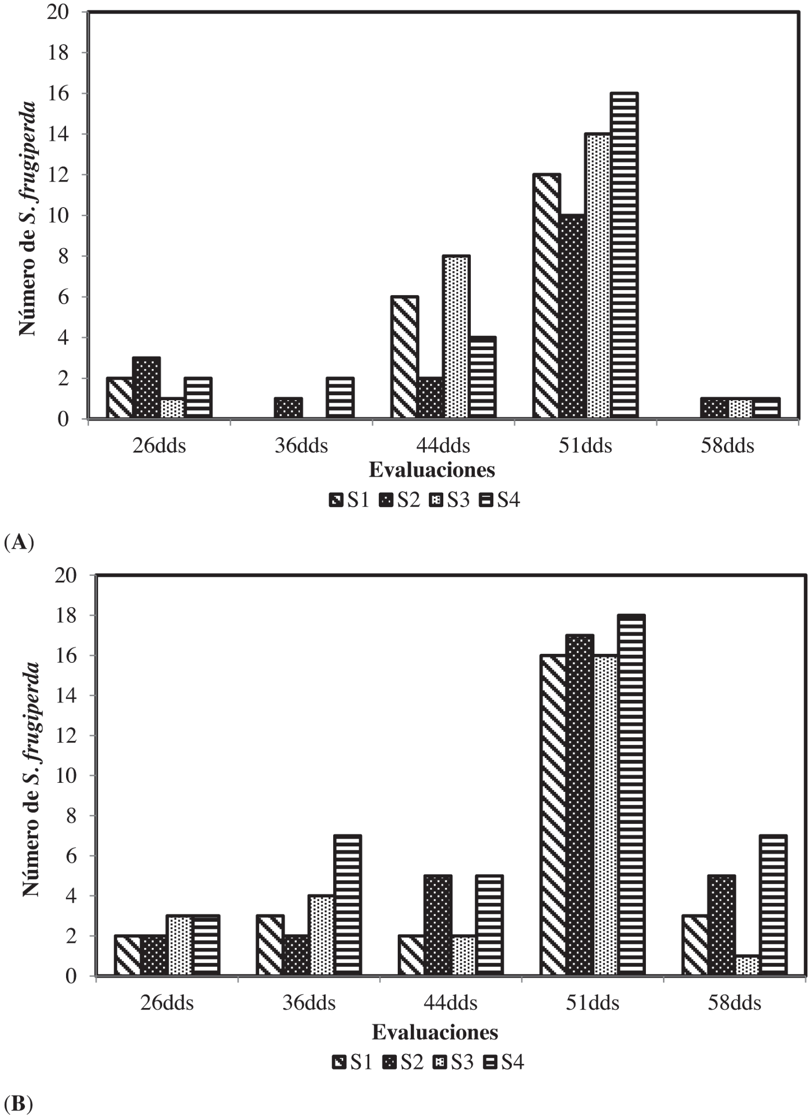


FIGURA 1. Número total de *S. frugiperda* (etapa larval) en el sitio de estudio: (A) labranza profunda con los cuatro sistemas de uso, (B) labranza liviana con los cuatro sistemas de usos.

habiendo diferencias significativas entre ellos. El tratamiento S4 presentó un mayor ataque de la plaga en comparación con el resto de los sistemas, lo que se pudo deber al vigor presente en las plantas de este sistema de monocultivo, las cuales recibieron un tratamiento en bandas con fórmula completa de fertilizantes.

Estos resultados se asemejan a los obtenidos por Piñango et al. (2001) quienes no se observaron diferencias significativas en la población de cogolleros al comparar diferentes métodos de labranzas. De igual manera se observó un pico con los valores máximos de poblaciones del cogollero a los 51 DDS y mínimos a los 58 DDS. Sin embargo, el estudio de Pérez y Andreu (1993) refleja que el mayor grado de daño de poblaciones de larvas de cogollero en el estado Guárico se presentó en tratamientos con labranza convencional.

En el Cuadro 4 se observa que hay diferencias significativas entre las evaluaciones efectuadas a los 51 DDS con labranza liviana y pro-

CUADRO 4.—*Dinámica poblacional de S. frugiperda (etapa larval) en la interacción entre la fecha de evaluación y el tipo de labranza y la interacción entre el tipo de labranza y el sistema de uso del suelo en el cultivo del sorgo.*

	Fecha de evaluación[1] y el tipo de labranza[2]	
Interacción	Valor	Grupo[3]
E4 x TLL	16.63	a
E4 x TLP	16.00	a
E3 x TLP	7.13	b
E2 x TLL	4.13	c
E5 x TLL	3.88	cd
E3 x TLL	2.75	cd
E1 x TLL	2.50	cd
E2 x TLP	2.50	cd
E1 x TLP	1.75	cd
E5 x TLP	0.63	d

	Tipo de labranza[2] y el sistema de uso del suelo[4]	
Interacción	Valor	Grupo[3]
TLL x S4	7.20	a
TLP x S1	6.80	ab
TLP x S2	6.00	ab
TLL x S2	6.00	ab
TLL x S3	5.50	ab
TLL x S1	5.20	ab
TLP x S3	4.90	ab
TLP x S4	4.70	b

[1] E1=26; E2=36; E3=44; E4=51; E5=58 días después de la siembra

[2] TLP= Tipo de labranza pofunda; TLL= Tipo de labranza liviana

[3] Valores seguidos de letras distintas difieren entre si según prueba de medias de Tukey ($P<0.05$)

[4] S1=Sorgo-*Stylosanthes capitata-Brachiaria dictyoneura*; S2=Sorgo-*Centrosema brasilianum-Brachiaria dictyoneura*; S3=Sorgo-*Brachiaria dictyoneura*; y S4=Sorgo monocultivo

funda con las efectuadas a los 44 DDS con labranza profunda, a los 36 DDS con labranza liviana y a los 58 días DDS con labranza profunda. Siendo el factor fecha de evaluación (DDS) el que tiene un mayor efecto en la dinámica poblacional del insecto.

Al respecto, las siembras comerciales del sorgo deben efectuarse durante el período comprendido: desde el mes de septiembre hasta finales de noviembre, con fecha tope al 15 de diciembre, lo cual garantiza, con mayor seguridad, la obtención de rendimientos de granos económicamente aceptables y el menor ataque de plagas o enfermedades en los llanos venezolanos (González y Velásquez, 2005). Se observaron diferencias significativas entre los dos tipos de labranza para S4 (Sorgo monocultivo) presentando la labranza liviana mayores valores en la dinámica poblacional que la labranza profunda. En el resto de las interacciones no se observaron diferencias significativas.

La Figura 2 muestra que la plaga hizo su aparición en la cuarta semana del cultivo con niveles poblacionales de 0.1 larvas por planta por área evaluada, observándose el mayor ataque en la séptima semana (0.81 larvas por planta) cuando hubo ausencia de precipitaciones.

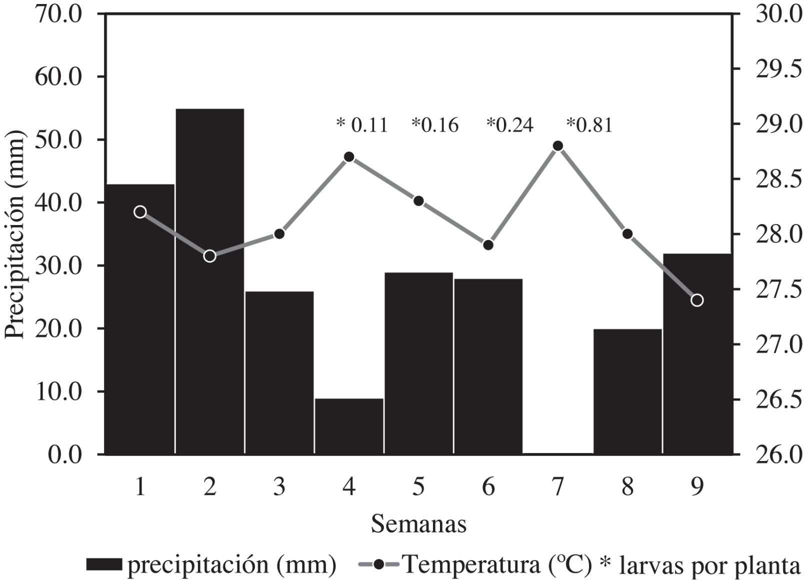


Figura 2. Comportamiento semanal de la precipitación total (mm) y temperatura media del aire (°C) en la zona de estudio durante la semana 1 (14/09/2012) hasta semana 9 (09/11/2012).

La duración del ataque de la plaga fue aproximadamente un mes y la presencia del insecto estuvo relacionada con las condiciones climáticas típicas del periodo seco. Los niveles de infestación a los 51 DDS correspondieron a la transición de época húmeda a seca, la cual pareciera suficiente para generar un grado de humedad en el suelo, que favorece la transformación del insecto de pupa a adulto incrementando las poblaciones en el campo (Piñango et al., 2001; Olivares et al., 2013).

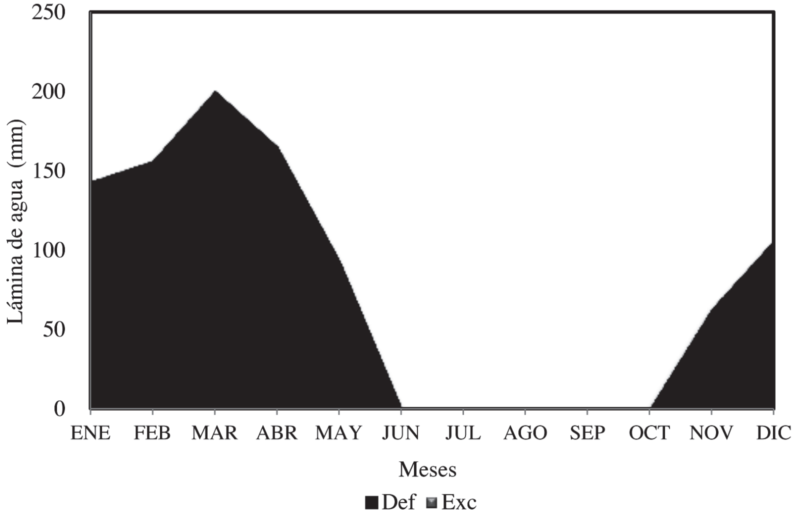
Este resultado es comparable donde las mayores poblaciones de la plaga ocurrieron en los periodos de menor precipitación, coincidiendo con los observados por Clavijo y Notz (1978) quienes aseveraron que las mayores poblaciones del insecto fueron favorecidas por las condiciones climáticas del periodo seco. Por otra parte, Marenco y Saunders (1993), no encontraron correlación entre la presencia de larvas de cogollero y las variables climáticas por ellos registradas, atribuyéndole la disminución de las poblaciones a parasitoides, depredadores y canibalismo.

La aparición de poblaciones de *S. frugiperda* puede ocurrir de manera extensa y persistir por periodos de tiempo cortos, donde su fluctuación depende esencialmente de las condiciones ambientales. Las poblaciones pueden aumentar después de un periodo de sequía, ya que, al incrementarse la concentración de los contenidos nutricionales en las plantas, se satisfacen más fácilmente los requerimientos alimenticios del insecto (Coyle et al., 2013; Martínez et al., 2018).

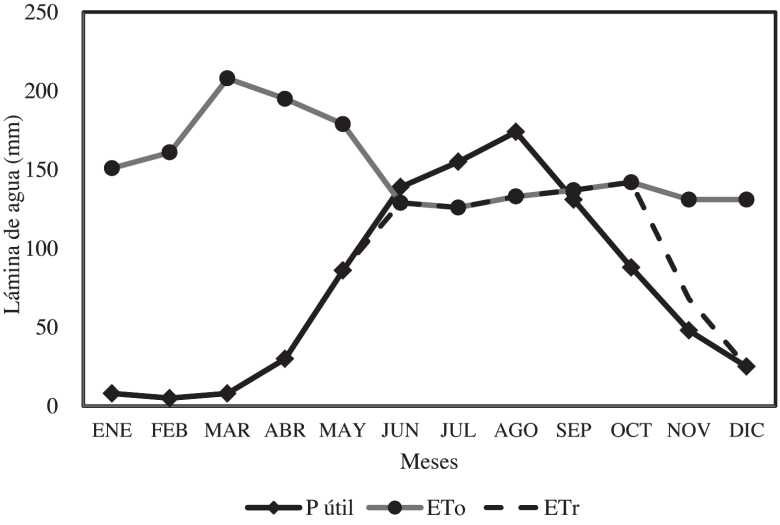
En condiciones naturales los insectos se encuentran expuestos a ciclos variables de temperatura, pero cuando las condiciones térmicas son constantes, el desarrollo de los insectos es diferente. Algunos insectos muestran un ciclo de crecimiento más corto cuando la temperatura fluctúa, que cuando permanece uniforme (Auad et al., 2015).

El balance hídrico para esta área permitió identificar periodos donde existe suficiente agua en el suelo para el desarrollo de los cultivos, describiendo cuantitativamente el comportamiento del régimen de humedad del área de interés. Asumiendo un porcentaje de lluvia útil de 90% y una capacidad máxima de almacenamiento del suelo de 100 mm; así mismo, la Evapotranspiración Potencial (Eto) se obtuvo mediante la utilización del coeficiente de tina (0.8) en función a las características de la zona de estudio (Olivares et al., 2015).

La distribución del déficit hídrico (Figura 3a) abarca el periodo desde octubre hasta junio, con un máximo de 212 mm en marzo, generalmente los cultivos que se siembran después en julio corren el riesgo de sufrir déficit hídrico en la fase de floración. Solo durante la época lluviosa existe almacenamiento de agua en el suelo disponible para la planta (Figura 3b). De acuerdo con las características físicas de los suelos en la Mesa de Guanipa y al régimen de precipitación en la zona, no se presentan láminas de excesos durante todo el año (Olivares et al., 2013, 2017a).



(A)



(B)

FIGURA 3. (A) Distribución anual de los déficit hídricos (mm) para El Tigre, Anzoátegui; (B) Comportamiento anual de la precipitación, Evapotranspiración potencial (Eto) y la Evapotranspiración real (Etr) obtenida mediante el balance hídrico en El Tigre, Anzoátegui.

Es importante mencionar que, durante los meses de septiembre y octubre se presenta la salida de la época de lluvias, las cuales favorecen la producción del sorgo. Pero si se siembra durante el mes de diciembre o en el transcurso del mes de enero, la humedad del suelo no será suficiente para satisfacer las demandas del cultivo. Además, se impediría el buen aprovechamiento del fertilizante, lo cual conlleva a la obtención de bajos rendimientos y mayor susceptibilidad del cultivo al ataque de plagas. Es decir, el efecto de la sequía se ve potenciado ante un estrés adicional, como el que ocasionan estos insectos (Paredes-Trejo y Olivares, 2018; Olivares et al., 2016c).

CONCLUSIONES

Los resultados obtenidos solo explican el comportamiento de las poblaciones de *S. frugiperda* en términos de las condiciones climáticas y dos tipos de labranza, mediante distintos sistemas de uso del suelo con el sorgo. Los valores de temperatura y precipitación para los cuales se presentó el mayor número de larvas capturadas hacen suponer que su actividad es mayor en estas condiciones, factor importante al momento de orientar estrategias en el manejo integrado de plagas.

Además de las condiciones climáticas, la acción de los enemigos naturales también afectó los cambios en la densidad poblacional del insecto. El número de larvas de cogollero del primer instar está significativamente correlacionado con la edad de la planta, es decir, las plantas maduras son menos atractivas para la oviposición por el cogollero. Por lo tanto, pocas o ningunas larvas de los primeros instares son encontradas en el follaje de plantas viejas.

Así mismo, estos resultados permitirían señalar que existe una tendencia benéfica de los sistemas de labranza conservacionistas favoreciendo el control natural de *S. frugiperda*, disminuyendo el uso de los plaguicidas tradicionales y propendiendo a la sostenibilidad de los sistemas de producción en la zona. Estos últimos aspectos hacen interesante al sistema de conservación del suelo, cuya adopción por parte de los productores de la zona ha sido lenta. Dicha situación se atribuye al desconocimiento de esta forma de producción, así como a la desatención en el manejo adecuado de las malezas y del comportamiento o preferencias alimenticias de esta plaga.

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Research Note

AQUATIC VEGETATION ASSESSMENT AT PORTUGUÉS AND CERRILLOS RESERVOIRS, PUERTO RICO[1,2]

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Waterbodies in Puerto Rico are constantly threatened by the introduction, establishment and spread of aquatic weeds. Their introduction is promoted by the aquarium and horticultural trade, as well as by travel and commerce via air and marine transportation. Once introduced, their dominance limits recreational use of waterbodies, and management operations in reservoirs used for potable water and flood control deteriorate. In Puerto Rico, the problem has worsened because of limited citizen awareness, wrong public perception of available management techniques and eutrophic man-made water bodies serving as suitable habitat for aquatic weeds. Currently the most troublesome aquatic weeds in Puerto Rico are waterhyacinth (*Eichhornia crassipes*), waterlettuce (*Pistia stratiotes*), alligatorweed (*Alternanthera philoxeroides*), hydrilla (*Hydrilla verticillata*) and giant salvinia (*Salvinia molesta*) (Robles, 2011). During the 1970s, botanists from the US Army Corps of Engineers (USACE) recognized the problem that waterhyacinth, waterlettuce and alligatorweed represented to several waterbodies in Puerto Rico (Gangstad, 1977; Rushing, 1974). Waterhyacinth and waterlettuce are free-floating aquatic weeds well established in such reservoirs as La Plata, Guayabal and Carraízo, and Cartagena Wildlife Refuge in Puerto Rico (Robles, 2011). Alligatorweed has been observed in many water bodies and drainage canals; however, its area of infestation has decreased due to successful biocontrol agents (Robles, 2011). Recent introductions of the submersed aquatic weed, hydrilla, and the aquatic floating fern, giant salvinia, are limited to a few locations in northern Puerto Rico (Robles, 2011). All five aquatic weeds cause negative ecological and economic impacts to water supply reservoirs, irrigation and drainage canals, private ponds, as well as estuaries and freshwater wetlands used as wildlife refuges (Robles and González, 2010). Specifically, waterhyacinth causes problems in the operation of hydroelectric plants, clogging dams and pumping facilities of aqueducts and sewer operations (Rushing, 1974; Gangstad, 1977). Other troublesome weeds like the facultative wetland species catclaw mimosa (*Mimosa pigra*) are wide-

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spread throughout many waterbodies in Puerto Rico, limiting their recreational use as well as shoreline access to water for humans and animals.

In the municipality of Ponce, two water reservoirs, Cerrillos and Portugués, were constructed in 1992 and 2015, respectively, by the USACE (2015) as flood risk management projects for southern Puerto Rico. According to González (2012), the Portugués reservoir alone could prevent flood risk to 40,000 people in southern Puerto Rico. Both reservoirs are currently being managed by the Department of Natural and Environmental Resources of Puerto Rico (DNER) and used for recreation and flood control programs. Cerrillos reservoir may be used for water supply with a water storage capacity of 59.1 million cubic meters. Its dam is located on the Cerrillos River and is part of the Bucaná River Basin. The smaller Portugués reservoir has a capacity of 15.2 million cubic meters, and its dam is located on the Portugués River as part of the Portugués River Basin. Aquatic vegetation assessments of these two reservoirs have not been performed. Considering the risk posed by aquatic weeds presence at water reservoirs, it was important to conduct an aquatic vegetation assessment to maintain the functionality of flood control programs at Cerrillos and Portugués reservoirs.

Hence, a reservoir survey was performed, between April and May of 2015, using the point-intercept method to assess aquatic vegetation (Madsen, 1999). The survey consisted of observing the presence of aquatic plant species in the water body by navigating by boat sequentially along an evenly spaced grid of points aided by global positioning systems (GPS). This survey method has been widely used in providing estimates of frequency of occurrence, temporal changes of plant communities, spatial distribution of aquatic plants within a waterbody as well as providing estimates of percent plant cover (Madsen and Wersal, 2018; Madsen and Wersal, 2017; Madsen et al., 2016; Madsen et al., 2015; Cox et al., 2014; Robles et al., 2011; Wersal et al., 2010; Madsen et al., 2008; Madsen et al., 2006; Wersal et al., 2006; Case and Madsen, 2004). Specifically, point-intercept has proven to be as accurate as line-intercept and requires less time and effort to perform (Madsen and Wersal, 2017; Floyd and Anderson, 1987).

The reservoir survey was set up using ArcGIS: ArcMap software version 10.2 (ESRI 2014) where a grid of survey points 100 meters apart was created over both reservoir boundaries (Figure 1). Both reservoir boundaries and survey points were transferred to a handheld GPS unit, Trimble model Juno[6], to perform the survey. The GPS unit was capable of 3-meter position accuracy, and all data were projected in Puerto Rico Universal Transverse Mercator (UTM) zone 20 N. One rake toss was performed at each survey point by deploying the rake over the side of the boat and allowing the rake to sink to the bottom to ensure documentation of submersed aquatic plant species. The presence of aquatic plant species observed at each point was recorded as “1”. Due to low water level and limiting navigation, only 112 and 30 survey points were surveyed in Cerrillos and Portugués reservoirs, respectively. For each aquatic plant species, points labeled as “1” were added to obtain their total count.

Water quality at the time of aquatic vegetation assessment was determined at the same aquatic plant survey points. At each point, an YSI EXO2 Multiparameter probe was launched to measure the following parameters at 30-cm depth below the water surface: conductivity (µS/cm), temperature (°C), dissolved oxygen (mg/L), pH, total dissolved solids (mg/L) and total algae (Chlorophyll µg/L). The probe was calibrated by the manufacturer prior to starting field surveys. A Secchi Disk was lowered within the water column to obtain

[6] 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.

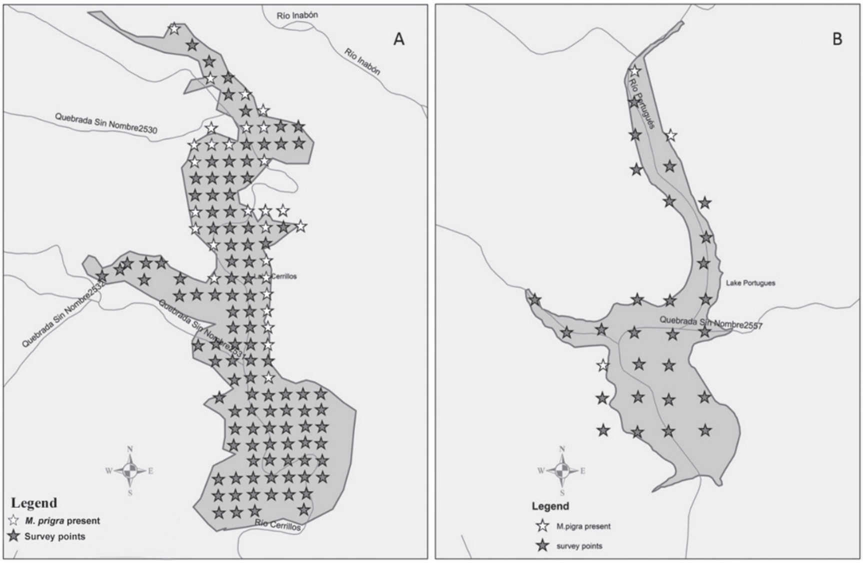

FIGURE 1. Geographic distribution of surveyed points (★) and presence of catclaw mimosa (*Mimosa pigra*) (☆) in Lake Cerrillos (A) and Lake Portugués (B).

water transparency data at each surveyed point and depth was recorded using a water depth finder. The same parameters were measured at the deepest point of each reservoir in order to describe the water column profile. This survey consisted of lowering the multiparameter probe into the water column every 30 cm up to a maximum depth of 10 m. Statistical analysis was performed using InfoStat v. 2020 software using a significance level of 0.05. Frequency of occurrence of aquatic plants was correlated to a water quality parameter measured at survey points using Spearman's correlation coefficient (r). The statistical analysis was used to determine which water quality parameter may predict aquatic plant presence.

According to the reservoir field survey performed, more than 75% of the area of both reservoirs is open water and free of aquatic vegetation. The most common aquatic plant species observed was catclaw mimosa with a frequency of occurrence of 10% and 25% at Portugués and Cerrillos reservoirs, respectively (Figure 1). Catclaw mimosa is widespread in Puerto Rico and currently listed on the Federal Noxious Weed List of the United States. This woody perennial is classified as Facultative Wetland Species (Lichvar et al., 2016) as it inhabits in wetland or non-wetland areas (Lichvar et al., 2012). Catclaw mimosa reproduces mainly by seed (Cronk and Fennessy, 2001; Langeland and Craddock-Burns, 1997) and is highly adapted to disturbance (CIEIPR, 2004) which facilitates its invasion (Center et al., 1995).

Among all water quality parameters measured, only water depth was significantly ($p < 0.05$) correlated to catclaw mimosa presence (r of -0.31 and -0.58 for Cerrillos reservoir and Portugues reservoir, respectively). This result supports field observations that catclaw mimosa was growing primarily along the shoreline, where water depth is below 5 m, and closer to boat ramps. Although catclaw mimosa can grow up to 4 m high (Lonsdale et al., 1988; Creager, 1992), it was also observed in both reservoirs, thriving under water in coves and off the shoreline. Only in the Portugués reservoir does catclaw mimosa coexist with aquatic plants from the genus *Colocasia* sp. and *Polygonum* sp.

Water quality parameters such as pH, transparency, specific conductance, total dissolved solids, dissolved oxygen and total algae are reported in Table 1 for both reservoirs. The first five parameters ranged within the same values in both reservoirs suggesting similarities between them and that they are not affected by the presence of catclaw mimosa By contrast, total algae at Portugues reservoir ranged up to 5.4 and 6.4 with or without catclaw mimosa as compared to Cerrillos reservoir that reported lower values (Table 1).

In general, the temperature throughout the water column in both lakes fluctuated between 26 and 28 °C. Data collected throughout the water column showed that dissolved oxygen in both reservoirs stratified at a water depth of 3.05 m where a decline from 8 to 1 mg/L was observed (Figure 2). Total algae in Cerrillos reservoir was relatively constant and ranged between 2 and 4 Chl µg/L throughout the water column. Portugués reservoir has similarly low values of total algae in shallow water (3 m or less); however, values increased up to 13 Chl µg/L in deeper water.

Although it is uncertain how catclaw mimosa was introduced to both reservoirs, it is suggested that disturbance events in reservoir construction, such as land alteration to construct levees that introduces soil from a different location, and further flooding promote catclaw mimosa invasion. In both reservoirs, catclaw mimosa plants were observed producing inflorescences and seedpods which means that this species is established, although its frequency of occurrence is less than 25% of the total reservoir area. This observation raises concern about further invasiveness and consequent nuisance issues related to water body management. In fact, according to Lonsdale et al. (1988) catclaw mimosa may form dense impenetrable thickets with thorny stems at plant densities as low as 0.12 plants per square meter. Moreover, once a seed germinates, plant height may increase at a rate of 2.4 to 2.6 cm per day (Creager, 1992) with a rate of spread up to 76 m per year (Lonsdale, 1993).

Management techniques need to be implemented soon in both reservoirs to prevent the spread of catclaw mimosa. Although the frequency of occurrence of catclaw mimosa is less than 25%, this seed producing species spreads rapidly. Water level fluctuations may lead to seed germination along the shoreline which promotes higher seed-bank density. Dredging operations must consider the presence of viable seed removed with the sediment to prevent the spread of catclaw mimosa.

Management tools that combine chemical and mechanical techniques are effective in limiting the spread of catclaw mimosa (Cook et al., 1996). Mechanical techniques consisting of cutting catclaw mimosa stems may be effective if plant material is removed from the

TABLE 1.—*Water quality parameters measured at surveyed points and those where catclaw mimosa was present at Cerrillos reservoir and Portugués reservoir.*

Reservoir	pH	Transparency (cm)	Specific Conductance (µS/cm)	Total Dissolved Solids (mg/L)	Dissolved Oxygen (mg/L)	Total algae (µg/L)
Catclaw mimosa not present						
Cerrillos	7.4-8.4	76-244	76-386	50-220	6.1-7.6	0.0-2.4
Portugués	8.4-8.5	91-183	319-324	208-211	7.1-7.7	1.1-6.4
Catclaw mimosa present						
Cerrillos	7.4-8.3	76-239	140-339	91-220	6.1-7.6	0.0-1.1
Portugués	8.4-8.5	91-122	319-322	208-210	7.1-7.5	1.1-5.4

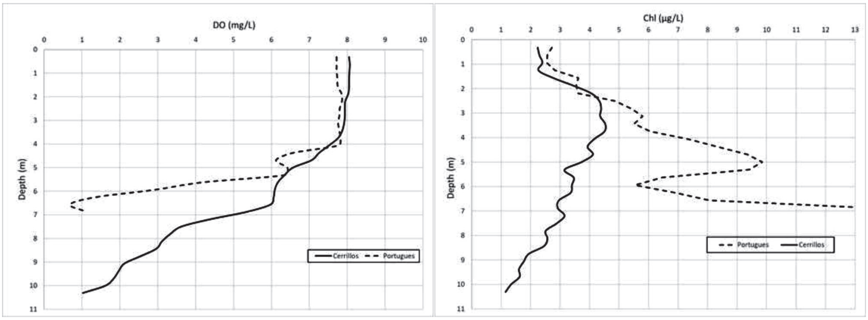

Figure 2. Distribution of dissolved oxygen (DO) and total algae (Chl) within the water column up to a 10-m depth of Cerrillos and Portugués reservoirs.

site to prevent seed establishment. Herbicide screening in greenhouse studies conducted in Florida have shown an acceptable efficacy of glyphosate and triclopyr in controlling catclaw mimosa (Creager, 1992). In Sri Lanka, Marambe et al. (2004) reported that glyphosate effectively controlled mimosa seedlings less than six months old, when applied three times repeatedly at four-month intervals on the same set of seedlings.

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Nota de Investigación

*CRECIMIENTO Y DESARROLLO DE ZARZA NEGRA (***Mimosa pigra** *L.)* *EN EL ESTE DE PUERTO RICO*[1,2]

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La zona este de Puerto Rico está categorizada como bosque húmedo sub-tropical y es reconocida como una de las zonas de mayor producción de carne y leche en la Isla. En esta zona, predomina la zarza negra (*Mimosa pigra* L.), considerada como una maleza invasora en los pastizales y llanos húmedos de Puerto Rico (Robles et al., 2010). Debido a su alta capacidad de desarrollo y crecimiento, es capaz de colonizar áreas nuevas con rapidez (Hall et al., 2012). Una vez establecida, la zarza negra puede tolerar inundaciones, sequías y otros disturbios. En la zona este de Puerto Rico no se han realizado trabajos de investigación sobre el desarrollo de la zarza negra. El objetivo de este estudio fue documentar los aspectos del desarrollo y crecimiento de la zarza negra en una localidad dentro de la zona ecológica del este de Puerto Rico.

En abril del 2013 se estableció un experimento de campo en la Estación Experimental Agrícola-Subestación de Gurabo para estudiar el crecimiento y desarrollo de la zarza negra. La subestación está localizada en el área este central de Puerto Rico, posee suelos del orden Inceptisol, serie Coloso (fino, mixto, activo, isohipertérmico Vertic Dystrudepts) (Muñoz et al., 2018), los cuales se componen de un 9% de arena, 42% de arcilla y 49% de limo con pH de 6.1 y 3% de materia orgánica. La subestación está localizada en la zona ecológica del bosque húmedo sub-tropical, con un rango de temperatura promedio entre 17 y 24 °C y una precipitación anual promedio de 1,000 a 1,100 mm (Miller, 2009).

Se cosecharon semillas de una población natural existente de zarza negra en la subestación, se colocaron en bandejas de germinación con sustrato a base de musgo de *sphagnum* y se germinaron en el invernadero. Luego de nueve semanas, 144 plantas se trasplantaron a tiestos de 15 cm. Luego de crecer durante 60 días más en el invernadero, estas plantas se trasplantaron a un predio experimental de 0.40 hectárea, el cual fue preparado con dos pases de arado tipo rastrillo. Las plantas se trasplantaron a una distancia de 3 m entre hileras y 3 m entre plantas. De la población plantada de zarza negra se escogieron diez plantas al azar (n=10), las cuales se identificaron con etiquetas de vinilo.

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A estas plantas se les realizaron muestreos cada tres semanas después de trasplantadas (SDT), comenzando 3 SDT. Los parámetros medidos fueron altura, diámetro del tallo, número de flores, número de vainas y número de semillas liberadas naturalmente por la planta. La altura se determinó desde la base del tallo hasta la altura de la copa. El diámetro del tallo se midió a partir de los primeros 15 cm de tallo (desde el suelo) utilizando un calibrador de medidas (“caliper”).

Para determinar materia seca de hojas y tallos, cada tres semanas se removieron cuatro plantas al azar del predio experimental, cortándolas a 2.54 cm del suelo. En el laboratorio cada planta removida se separó en hojas y tallos. El tallo, incluyendo ramas principales y secundarias se cortó en pedazos de 5 cm aproximadamente. Los tallos y hojas de cada planta se colocaron en bolsas de papel separadas e identificadas y luego se secaron en un horno a 50° C por 72 h. Cada muestra se pesó para determinar su materia seca. Los datos obtenidos de materia seca total por planta se utilizaron para determinar la tasa de crecimiento de la zarza negra cada tres semanas a través del periodo de muestreo utilizando la siguiente fórmula:

$$\text{TCR} = \frac{(\text{In W2} - \text{In W1})}{(\text{T2} - \text{T1})}$$

Esta fórmula calcula el incremento del peso de la planta por unidad de tiempo en donde W2 y W1 representa el peso de la planta en el periodo de Tiempo 2 y Tiempo 1, respectivamente (Gardner et al., 1985). Esta fórmula indica el incremento en materia seca en un intervalo de tiempo en relación al peso inicial. Para determinar la producción de semillas liberadas naturalmente por la planta, a cada una de las 10 plantas de zarza negra etiquetadas se les colocó una malla plástica en la parte inferior del dosel al momento que comenzó la producción de vainas. La malla se fijó a una estructura de forma cuadrada hecha de tubos de plástico de cloruro de polivinil (PVC) arreglados para formar un área de 4 m^2 (Figura 1).

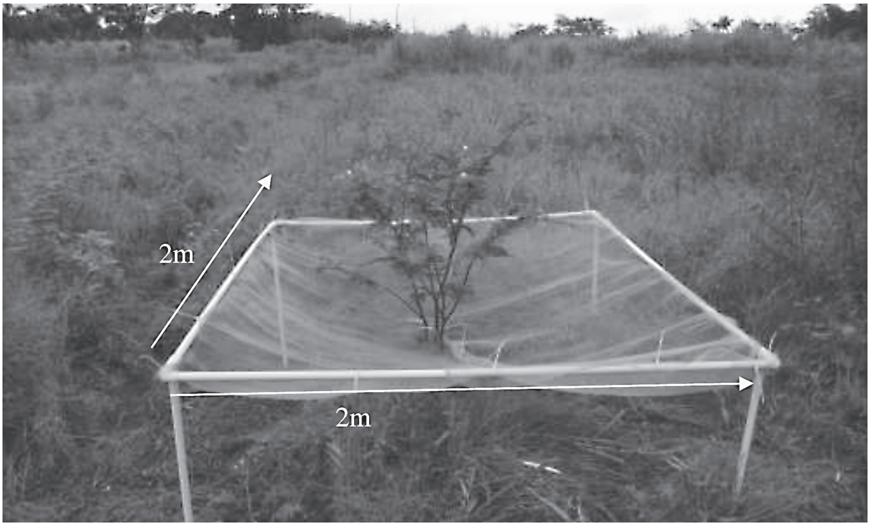

FIGURA 1. Malla en el marco de PVC utilizada para recolectar las semillas de la zarza negra durante el periodo de muestreos.

Las semillas recolectadas se identificaron según la fecha de muestreo y luego se sembraron en bandejas de germinación y se colocaron en un invernadero localizado en la finca Alzamora en el Recinto Universitario de Mayagüez para determinar el porcentaje de germinación. Se utilizó un sustrato a base de musgo *sphagnum* y se regaron tres veces al día durante diez minutos. Se determinó el porcentaje de germinación para cada fecha de muestreo, a los 2, 3, 4, y 5 semanas después de sembradas. El periodo de germinación comenzó el 24 noviembre 2014 y se extendió hasta el 29 diciembre 2015 (cinco semanas). Para cada observación, se contó el número de semillas germinadas por cada grupo y se determinó el porcentaje de germinación. Se contaron solo aquellas semillas que germinaron y desarrollaron su primera hoja verdadera u hoja pinnada ya que se consideraba que éstas podrían desarrollarse en el campo. Se utilizó el programa estadístico InfoStat® versión 2008 para calcular el error estándar de cada media de los parámetros medidos. Además, se ajustaron modelos de regresión lineal para obtener el coeficiente de determinación (R^2) y definir el cambio en la tasa de crecimiento relativo sobre el tiempo de muestreo.

Durante la época de desarrollo y crecimiento de la zarza negra en el campo, la precipitación fluctuó entre 23 y 253 mm. Los meses de julio de 2013 y marzo 2014 fueron los de mayor y menor precipitación, respectivamente (Figura 2). La precipitación acumulada durante el año de estudio fue de 1,685 mm, lo cual concuerda con la precipitación anual promedio de esta área durante los años 1981 al 2010, la cual fue de 1,727 mm (NWS, 2016). La temperatura promedio fluctuó entre 19° C y 31 °*C* (Figura 2**).** Durante el año de estudio la temperatura máxima observada fluctuó entre 29 y 32 °C mientras la temperatura mínima fluctuó entre 13 y 26 °C (Figura 2).

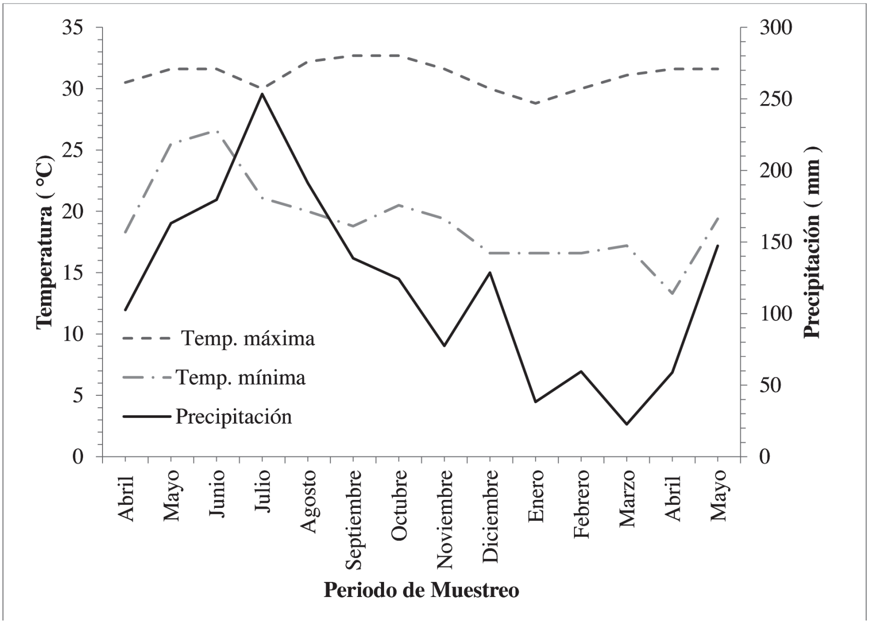

FIGURA 2. Precipitación y temperatura promedio mensual durante el periodo de muestreo en la subestación de Gurabo, Puerto Rico, de abril 2013 a mayo 2014.

La altura de la zarza negra a las 14 semanas después de germinación (SDG) fue de 1.34 m. Bajo las condiciones climáticas existentes, la planta aumentó en altura a razón de 0.05 m por día (Figura 3). Durante un año de crecimiento, alcanzó una altura máxima de 6.66 m en promedio por planta. El diámetro del tallo observado a las 14 SDG fue de 1.4 cm y alcanzó un máximo de 10.6 cm a las 71 SDG. El diámetro aumentó a razón de 0.20 cm cada 22 días (tres semanas) en promedio por planta (Figura 3).

Hasta las 46 SDG, la materia seca de hojas y tallos fue consistentemente menor de 200 g (Figura 4). Luego de este periodo, la materia seca de las hojas y tallos aumentó hasta alcanzar los 300 g y 1,500 g, respectivamente. Estos valores se alcanzaron luego de las 26 semanas de edad de la planta. A las 26 semanas de edad, la zarza negra alcanzó los 29.1 g de materia seca en promedio por planta. Los patrones de aumento de peso seco de hojas y tallos fueron similares uno del otro, lo cual indica que durante este estudio, las plantas de zarza negra se mantuvieron en constante crecimiento.

En la Figura 5 se muestra una correlación significativa (R^2=0.87) entre el crecimiento relativo promedio (g por semana) y la edad de la planta (SDG). Al momento de culminar este estudio en mayo 2014, se observó que las plantas de zarza negra continuaban creciendo y desarrollándose. La tasa de crecimiento relativo en promedio para zarza negra en Puerto Rico fue de 6.80 g por semana (Figura 5). En nuestro estudio pudimos observar que esta planta se desarrolló lentamente y no es hasta las 38 semanas de edad que mostró un desarrollo agresivo. Este comportamiento se puede atribuir a que hubo dos periodos en donde la precipitación fue mayor durante los

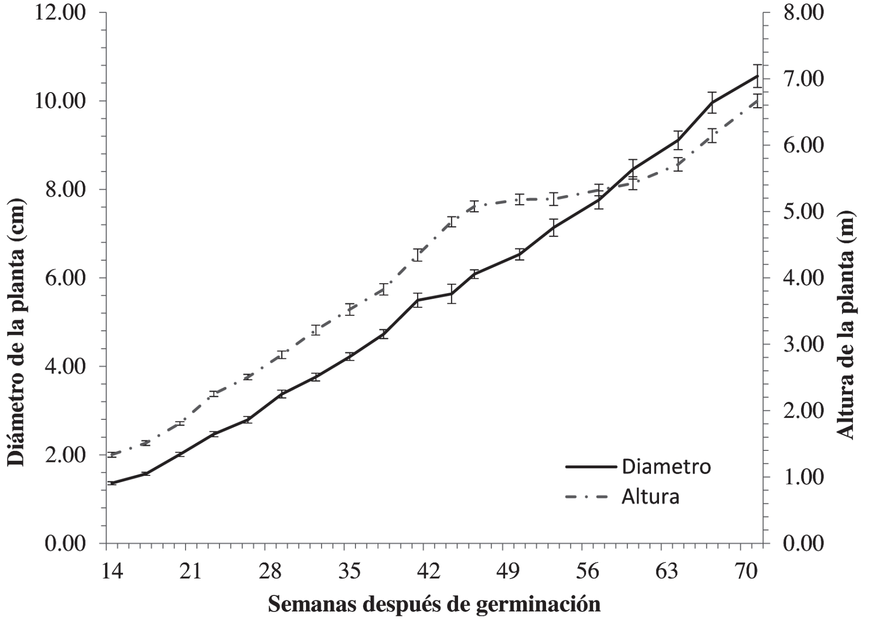

FIGURA 3. Diámetro y altura de la zarza negra según la edad del arbusto (los valores representan medias ± 1 ES para cada semana después de la germinación).

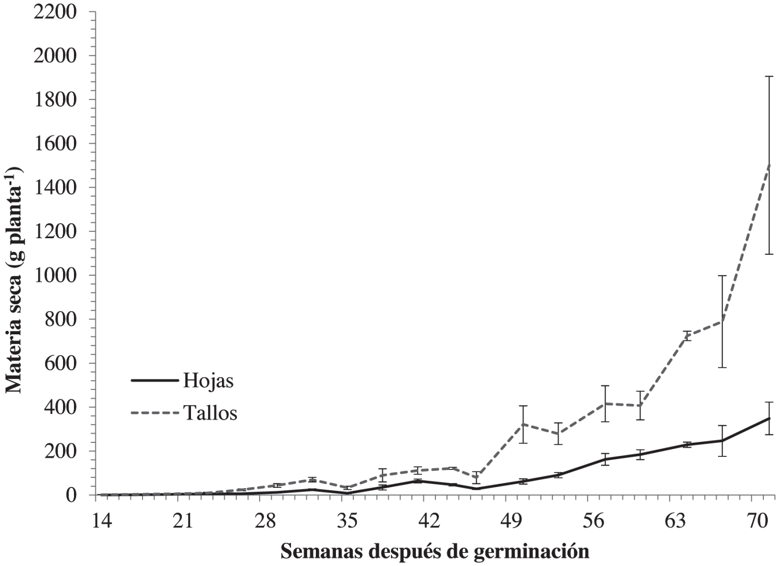

FIGURA 4. Materia seca de hojas y tallos de zarza negra según la edad de la planta (los valores representan medias ± 1 ES para cada semana después de germinación).

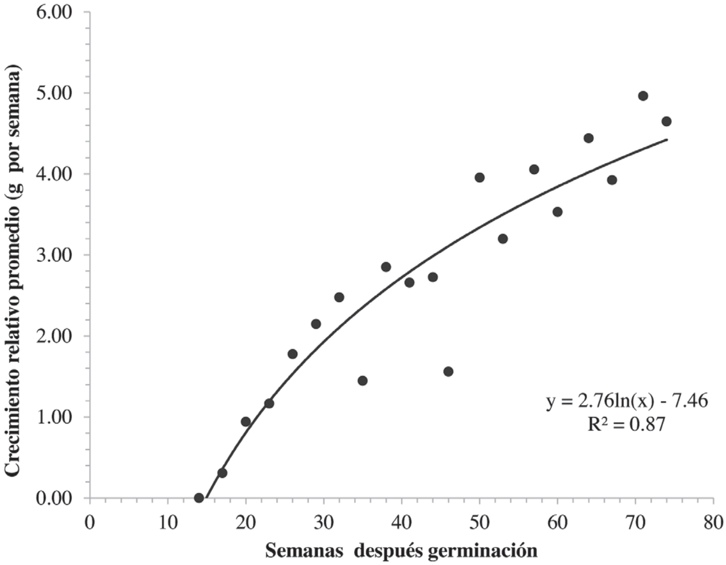

FIGURA 5. Tasa de crecimiento relativo (TCR) promedio semanal para la zarza negra creciendo en la zona este de Puerto Rico.

periodos de muestreo (Figura 2). Consecuentemente, la zarza negra respondió a estos cambios aumentando su materia seca de hoja y tallo.

Según las observaciones de campo, a las 26 SDG las plantas comenzaron a florecer. La producción inicial fue de menos de cinco inflorescencias por planta y continuó aumentando hasta alcanzar 53 inflorescencias a las 71 SDG (Figura 6).

La producción de vainas comenzó a observarse a partir de las 29 SDG. Cada 22 días se observó una producción de tres vainas por planta (Figura 6). Luego, desde las 35 SDG se observó un aumento en la producción de vainas. Este aumento coincidió con el de la producción de inflorescencias durante el periodo observado. No fue hasta luego de las 67 SDG que la zarza negra comienza otro periodo de producción de vainas en respuesta al aumento en la producción de inflorescencias de las 64 SDG (Figura 6).

Luego de la producción de flores, se comenzó a observar la producción de vainas, que a su vez propicia la producción de semillas. La producción de semillas comenzó a observarse a las 35 SDG. La etapa de producción máxima de semillas comenzó desde 41 SDG hasta 52 SDG, donde se colectaron desde 183 hasta 319 semillas por metro cuadrado en promedio por planta (Figura 6). A las semillas recolectadas en este estudio se les determinó el porcentaje de germinación según la edad de la planta. Estas semillas estuvieron en un invernadero a un rango de temperatura de 26 a 28 °C (Figura 7).

En la Figura 8 se observan fluctuaciones en germinación. Las semillas colectadas de plantas jóvenes (<46 SDG) demostraron menos de un 20% de germinación. En contraste, las semillas colectadas de plantas de 50 a 53 SDG, así como de aquellas de 71

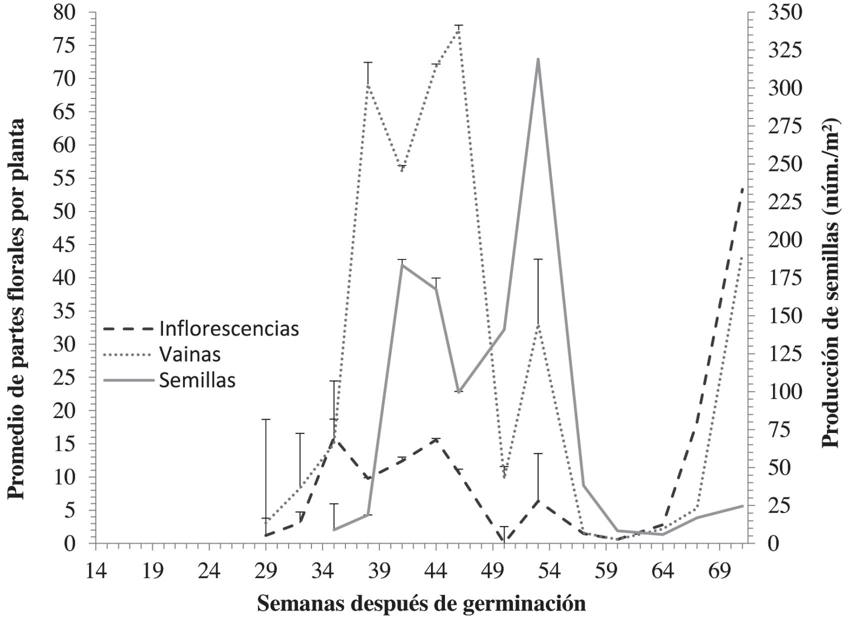

FIGURA 6. Producción de inflorescencias, vainas y semillas (número por m^2) de zarza negra según la edad de la planta en semanas después de la germinación (los valores representan medias ± 1 ES para cada semana después de la germinación).

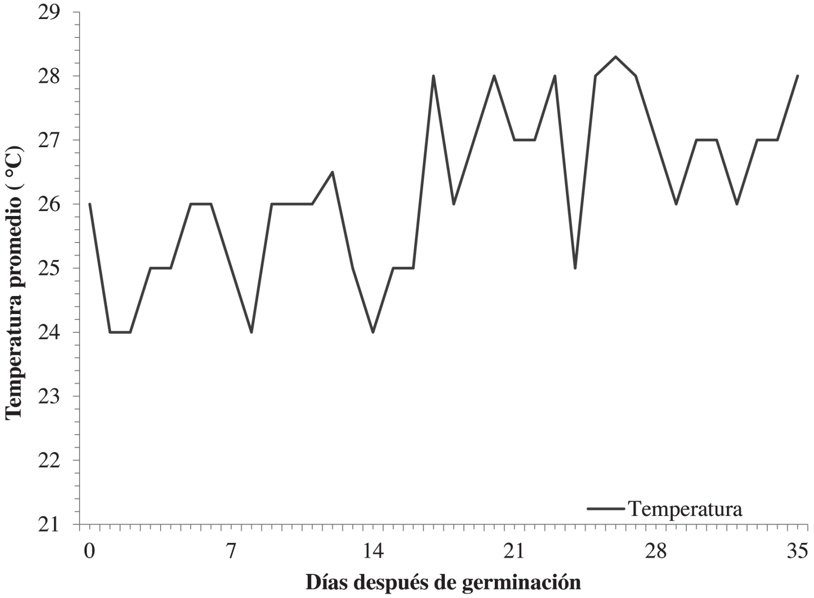

FIGURA 7. Temperatura promedio diaria del invernadero durante el periodo de germinación de la zarza negra.

SDG, mostraron mayor germinación, alcanzando valores de hasta 45%. En el periodo de 57 a 67 semanas de edad de la planta se observa una disminución en el porcentaje de germinación, aquí hubo un segundo periodo de producción de flores. Además, se observa que el porcentaje de germinación aumenta según aumenta el periodo de evaluación. A las cinco semanas de ser plantadas se alcanzó el mayor porcentaje de germinación (Figura 8). Estos valores son superiores a los reportados por Plaza (2014) en una población natural de zarza negra en la Laguna Cartagena en Lajas, donde la germinación de las semillas alcanzó solo un 34% de germinación a las doce semanas de ser plantadas. Estas diferencias se pueden atribuir a que las semillas colectadas por Plaza (2014) fueron recogidas del suelo sin conocer el tiempo que llevaban sobre el mismo, mientras que las semillas de este estudio fueron colectadas en mallas al momento de ser liberadas por la planta.

En un estudio realizado por Sheded y Hassan en Egipto (1999), las semillas de la zarza negra escarificadas mecánicamente y expuestas a temperaturas de entre 15 y 40 °C lograron alcanzar una tasa de germinación de un 100 por ciento. En nuestro estudio, las semillas que se sembraron no se escarificaron, y se alcanzó un 45 por ciento de germinación. Esto indica que la escarificación de la semilla podría ser un factor importante en la germinación.

El presente estudio confirma que la zarza negra tiene un crecimiento y desarrollo agresivo en la zona este de Puerto Rico. Al igual que en Gurabo, municipios localizados en la zona ecológica de bosque húmedo subtropical son hábitat susceptibles a la colonización de la zarza negra. Se recomienda a los municipios en esta zona que producen ganado de leche y forraje, utilizar los hallazgos del presente estudio para establecer un plan de manejo de la zarza negra.

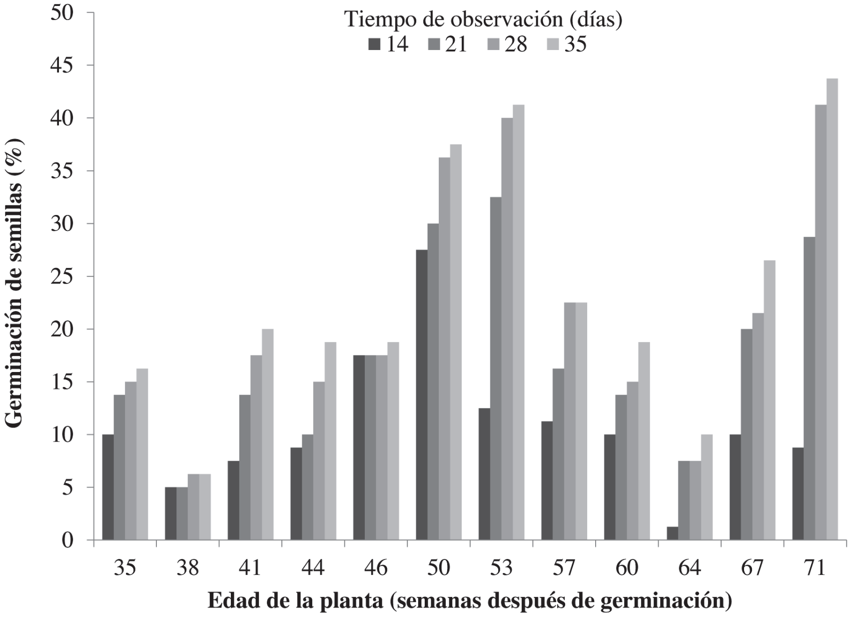

FIGURA 8. Porcentaje de semillas de zarza negra germinadas de acuerdo a su tiempo de observación, según la edad de la planta en semanas después de la germinación.

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Research Note

EVALUATION OF A COMMERCIAL ACCELEROMETER FOR REMOTE MONITORING OF LYING AND STANDING EVENTS IN DAIRY CALVES IN PUERTO RICO[1,2]

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Behavior monitoring provides important information about the impact of environment (Bonk et al., 2013), food (DeVries et al., 2003; González et al., 2008; Nielsen, 2013; Rayas-Amor et al., 2017) and water intake (Lukas et al., 2008; McDonald et al., 2019), welfare (Müller and Schrader, 2003), health (González et al., 2008; Lukas et al., 2008) and comfort status (Lomb et al., 2018) on dairy cattle. Thus, two methods have been commonly used to evaluate animal behavior for scientific research: direct visual observations and video footage analysis (Nielsen et al., 2010). However, both monitoring systems are time consuming (Ledgerwood et al., 2010; Bonk et al., 2013; Nielsen, 2013) and labor consuming (Ito et al., 2009; Ledgerwood et al., 2010), which limits considerably their feasibility. For these reasons livestock research has incorporated automated tools, such as data loggers, to remotely monitor animal behavior (Ledgerwood et al., 2010; Bonk et al., 2013).

Even though multiple studies have confirmed the effectiveness of using data loggers as indicators for lying events in mature dairy cows (Ito et al., 2009; Ledgerwood et al., 2010; Swartz et al., 2016), to our knowledge there is only one study (Bonk et al., 2013) that has validated these sensors for the study of lying activity in Holstein calves. However, the Bonk et al. (2013) study was performed in Germany, a country with very different environmental conditions than Puerto Rico (temperate vs. tropical weather, respectively). Thus, although their data loggers' validation may be highly useful as a guide when evaluating similar behavior in other countries, their results may not be directly extrapolated to Puerto Rico's environmental conditions.

Since Puerto Rico is located in the tropical region, the chronic nature of its high relative humidity and environmental temperature (Daly et al., 2003; PRCCC, 2013) can lead to heat stress in dairy cattle (West, 2003; Chen et al., 2013). This problem is exacerbated because Holstein cattle, the most common dairy breed on the island (Cortés et al., 2010), are highly adapted to temperate climates (Javed et al., 2004). This is important because heat stress has been reported to significantly affect cattle behavior, including their lying and standing patterns (Schütz et al., 2010; Chen et al., 2013; Polsky and von Keyser-

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lingk, 2017). Given that the results reported by Bonk et al. (2013) may not be representative for animals raised under tropical conditions, this study aimed to evaluate the use of the aforementioned data loggers (i.e., accelerometers) for the remote monitoring and study of lying and standing behavior in Puerto Rican dairy calves.

Five pre-weaned Puerto Rican Holstein heifers (53±20-day-old) from the Agricultural Experiment Station dairy herd at Lajas (Puerto Rico) were evaluated. Calves were individually housed (from birth) in pens with wire mesh floors and galvanized steel side panels located in the calves' barn at the dairy facilities. Ad libitum access to starter feed and water was provided during the study. During data collection each calf was fed approximately three liters of milk at around 0800 h, as part of the normal farm management practices. Each calf pen was 122 cm long and 46 cm wide.

A HOBO pendant G accelerometer (which records the g- force on the x-, y- and z-axis; Onset Computer Corporation, Bourne, MA)[6] was tied to the right hind leg of each calf, making sure that its y-axis was perpendicular to the ground when the calves were standing (Figure 1). Using two plastic cable ties (20.32 cm in length; Commercial Electric, Thailand), these sensors were attached to a previously perforated 3.81 x 30.48 cm flagger leg band (Nasco, Fort Atkinson, WI). To avoid any friction that the sensor may cause on the leg skin, a Vet Wrap bandage (Co-Flex, Andover Healthcare, Salisbury, MA) was placed three times around the sensor and leg band. The data loggers were programmed to collect g- force values on the y-axis at one-second intervals from 0700 to 0836 h in one day. Data loggers were installed on the calves' legs at 0600 h with no sign of associated distress being observed. One technician per calf was assigned to visually collect the specific time periods the calves were lying or standing during sampling. These visually collected lying and standing events were used as the gold standards (or standards for comparison) for the evaluation of the data loggers' recordings as possible indicators of these behaviors.

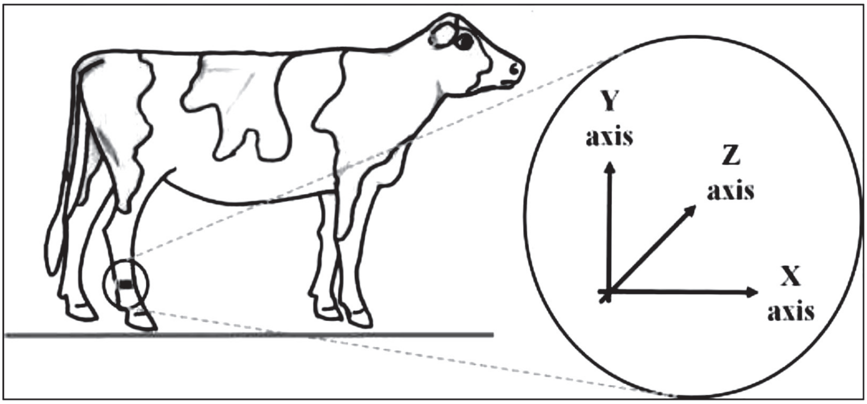

FIGURE 1. Image illustrating the placement of the HOBO Pendant G data logger on the calf's leg. Note that the data logger was placed so the y-axis points towards the calf's thurl, perpendicular to the floor surface.

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[6] 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.

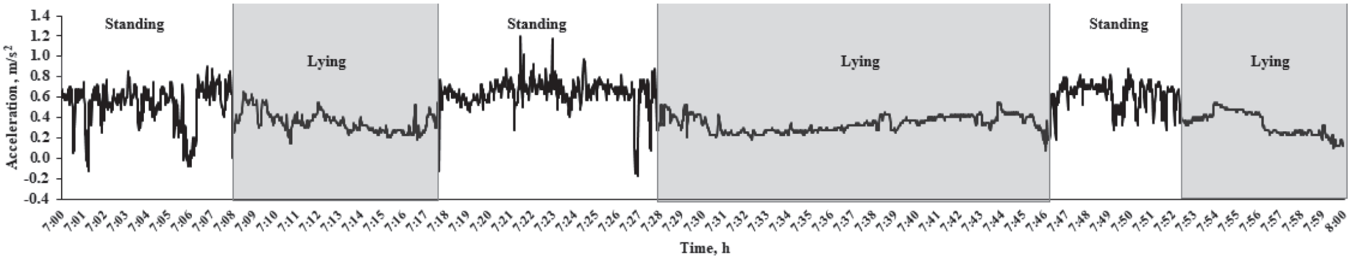

FIGURE 2. Illustration of the y-axis acceleration values recorded from 0700 to 0800 h by the HOBO Pendant G data logger on calf # 2231. The white spaces represent the time spent standing by the calf, while the gray boxes identify the time spent lying.

In order to classify the y-axis acceleration values recorded by the HOBO Pendant G accelerometers as lying or standing events, the overall mean and standard deviation values during the respective visually recorded events were calculated. The transitional movements between the lying and standing events were excluded from the data set. Using the obtained mean and standard deviation for each visually recorded behavior (i.e., lying and standing), a series of respective accelerometer categories were created, including the acceleration values ranging between 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations below and above the mean (Table 1). Using the IF-THEN statement in SAS, a dataset containing columns with all the created categories was made. The FREQ procedure in SAS was used to determine the probability that a visually recorded event was successfully classified by the data logger categories (sensitivity; Bonk et al., 2013), as well as the visual-accelerometer misclassifications and observations not receiving a classification in each category. The visual-accelerometer misclassifications included situations where a visually recorded lying event was classified as standing by the created category (lying-standing misclassification) or when a visually recorded standing event was classified as lying (standing-lying misclassification). Data outside the created ranges failed to be categorized and are referred to as non-classified data. Four more data sets were created for evaluating larger sampling intervals, including 30 seconds, as well as one, two, and five minutes. The IF-THEN statement of SAS was used to temporarily eliminate the undesired data in each new sampling interval.

Tables 2 to 6 present the sensitivities, misclassifications and non-classified values in each evaluated category and sampling interval. At the one-second sampling interval, all evaluated categories presented sensitivity values exceeding 99% (Table 2). However, the smaller the created classification category, the larger the amount of missed data due to non-classified values (from 0.46 to 55% of non-classified values in the 2.6 to 0.4 standard deviation categories, respectively). Thus, the categories that better classified the calves' behavior when sampling every second were 2, 2.2, 2.4, and 2.6 standard deviations below and above the mean. There, the lying and standing events were successfully

TABLE 1.—*Lying and standing categories with their y-axis acceleration values.*

Created Category[1] (Standard Deviations)	Lying		Standing	
	Minimum	Maximum	Minimum	Maximum
0.4	0.06741912	0.17132488	-1.05276992	-0.94686968
0.6	0.04144268	0.19730132	-1.07924498	-0.92039462
0.8	0.01546624	0.22327776	-1.10572004	-0.89391956
1	-0.01051020	0.24925420	-1.13219510	-0.86744450
1.2	-0.03648664	0.27523064	-1.15867016	0.84096944
1.4	-0.06246308	0.30120708	-1.18514522	-0.81449438
1.6	-0.08843952	0.32718352	-1.21162028	-0.78801932
1.8	-0.11441596	0.35315996	-1.23809534	-0.76154426
2	-0.14039240	0.37913640	-1.26457040	-0.73506920
2.2	-0.16636884	-0.40511284	-1.29104546	-0.70859414
2.4	-0.19234528	0.43108928	-1.31752052	-0.68211908
2.6	-0.21832172	0.45706572	-1.34399558	-0.65564402

[1] The overall acceleration mean and standard deviations for the standing (-0.2338588±0.0831065) and lying (0.1820495±0.2282673) events were calculated and used to create a series of respective classification categories ranging between 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations below and above the mean.

TABLE 2.—*Probability that a visually recorded event was successfully classified by the data logger categories (sensitivity) of a commercial data logger as an indicator of lying and standing activity in five pre-weaned Holstein heifers. Data were recorded at 1-second intervals from 0700 to 0836 h (N=23,408) during one morning.*[1]

	Visual Observations: Lying	*Visual Observations:* Standing	*Visual Observations:* Lying	*Visual Observations:* Standing	
Y-Axis[2]	*Data Logger:* Lying %	*Data Logger:* Standing %	*Data Logger:* Standing %	*Data Logger:* Lying %	Frequency Missing
± 0.4 SD	99.95	100.00	0	0.05	12866
± 0.6 SD	99.92	100.00	0	0.08	9636
± 0.8 SD	99.92	100.00	0	0.08	7849
± 1 SD	99.90	100.00	0	0.10	6735
± 1.2 SD	99.92	100.00	0	0.08	3468
± 1.4 SD	99.91	99.99	0.01	0.09	2202
± 1.6 SD	99.91	99.99	0.01	0.09	892
± 1.8 SD	99.90	99.98	0.02	0.10	705
± 2 SD	99.89	99.98	0.02	0.11	287
± 2.2 SD	99.88	99.97	0.03	0.12	139
± 2.4 SD	99.86	99.97	0.03	0.14	122
± 2.6 SD	99.85	99.97	0.03	0.15	109

[1] Only data with a definite visually recorded behavior (lying or standing) were used for the statistical analysis.

[2] The data logger categories were created in the ranges of 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations (SD) below and above the mean.

classified by the data loggers close to 100% of the time, while missing less than 1.24% of the dataset due to non-classifications. However, sampling at 1-second intervals will only allow for six hours of data collection due to the memory limitations of the sensors. As a result, multiple researchers have used larger intervals to study similar behaviors in cattle (Ledgerwood et al., 2010). Thus, the evaluation of greater sampling intervals is required. Sampling times of 30 seconds, one, two, and five minutes allow for data storage capacities of 7.5, 15.1, 30.1, and 75.3 days, respectively.

For the 30-second measuring interval, all categories reported sensitivity values above 99% (Table 3). Nevertheless, the smaller the data logger classification category, the larger the amount of missed data as a result of non-classified values (from 0.38 to 54% of non-classified values for the 2.6 to 0.4 standard deviations below and above the mean categories, respectively). For this reason, the categories that best represented the lying and standing events were also those using 2, 2.2, 2.4 and 2.6 standard deviations below and above the mean. In such categories the data loggers successfully identified the lying and standing events close to 100% of the time, while the misclassified values represented less than 1.15% of the dataset.

In the one-minute sampling interval all the lying and standing events were accurately identified (sensitivities of 100%) for all the evaluated categories (Table 4). Therefore, the determination of the most efficient classifications was focused on the amount of missing data due to non-classified values. Such categories were also the 2, 2.2, 2.4 and 2.6 standard deviations below and above the mean, which only presented less than 1.54% of non-classified data.

As previously mentioned, increasing the sampling interval to two or five minutes will allow for considerably greater sampling periods. However, as stated in Table 5, when data was collected every two minutes, a considerable decrease in the sensitivity of the evaluated classification categories was observed, only ranging between 70 to 91%. Even in the 2, 2.2, 2.4, and 2.6 standard deviations classifications, where only less than 2.6% of the data was lost due to non-classified values, the observed sensitivity ranged between 74 and 87%.

Similar to the sampling interval of two minutes (Table 5), the collection of data every five minutes (Table 6) resulted in a decline in the sensitivity of the evaluated classifications, when compared to the sampling intervals of one second (Table 2), 30 seconds (Table 3), and one minute (Table 4). In the five-minute sampling interval (Table 6), even when the sensitivity of classifying the lying events reached 100% in the 0.4, 0.6, and 0.8 standard deviations below and above the mean categories, the respective misclassifications ranged between 12 and 18%. Moreover, when using such categories, 37 to 55% of the data was lost due to non-classified values. Even in the categories from 1.6 to 2.6 standard deviations below and above the mean, where only less than 2.7% of the data was lost due to non-classifications, the obtained sensitivities only ranged between 84.38 and 93.48%.

The evaluated sensors were able to accurately identify the calves' lying and standing events when the sampling interval was maintained below or equal to one minute (Tables 2, 3, and 4). These findings are in agreement with the lying activity studies carried out by Bonk et al. (2013) in dairy calves, as well as Ito et al. (2009) and Ledgerwood et al. (2010) in dairy cows. Rayas-Amor et al. (2017) reported a similar trend when evaluating grazing activity with the same data loggers in dairy cows. Moreover, even when all the evaluated sampling intervals below or equal to one minute showed sensitivities exceeding 99% (Tables 2, 3, and 4), only the one-minute interval showed sensitivity values of 100%, suggesting this is the most accurate sampling interval. These results are similar to those reported by Bonk et al. (2013), Ito et al. (2009), and Ledgerwood et al. (2010). However, it is important to consider that going from a sampling interval of 30 seconds

TABLE 3.—*Probability that a visually recorded event was successfully classified by the data logger categories (sensitivity) of a commercial data logger as an indicator of lying and standing activity in five pre-weaned Holstein heifers. Data were recorded at 30-second intervals from 0700 to 0836 h (N=780) during one morning.*[1]

	Visual Observations: Lying	*Visual Observations:* Standing	*Visual Observations:* Lying	*Visual Observations:* Standing	Frequency Missing
Y-Axis[2]	*Data Logger:* Lying %	*Data Logger:* Standing %	*Data Logger:* Standing%	*Data Logger:* Lying%	
± 0.4 SD	99.34	100.00	0.00	0.66	425
± 0.6 SD	99.50	100.00	0.00	0.50	321
± 0.8 SD	99.52	100.00	0.00	0.48	264
± 1 SD	99.57	100.00	0.00	0.43	221
± 1.2 SD	99.70	100.00	0.00	0.30	112
± 1.4 SD	99.73	100.00	0.00	0.27	69
± 1.6 SD	99.76	100.00	0.00	0.24	24
± 1.8 SD	99.76	99.70	0.30	0.24	19
± 2 SD	99.77	99.70	0.30	0.23	9
± 2.2 SD	99.77	99.71	0.29	0.23	4
± 2.4 SD	99.77	99.71	0.29	0.23	3
± 2.6 SD	99.77	99.71	0.29	0.23	3

[1] Only data with a definite visually recorded behavior (lying or standing) were used for the statistical analysis. [2] The data logger categories were created in the ranges of 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations (SD) below and above the mean.

TABLE 4.—*Probability that a visually recorded event was successfully classified by the data logger categories (sensitivity) of a commercial data logger as an indicator of lying and standing activity in five pre-weaned Holstein heifers. Data were recorded at 60-second intervals from 0700 to 0836 h (N=390) during one morning.*[1]

	Visual Observations: Lying	*Visual Observations:* Standing	*Visual Observations:* Lying	*Visual Observations:* Standing	
Y-Axis[2]	*Data Logger:* Lying%	*Data Logger:* Standing%	*Data Logger:* Standing%	*Data Logger:* Lying%	Frequency Missing
± 0.4 SD	100.00	100.00	0.00	0.00	211
± 0.6 SD	100.00	100.00	0.00	0.00	158
± 0.8 SD	100.00	100.00	0.00	0.00	133
± 1 SD	100.00	100.00	0.00	0.00	112
± 1.2 SD	100.00	100.00	0.00	0.00	55
± 1.4 SD	100.00	100.00	0.00	0.00	34
± 1.6 SD	100.00	100.00	0.00	0.00	14
± 1.8 SD	100.00	100.00	0.00	0.00	12
± 2 SD	100.00	100.00	0.00	0.00	6
± 2.2 SD	100.00	100.00	0.00	0.00	3
± 2.4 SD	100.00	100.00	0.00	0.00	2
± 2.6 SD	100.00	100.00	0.00	0.00	2

[1] Only data with a definite visually recorded behavior (lying or standing) were used for the statistical analysis. [2] The data logger categories were created in the ranges of 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations (SD) below and above the mean.

TABLE 5.—*Probability that a visually recorded event was successfully classified by the data logger categories (sensitivity) of a commercial data logger as an indicator of lying and standing activity in five pre-weaned Holstein heifers. Data were recorded at 2-minute intervals from 0700 to 0836 h (N=195) during one morning.*[1]

	Visual Observations: Lying	*Visual Observations:* Standing	*Visual Observations:* Lying	*Visual Observations:* Standing	
Y-Axis[2]	*Data Logger:* Lying%	*Data Logger:* Standing%	*Data Logger:* Standing%	*Data Logger:* Lying%	Frequency Missing
± 0.4 SD	90.70	70.00	30.00	9.30	102
± 0.6 SD	89.09	72.58	27.42	10.91	78
± 0.8 SD	89.47	75.36	24.64	10.53	69
± 1 SD	88.89	76.39	23.61	11.11	60
± 1.2 SD	86.52	76.71	23.29	13.48	33
± 1.4 SD	86.73	75.00	25.00	13.27	21
± 1.6 SD	86.11	75.00	25.00	13.89	11
± 1.8 SD	86.24	75.00	25.00	13.76	10
± 2 SD	86.73	74.03	25.97	13.27	5
± 2.2 SD	86.84	74.03	25.97	13.16	4
± 2.4 SD	86.84	74.03	25.97	13.16	4
± 2.6 SD	86.09	74.03	25.97	13.91	3

[1] Only data with a definite visually recorded behavior (lying or standing) were used for the statistical analysis. [2] The data logger categories were created in the ranges of 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations (SD) below and above the mean.

TABLE 6.—*Probability that a visually recorded event was successfully classified by the data logger categories (sensitivity) of a commercial data logger as an indicator of lying and standing activity in five pre-weaned Holstein heifers. Data were recorded at 5-minute intervals from 0700 to 0836 h (N=78) during one morning.*[1]

	Visual Observations: Lying	*Visual Observations:* Standing	*Visual Observations:* Lying	*Visual Observations:* Standing	
Y-Axis[2]	*Data Logger:* Lying%	*Data Logger:* Standing%	*Data Logger:* Standing%	*Data Logger:* Lying%	Frequency Missing
± 0.4 SD	100.00	85.00	15.00	0.00	43
± 0.6 SD	100.00	88.00	12.00	0.00	33
± 0.8 SD	100.00	82.14	17.86	0.00	29
± 1 SD	95.83	83.87	16.13	4.17	23
± 1.2 SD	94.12	84.38	15.63	5.88	12
± 1.4 SD	92.50	84.38	15.63	7.50	6
± 1.6 SD	93.18	84.38	15.63	6.82	2
± 1.8 SD	93.18	84.38	15.63	6.82	2
± 2 SD	93.48	84.38	15.63	6.52	0
± 2.2 SD	93.48	84.38	15.63	6.52	0
± 2.4 SD	93.48	84.38	15.63	6.52	0
± 2.6 SD	93.48	84.38	15.63	6.52	0

[1] Only data with a definite visually recorded behavior (lying or standing) were used for the statistical analysis. [2] The data logger categories were created in the ranges of 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 standard deviations (SD) below and above the mean.

to 60 seconds (Tables 3 and 4) will result in the loss of a considerable amount of behavior recordings (including misclassified data), subsequently giving a false idea of greater sensitivity. Similarly, the limitation of the sampling intervals of two and five minutes, which obtained considerably lower sensitivity values (Tables 5 and 6), may be the loss of important behavior data due to the increased sampling interval. In fact, Ito et al. (2009) suggested that the greater the data collection frequency (smaller sampling interval), the greater the accuracy in behavior identification.

The present study validated the use of accelerometers as viable tools for the assessment of lying and standing behavior in dairy calves raised in the tropics (Puerto Rico). The evaluated sensors were able to successfully identify the lying and standing events when the sampling interval was maintained ≤ one minute, and the classification category included the acceleration values between 2 to 2.6 standard deviations below and above the mean. Inside these values, the ideal sampling interval will depend on the required duration of the study, since more frequent sampling implies a shorter memory life of the sensor. Future studies should be directed towards evaluating the feasibility of these sensors as indicators of other behaviors in cattle raised in the tropics.

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Feature

Contributions in Puerto Rico to Bean, *Phaseolus* spp., Research[1,2]

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Most literature reviews focus on a specific topic. The purpose of this paper, however, is to review the contributions made by a multidisciplinary team of researchers at a specific location (Puerto Rico) over a period of several decades. This paper documents bean research of the University of Puerto Rico and the USDA-ARS Tropical Agriculture Research Station during the past century. The following illustrates the merits of continuity of effort in research and shows that research often follows a logical sequence of actions to solve problems related to genetic improvement as well as to study biotic and abiotic factors that affect common bean production in Central America and the Caribbean. Finally, this narrative demonstrates that the ongoing development of improved bean germplasm lines and cultivars is cyclical and builds upon previous research achievements.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an important component of the traditional diet in Puerto Rico. The 1900 report of the USDA Puerto Rico Agricultural Experiment Station (USDA-PRAES) noted that rice and beans were staple crops in Puerto Rico and found on the tables of both the rich and poor. Smit et al. (2007) noted that annual per capita consumption of grain legumes, mostly dry beans, in

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Puerto Rico (6.4 kg) is almost double the per capita consumption in the U.S. (3.4 kg; US Dry Bean Council, 2019).

Common and lima beans (*Phaseolus lunatus* L.) have been produced in the Caribbean for at least 500 years. The grain legume fits well into rotations and is often intercropped with other longer season crops such as plantains and bananas. Small white beans, locally known as 'Blanca del País' and striped pink beans known as 'Colorado del País' are traditional market classes in Puerto Rico (Koenig, 1953). Consumers in Puerto Rico today consume a wide array of market classes including white, red, pink, pinto, black and kidney beans (Bean Institute, 2018). Although Puerto Ricans represent roughly 1% of the U.S. population, they consume 3.1% of the common and lima beans in the U.S. (Lucier et al., 2000).

Snyder noted in the 1916 report of the USDA-PRAES that 47,200 ha of beans and cowpeas were planted (Snyder, 1918). Approximately 50% of the plantings were white beans, 25% were red beans and 25% were cowpeas [*Vigna unguiculata* (L.) Walp]. Picó (1937) reported that in 1929, a total of 13,360 ha of dry beans were planted in Puerto Rico. In 1950-1951, dry beans were harvested on the island from 11,429 ha, although seed yields only averaged 375 kg/ha (Koenig, 1953).

Puerto Rico has imported grain legumes for over 100 years. In 1891, Puerto Rico imported from the U.S. 15,751 bushels of beans and peas having a value of $28,497 (U.S. Treasury Dept., 1893). Almost all the dry beans currently consumed in Puerto Rico are imported, although the potential for increased local production exists. Using recommended practices and improved cultivars, it is currently feasible to produce an average bean seed yield of 2,000 kg/ha. Therefore, it would be possible to produce on approximately 2,000 ha the same amount of dry beans that was produced in Puerto Rico in 1950-1951. Most beans in Puerto Rico are currently harvested near physiological maturity as green-shelled beans. In 2012, a total of 573 farms reported planting green-shelled or dry beans on 320 ha resulting in a production of 301 MT (USDA-NASS, 2012).

González-Ríos and Riollano (1951) noted that the evaluation of bean germplasm in Puerto Rico was initiated by McClelland in 1918. Nineteen bean cultivars from the U.S., the Dominican Republic, Venezuela and Puerto Rico were evaluated at the USDA-PRAES. Cultivars from the tropics were reported to be better adapted than cultivars from the U.S.; 'Blanca del País' and 'Negra de Venezuela' were among the most productive cultivars. González-Ríos and Riollano (1951) reported that in 1922 white bean breeding lines were developed from crosses between those cultivars. Seed of the most promising breeding lines from those crosses were distributed to farmers in Puerto Rico. After 1923, the focus of bean research at the USDA-PRAES in Mayagüez switched to snap bean improvement.

In the 1930s, the University of Puerto Rico Agricultural Experiment Station (UPR AES) initiated a project to improve the yield potential and quality of white and striped pink bean cultivars for Puerto Rico (González-Ríos and Riollano, 1951). Seed samples of bean landraces were obtained from farmers. The collection of bean landraces was initially evaluated in plant rows. Between 1938 and 1941, approximately 2,000 selections were evaluated. Three lines were identified as having superior seed yield and agronomic characteristics. During the first year of World War II, 2,250 kg of seed of these lines were distributed to farmers to help alleviate food shortages. In 1945, seven other lines were selected for evaluation in yield trials. The selection No. 1632, which had the highest seed yield potential, was released as 'Bonita' (González-Ríos and Riollano, 1951). This remained the standard white bean variety in Puerto Rico until the 1980s.

Walter J. Kaiser and Nader Vakili, plant pathologists, were transferred from the USDA-ARS Regional Pulse Improvement Project in Tehran, Iran, to the USDA-ARS Mayagüez Institute of Tropical Agriculture (ARS-MITA) in the early 1970s. During the 1970s, the University of Puerto Rico and the ARS-MITA participated in a collaborative research project supported by the U.S. Agency for International Development (USAID). The project was entitled "Improvement of Tropical Production of Beans and Cowpeas through Disease and Insect Control". Nader Vakili and George Freytag, USDA-ARS plant geneticist, developed and released more than 25 improved bean germplasm lines (http://arsftfbean.uprm.edu/bean/?p=893). The release of improved germplasm by USDA-ARS bean research geneticists in Puerto Rico has continued to date (Table 1).

Freytag maintained and evaluated a collection of many different species of the genus *Phaseolus*. These evaluations were used in the development of the comprehensive book titled "Taxonomy, Distribution, and Ecology of the Genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America", which was published in collaboration with Daniel Debouck, curator of the bean germplasm collection at the International Center for Tropical Agriculture (CIAT) (Freytag and Debouck, 2002).

In 1981, The University of Puerto Rico Agricultural Experiment Station and the USDA ARS-MITA, renamed Tropical Agriculture Research Station (ARS-TARS), received a grant from the Bean/Cowpea CRSP (Collaborative Research Support Program) which was financed by USAID. The objective of this research and training project was to improve bean research capabilities in Honduras and the Dominican

TABLE 1.—*List of cultivars and germplasm released by the USDA-ARS-Tropical Agriculture Research Station in collaboration with the University of Puerto Rico (1973-2014).*

Cultivar, germplasm	Seed type	Year	Traits	Participating institutions	Reference
La Vega (MA)	Black	1973	Slow rusting, root rot	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
Violeta (Andean)	Purple speckled	1976	BCMV, CMV, root rot	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
Palmarejo (Andean)	Maroon and pink mottled	1976	Rust, BCMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
Oro Rico (MA)	Pink	1976	BCMV, Rhizoctonia root rot	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
Congo Rico (Andean)	Pink striped	1976	BCMV, tolerant to BGYMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
11 germplasms (*P. coccineus*)		1979	Root rot, rust, ALS, powdery mildew, Cercospora, CBB, CMV, BCMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
MITA-76-R287 (MA)	Black	1979	Rust, BCMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
MITA-RSP-1,-2,-3,-4 (MA)	White, Black, Cream speckled, Tan	1979	Rust	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
Chorro (MA)	Brown	1979	Root rot, ASB, Rust, BCMV, CPMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
Carbon (MA)	Black	1979	Root rot, ASB, Rust, BCMV, Leaf hopper	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
B-128, B-190, B-351, 2B-5-1 (MA)	Dull black and shiny black	1979	Rust, BCMV, upright architecture combinations	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893
W-117, W-142, 2W-33-2 (MA)	Small white	1979	BCMV, slow rusting combinations	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/bean/?p=893

TABLE 1.— (Continued 1) *List of cultivars and germplasm released by the USDA-ARS-Tropical Agriculture Research Station in collaboration with the University of Puerto Rico (1973-2014).*

Cultivar, germplasm	Seed type	Year	Traits	Participating institutions	Reference
MITA-6383	Small white	1979	Rust, CBB, root rot, ASB, BCMV(I), CMV, CPMV, Cercospora	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/ bean/?p=893
XR-235-1-1 Interspecific (Pv/Pc)	Cream	1981	CBB	USDA-ARS-TARS, UPR, U. of Florida	Freytag et al., 1982
4M-99 (Andean)	Red mottled	1983	Root rot, ASB, Rust, BCMV, Leaf hopper	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/ bean/?p=893
3M-150, 3M-152 (Andean)	Cranberry, Kidney	1983	Root rot, ALS, BCMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/ bean/?p=893
Mogote (MA)	Brown	1983	Root rot, ASB, CBB, Rust, Cercospora, BCMV, CPMV, CMV	USDA-ARS-TARS, UPR	http://arsftfbean. uprm.edu/ bean/?p=893
L-266-10, L-227-1 (MA)	Small white	1983	Rust, I gene, Root rot, ASB	USDA-ARS-TARS, UPR, MSU	Freytag et al., 1985
TARS-VCI-4B Interspecific (Pv/Pc)	Small pinto	1994	CBB, Rhizoctonia root rot	USDA-ARS-TARS, UPR, NDSU	Miklas et al., 1994
TARS-HP70, -HP140, -HP146 (MA)	Small white	1994	High protein	USDA-ARS-TARS, UPR	Miklas et al., 1995a
TARS-VR1s, -VR7s, -VR8s (MA)	Small white	1995	BCMV, BCMNV (all strains) I + bc-3	USDA-ARS-TARS, UPR	Miklas et al., 1995b
ICB-3, ICB-6, ICB-8, ICB-10 Interspecific (Pv/Pc)	Black, red, white	1999	CBB, ASB, Rust, I gene	USDA-ARS-TARS, UPR, NDSU	Miklas et al., 1999
TARS-PT03-1 (MA)	Small pinto	2005	CBB, Root rot, ASB	USDA-ARS-TARS, UPR, Agri-Food Canada, U. Tennessee	Smith et al., 2005

Table 1.—(Continued 2) *List of cultivars and germplasm released by the USDA-ARS-Tropical Agriculture Research Station in collaboration with the University of Puerto Rico (1973-2014).*

Cultivar, germplasm	Seed type	Year	Traits	Participating institutions	Reference
TARS-SR05 (MA)	Small red	2007	Root rot, ASB, CBB, BCMV (I)	USDA-ARS-TARS, UPR, Agri-Food Canada, U. Tennessee	Smith et al., 2007
TARS-HT1, HT2 (Andean)	Dark and light red kidney	2010	Heat	USDA-ARS-TARS, UPR, U. Tennessee	Porch et al., 2010
TARS-MST1, SB-DT1 (MA)	Black	2012	Heat, drought, root rot, CBB combinations	USDA-ARS-TARS, UPR, U. Nebraska	Porch et al., 2012
TARS-Tep 22, Tep 32 (*P. acutifolius*)	Small white, yellow	2013	Heat, drought, CBB, rust, architecture	USDA-ARS-TARS, UPR, CSU	Porch et al., 2013a
TARS-LFR1 (MA)	Small red	2014	Root rot, CBB, low fertility	USDA-ARS-TARS, UPR, Cornell U.	Porch et al., 2014a
TARS-LH1 (MA)	Pinto	2020	Leafhopper resistance, drought	USDA-ARS-TARS, UPR, Michigan State U.	Porch et al., 2020

Republic. The Bean/Cowpea CRSP project initially collaborated with researchers at the 'Escuela Agrícola Panamericana' (EAP) in Zamorano, Honduras, and the 'Secretaría de Agricultura' in the Dominican Republic. Graduate training in plant breeding and plant pathology was an important objective of this project and, since 1981, more than 60 students from Central and South America, the Caribbean and the U.S. have received M.S. degree training at the University of Puerto Rico, Mayagüez Campus, with full or partial support from projects funded by USAID (Table 2). A more regional focus evolved as the Bean/Cowpea CRSP project matured. Collaboration with bean researchers in Haiti and Central America was strengthened (Beaver et al., 2003). The University of Puerto Rico and the ARS-TARS bean research programs participated in the development and release of numerous cultivars that are widely used in Central America and the Caribbean and as parental materials in North America (Table 3). The UPR bean research program also received long-term support from Regional Hatch Projects W-150, W-1150, W-2150, W-3150 (https://www.nimss.org/) that facilitated collaborations with *Phaseolus* researchers in the U.S. and led to the development of germplasm and scientific findings. The long-term commitment of the USDA-ARS to support a bean research geneticist at the Tropical Agriculture Research Station provided continuity of effort in the improvement of *Phaseolus* spp. germplasm for the tropics and the United States.

Since the 1970s, disease resistance has been a major focus of bean research in Puerto Rico. Climatic differences during the year and contrasting ecological zones permit bean researchers in the Caribbean to screen for resistance to different diseases and pests in different seasons of the year (Beaver et al., 1985b). Koenig (1953) reported that anthracnose caused by *Colletotrichum lindemuthianum* was the most common bean disease in Puerto Rico in the mid-20th century. Warmer weather patterns and the production of disease-free seed has eliminated this disease as a constraint to bean production in Puerto Rico. Researchers have responded to the emergence of new diseases. The study of virulence patterns of pathogens, the development of improved screening techniques and, in recent years, the development of molecular markers linked to disease resistance genes supported the effort to develop beans with enhanced levels of disease resistance (Beaver and Osorno, 2009). The development of bean germplasm with enhanced levels of tolerance to abiotic stresses, such as high temperature, drought and low soil fertility, has become an important breeding objective for the bean research programs in Puerto Rico. The following sections will summarize the achievements of researchers in Puerto Rico dealing with some of the most important constraints to bean production in the tropics.

TABLE 2.—*List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Carl Didier-Joseph	2018	Haiti	Caracterización morfológica y fisiológica de muestras de semillas de habichuela (*Phaseolus vulgaris* L.) recolectadas en fincas y mercados y de distribuidores de semillas en Haití
Iveth Yassmin Rodríguez	2017	Honduras	Análisis fenotípico y genético de frijol tépari (*Phaseolus acutifolius* A. Gray) para tolerancia a factores bióticos y abióticos
Diego Rodríguez-Ortega	2017	Ecuador	Análisis fenotípico y genético de la resistencia a mancha angular (*Pseudocercospora griseola*) en el cultivo de frijol común (*Phaseolus vulgaris*)
Héctor Martínez-Figueroa	2017	Guatemala	Desarrollo de líneas de frijol (*Phaseolus vulgaris* L.) que combinen resistencia a mustia hilachosa [*Thanatephorus cucumeris* Frank (Donk)], bacteriosis común (*Xanthomonas axonopodis* pv. *phaseoli*) y los virus BGYMV, BCMV y BCMNV
Ana Gabriela Vargas-Palacios	2016	Nicaragua	Estudio de la reacción al Virus del Mosaico Necrótico Común del Frijol (BCNMV) y la habilidad de fijación biológica del nitrógeno (FBN) en frijol tépari (*Phaseolus acutifolius* A. Gray) e introgresión de la FBN al frijol común (*Phaseolus vulgaris* L.)
Giovanni Lorenzo Vázquez	2016	Puerto Rico	Evaluación del ensayo BASE 120 para adaptación al estrés abiótico en habichuela (*Phaseolus vulgaris* L.)
Bernardo Mateo	2016	Dominican Republic	Selección de líneas de frijol (*Phaseolus vulgaris* L.) que combinan resistencia al gorgojo común (*Acanthoscelides obtectus* Say) con resistencia a los virus BGYMV, BCMV y BCMNV

TABLE 2.— (Continue 1) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Marcelino Guachambala-Cando	2013	Ecuador	Estudio de la herencia de los altos niveles de resistencia al tizón bacteriano, causado por *Xanthomonas axonopodis* pv. *phaseoli*, en el frijol común (*Phaseolus vulgaris* L.)
Abiezer González-Vélez	2012	Puerto Rico	Desarrollo de líneas de habichuela (*Phaseolus vulgaris* L.) con resistencia al gorgojo común (*Acanthoscelides obtectus* Say)
Velcy R. Palomino-Lescano	2012	Peru	Análisis Bayesiano de un modelo lineal mixto para medir el impacto del cambio climático en el rendimiento del frijol para el año 2030 a nivel mundial
Monica Mmbui-Martins	2011	Angola	Desarrollo de líneas de frijol (*Phaseolus vulgaris* L.) para Angola con mayores niveles de resistencia a las enfermedades
Antonio Ndengoloka-David	2011	Angola	Estudio de la diversidad fenotípica de germoplasma de Caupí [*Vigna unguiculata* (L.) Walp.] de Angola y un grupo de germoplasma que representa otras regiones del mundo
Suheidy Valentín-Torres	2010	Puerto Rico	ITS-5.8S-rDNA region and disease severity comparison of *Rhizoctonia solani* anastomosis groups isolated from common bean (*Phaseolus vulgaris* L.) at Isabela, Puerto Rico
Luis Ruiz-Quiles	2010	Puerto Rico	Desarrollo de líneas de habichuela (*Phaseolus vulgaris* L.) con resistencia a BCMV, BGYMV y mejor adaptación en suelos bajos en N
Axel Ramírez-Madera	2009	Puerto Rico	Response of common bean (*Phaseolus vulgaris* L.) to Rhizobium inoculation and nitrogen fertilization
Roland Dorcinvil	2009	Haiti	Respuesta agronómica de líneas de habichuela (*Phaseolus vulgaris* L.) a la fertilidad de un Oxisol

TABLE 2.— (Continued 2) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Luwbia Aranda	2007	Bolivia	Desarrollo de métodos para la transformación genética de frijol común a través de electroporación
Gasner Demosthene	2007	Haiti	Desarrollo de líneas de habichuela (*Phaseolus vulgaris* L.) adaptadas a las condiciones ecológicas de Haití con resistencia a enfermedades e insectos
Víctor Ramírez-Builes	2007	Colombia	Plant-water relationships for several common bean genotypes (*Phaseolus vulgaris* L.) with and without drought stress conditions
William Suárez-Buitrago	2006	Colombia	Validación y aceptación de la variedad de habichuela (*Phaseolus vulgaris* L.) 'Morales' y la variedad de maíz (*Zea mays* L.) 'Mayorbela'
Marcos Saliceti-Rivera	2005	Puerto Rico	Evaluación de dos poblaciones de habichuela (*Phaseolus vulgaris* L.) para ser utilizadas en el modelo de simulación CROPGRO
José Aponte-Rivera	2004	Puerto Rico	Selección recurrente para resistencia a la mustia hilachosa de habichuela (*Phaseolus vulgaris* L.)
Nancy González-Martínez	2004	Puerto Rico	Identification of new sources of resistance to web blight of common bean
Juan Manuel Osorno	2003	Colombia	Herencia de una nueva fuente de resistencia al virus del mosaico dorado amarillo de la habichuela (*Phaseolus vulgaris* L.)
Angel Murillo-Ilbay	2003	Ecuador	Uso de germoplasma exótico para ampliar la base genética para resistencia a roya (*Uromyces appendiculatus*) y otras enfermedades de fréjol común (*Phaseolus vulgaris* L.) de Ecuador
Luis Durán-Ortiz	2002	Puerto Rico	Caracterización morfológica y molecular de variedades criollas de habichuela (*Phaseolus vulgaris* L.) de origen andino procedentes del Caribe

TABLE 2.—(Continued 3) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Carlos Germán Muñoz-Perea	2002	Colombia	Estudio de herencia e identificación de marcadores moleculares para genes de resistencia al virus del mosaico dorado amarillo del frijol (*Phaseolus vulgaris* L.)
Maricelis Acevedo-Román	2002	Puerto Rico	Estudio de herencia y búsqueda de un marcador molecular para la resistencia a la deformación de vainas causada por el virus del mosaico dorado amarillo de la habichuela
Belinda Román-Avilés	2001	Puerto Rico	Heredabilidad de tolerancia al calor en una población de habichuela (*Phaseolus vulgaris* L.) de origen andino
Juan Carlos Takegami	2000	Colombia	Estudio de herencia de resistencia a mustia hilachosa (*Rhizoctonia solan*i Kuehn.) en habichuela común (*Phaseolus vulgaris* L.)
Vilmaris Bracero-Acosta	2000	Puerto Rico	Métodos serológicos y moleculares utilizados en el diagnóstico del Virus del Mosaico Dorado de la habichuela
Juan Carlos Angel-Sánchez	1999	Colombia	Uso de marcadores moleculares para la selección de líneas resistentes al mosaico dorado y mosaico común del frijol
Manuel de Jesús Bautista-Pérez	1998	El Salvador	La mustia hilachosa de la habichuela en Puerto Rico y su control
Aracely Castro-Zuniga	1998	Honduras	Estudio de la productividad y aceptación de variedades mejoradas de frijol (*Phaseolus vulgaris* L.) en la región centro-oriental de Honduras
Salvador Baigés-Ramírez	1997	Puerto Rico	Evaluación y selección de habichuela (*Phaseolus vulgaris* L.) para adaptación a las altas temperaturas

TABLE 2.—(Continued 4) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Albeiro Molina-Castañeda	1997	Colombia	Expresión y herencia de la resistencia al virus del mosaico dorado del frijol (VMDF)
Valerie Stone	1996	U.S.	Interspecific hybridization within the genus *Phaseolus* using conventional plant breeding and biotechnical techniques
Carlos Urrea-Florez	1996	Colombia	Genetics of resistance to common bacterial blight in tepary bean and identification of RAPD markers linked to genes that confer resistance
Francisco Fernández-Toledo	1996	Puerto Rico	Evaluación de treinta genotipos de habichuela común (*Phaseolus vulgaris* L.) con tolerancia al calor para potencial de fijación de nitrógeno
Ramon Nina Pérez	1995	Dominican Republic	Resistencia del frijol común a *Macrophomina phaseolina* en pruebas de inoculación artificial y natural
Elizabeth Johnson	1994	Trinidad & Tobago	RAPD marker for resistance to rust and common bacterial blight in *Phaseolus vulgaris* L.
Danna de la Rosa	1994	Dominican Republic	Evaluación de plantas de habichuela (*Phaseolus vulgaris* L.) transgénicas para resistencia al virus del mosaico dorado de la habichuela
Máximo Halpay García	1994	Dominican Republic	Evaluación de cinco poblaciones de habichuela (*Phaseolus vulgaris* L.) para su reacción a diferentes aislamientos de roya (*Uromyces appendiculatus*)
Carlos Montoya-Marmolejo	1994	Colombia	Herencia de la resistencia de frijol (*Phaseolus vulgaris* L) a la mustia hilachosa (*Thanatephorus cucumeris*)
Marcos Obando-Rivas	1993	Ecuador	Manejo integrado de *Meloidogyne incognita* en el cultivo de la habichuela común *Phaseolus vulgaris* L.

TABLE 2.—(Continued 5) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Cristóbal Adames-Mora	1993	Dominican Republic	Estudio de la expresión de síntomas al virus del mosaico dorado de la habichuela (*Phaseolus vulgaris* L.)
Tania Polanco	1993	Dominican Republic	Desarrollo de una metodología de investigación para la identificación de genotipos de habichuela resistentes a la mustia hilachosa
Matthew W. Blair	1992	U.S.	Heritability of field resistance to bean golden mosaic virus and the sweetpotato whitefly (*Bemisia tabaci* Genn.) in dry bean (*Phaseolus vulgaris* L.)
Oswaldo Díaz-Arrazola	1992	Honduras	Evaluación de plantas transgénicas de frijol (*Phaseolus vulgaris* L.) por su reacción al virus del mosaico dorado transmitido por *Bemisia tabaci* (Genn.)
Oswaldo Varela	1992	Honduras	Evaluación de frijol (*Phaseolus vulgaris* L.) en generaciones tempranas para resistencia a bacteriosis común
Rodrigo O. Campo-Aranda	1992	Colombia	Control biológico de *Macrophomina phaseolina* (Tassi.) Goid en frijol (*Phaseolus vulgaris* L.) con rizobacterias
Obed Román-Hernández	1991	Puerto Rico	Efectos de época de siembra y etapa de cosecha en el comportamiento de cinco genotipos de habichuelas (*Phaseolus vulgaris* L.)
Roldán Echeverría	1991	Honduras	Aptitud combinatoria para tolerancia a calor en frijol común (*Phaseolus vulgaris* L.) grano rojo pequeño
Alfonsina Sánchez-Paniagua	1991	Dominican Republic	Biocontrol de hongos patógenos del frijol (*Phaseolus vulgaris* L.) con *Pseudomonas cepacia*
Herminia Cataño-Cataño	1990	Dominican Republic	Selección de genotipos de *Phaseolus vulgaris* capaces de nodular en presencia de fertilizante nitrogenado

TABLE 2.— (Cintinued 6) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Froilán Perdomo-Valerio	1990	Dominican Republic	Evaluación in vitro de cepas de *Rhizobium* spp. y otros géneros bacterianos para el control biológico de *Macrophomina phaseolina*
Eduardo Peña-Rojas	1989	Colombia	Determinación de la pubescencia de cuatro genotipos de habichuela *Phaseolus vulgaris* L. y su efecto sobre el insecto *Bemisia tabaci* Gennadius
Fernando Oviedo-Terrero	1989	Dominican Republic	Caracterización y herencia de la pubescencia erecta en genotipos de habichuela (*Phaseolus vulgaris* L.)
Jonathan Cerna-García	1989	Honduras	Inheritance of early maturity of indeterminate dry beans (*Phaseolus vulgaris* L.)
Bryan Brunner	1988	U.S.	Estimation of outcrossing of the common bean (*Phaseolus vulgaris* L.) in Puerto Rico
Hiram Vélez-Martínez	1988	Puerto Rico	Determinación de pérdida en rendimiento causado por la roya *Uromyces phaseoli* (Reben) Wint. en la habichuela (*Phaseolus vulgaris* L.) en Puerto Rico
Luis del Río	1987	Peru	Microflora de la semilla de frijol (*Phaseolus vulgaris* L.) en Honduras
Jacqueline Lazú-Laboy,	1987	Puerto Rico	Efecto del genotipo de *Phaseolus vulgaris* L. en la fijación biológica de nitrógeno por *Rhizobium phaseoli*
Manuel de Jesús Mateo-Solano	1986	Dominican Republic	Heredabilidades y correlaciones para componentes de rendimiento y caracteres morfológicos en poblaciones de habichuela derivadas de cruzamientos entre genotipos determinados e indeterminados evaluadas en ambientes tropicales
Hugo Deschamps-Pimentel	1986	Dominican Republic	Hospedantes alternos del mosaico dorado de la habichuela (*Phaseolus vulgaris* L.)

TABLE 2.— (Continued 7) *List of Master's (M.S.) theses at the University of Puerto Rico with research topics related to* Phaseolus *spp. (1976-2018).*

Name	Year of graduation	Country of origin	Title of thesis
Miguel Martínez	1984	Dominican Republic	Estudio del efecto de las cuatro razas del nematodo nodulador (*Meloidogyne incognita*) sobre cultivares de habichuela
Elsie I. Carrillo	1984	Puerto Rico	Efecto de los componentes de acidez del suelo en el desarrollo y producción de habichuela (*Phaseolus vulgaris*)
Yovanny Velázquez-Báez	1984	Dominican Republic	Respuesta de la habichuela (*Phaseolus vulgaris* L.) variedad Arroyo Loro No. 1 a la inoculación con *Rhizobium phaseoli* en Lajas, Puerto Rico
Mildred Zapata-Serrano	1982	Puerto Rico	Virulencia de *Xanthomonas phaseoli* (E.F. Smith) Dowson y de *X. phaseoli* pv. *fuscans* (Burkh.) Starr y Burkh., agentes etiológicos de los tizones común y fusco de la habichuela (*Phaseolus vulgaris* L.) y *P. coccineus* L.
Mirta E. Rivera-de la Torre	1980	Puerto Rico	Relación entre la época de cosecha y la calidad e incidencia de hongos en semilla de habichuela (*Phaseolus vulgaris* L.)
Moisés Cordero-García	1979	Puerto Rico	Efecto de la inoculación con *Rhizobium phaseoli* y la fertilización nitrogenada de la habichuela seca
Rubén Guadalupe Luna	1977	Puerto Rico	Influencia de la época de siembra en el comportamiento de la habichuela seca. M.S. Thesis. Univ. of Puerto Rico. Mayagüez, PR.
M.A. López-Guadamuz	1976	Puerto Rico	Identificación de las razas fisiológicas más comunes de la roya [*Uromyces appendiculatus* (Pers.) Unger] del frijol (*Phaseolus vulgaris* L.) en Puerto Rico

Table 3.—*List of some BGYMV resistant cultivars and breeding lines released in Puerto Rico or developed and released in collaboration with the USDA-ARS, U.S. universities and bean research programs in Central America and the Caribbean.*

Cultivar or breeding line	Seed type	Countries of release	Traits in addition to BGYMV resistance	Participating Institutions	Reference
Bella	White	Puerto Rico	*I* and *bc-3* genes, common blight resistance, tolerance to low N soils	UPR, USDA-ARS-TARS	Beaver et al., 2018a. *J. Plant Reg.* 12:190-193.
Beníquez	White	Puerto Rico	*I* and *bc-3* genes, erect plants	UPR, USDA-ARS-TARS	Beaver et al., 2011. *J. Agric. Univ. P. R.* 95: 237-240
Verano	White	Puerto Rico	*I* gene, common blight resistance and heat tolerance	UPR, USDA-ARS-TARS	Beaver et al., 2008. *J. Plant Reg.* 2:187-189.
Morales	White	Puerto Rico	*I* gene, *Ur-5* rust resistance, and leafhopper tolerance	UPR, USDA-ARS-TARS	Beaver and Miklas, 1999. *Crop Sci.* 39:1259.
PR0806-80 PR0806-81	White	Puerto Rico	*I* and *bc-3* virus*, Ur-4, Ur-5* and *Ur-11* rust resistance genes.	UPR, USDA-ARS-TARS, Zamorano	Beaver et al., 2015. *J. Plant Reg.* 9:208-211
Sankara (XRAV-40-4)	Black	Haiti, Honduras	*I* and *bc-3* genes	NSS, UPR, Zamorano, USDA-ARS-TARS, Univ. Nebraska	Beaver et al., 2014. *J. Agric. Univ. P.R.* 98:83-87.
DPC-40	Black	Dominican Republic	*I* and *bc-3* genes	IDIAF, UPR, USDA-ARS-TARS	Arias et al., 2010. Proceedings of the 2010 CFCS Meeting.
PR9443-4	Light red kidney	Puerto Rico	*I* gene, common blight and rust resistance	UPR, USDA-ARS-TARS	Beaver et al., 1999b. *Crop Sci.* 39:1262

TABLE 3.—(Continued) *List of some BGYMV resistant cultivars and breeding lines released in Puerto Rico or developed and released in collaboration with the USDA-ARS, U.S. universities and bean research programs in Central America and the Caribbean.*

Cultivar or breeding line	Seed type	Countries of release	Traits in addition to BGYMV resistance	Participating Institutions	Reference
PR0737-1	Red mottled	Haiti	*I* and *bc-3 genes*	NSS, UPR, USDA-ARS-TARS	Prophete et al., 2014. *J. Plant Reg*. 8: 49-52
PR1146-138	Yellow	Haiti	*I* gene and leafhopper resistance	NSS, UPR, USDA-ARS-TARS	Beaver et al., 2016b. *J. Plant Reg*. 10:145-148
PR0401-259	Pink	Puerto Rico	*I* gene, common bacterial blight and web blight resistance, heat tolerance	UPR, Zamorano, USDA-ARS-TARS	Beaver et al., 2012. *J. Plant Reg*. 6:81-84.
PR9771-3-2, PR0247-49, PR0157-4-1	Small red Black White	Puerto Rico	*bgm-2* gene derived from *P. coccineus* line G35172	UPR, USDA-ARS	Beaver et al., 2005. *Crop Sci.* 45:2126.
Amadeus 77	Small red	Central America	*I* gene, common bacterial blight and web blight resistance	Zamorano, DICTA, CENTA, UPR	Rosas et al., 2004b. *Crop Sci.* 44:1867-1868.
Tio Canela 75	Small red	Central America	*I* gene	Zamorano, DICTA, UPR	Rosas et al., 1997. *Crop Sci*. 37:1391.
PR9357-107	Small red	Central America	*I* + *bc-3* genes	Zamorano, USDA-ARS-TARS, UPR, MSU, UNL	Beaver et al., 1998. *Crop Sci*. 38:1406-1407.
Carrizalito	Small red	Central America	*bgm, I* genes	UPR, Zamorano, DICTA	Rosas et al., 2005. *Crop Sci*. 45:2656-2657.

Bean Golden Yellow Mosaic Virus

Bean golden yellow mosaic virus (BGYMV) is a whitefly [*Bemisia tabaci* (Gennadius)] transmitted Begomovirus that represents a serious threat to dry and snap bean production in southern Florida, Mexico, Central America and the Caribbean (Singh and Schwartz, 2010; Blair et al., 1995). Bird and Maramorosch (1978) and Bird and Sánchez (1971) were pioneers in the study of viral diseases associated with whiteflies. Bird, a plant virologist of UPR AES, reported a whitefly-transmitted golden-yellow mosaic infecting lima beans in fields in northwestern and north-central Puerto Rico (Bird et al., 1972). Research followed using viruliferous whiteflies to infect common beans to determine the time needed to acquire, retain and transmit the virus (Bird et al., 1972, 1975), which based on physical properties was identified as BGYMV (Bird et al., 1977). Goodman et al. (1977) reported that infectivity of BGYMV was associated with a virus-like nucleoprotein particle. Bird et al. (1972) noted that BGYMV symptoms in Puerto Rico were very similar to the whitefly-transmitted bean golden mosaic virus (BGMV) infecting beans in Brazil. Not until nucleotide sequencing of the virus components was conducted, were they identified as distinct BGYMV and BGMV viruses (Faria et al., 1994; Maxwell et al., 1994).

Bird et al. (1972) also used viruliferous whiteflies to study host range in Puerto Rico and observed that only common bean and lima bean produced BGYMV symptoms. The host range was expanded to *Macroptilium lathyroides* by Rivera-Vargas et al. (2001) who observed symptoms in this weed species when they inoculated it with viruliferous whiteflies. Polymerase chain reaction (PCR) techniques, using primers from highly conserved regions of the viral genome, were used to confirm that *M. lathyroides* plants were infected with a geminivirus. Rivera-Vargas et al. (2007) studied the dissemination of BGYMV from infection foci in a bean field using statistical models. Martínez et al. (1991) did not find BGYMV to be transmitted in seed.

After several cycles of selection utilizing parents having different types of partial resistance to BGYMV, the International Center for Tropical Agriculture (CIAT) developed breeding lines that expressed high levels of resistance (Singh and Schwartz, 2010). A bean breeding line from CIAT, DOR 482, released in Honduras as ‘Don Silvio’ (Rosas et al., 2004a), was used as a parent in Puerto Rico to develop the BGYMV resistant white bean cultivar ‘Morales’ (Beaver and Miklas, 1999). Incrementally, other traits were added to cultivars harboring BGYMV resistance in subsequent releases of white beans for Puerto Rico (Table 2): ‘Verano’ added common bacterial blight resistance and greater tolerance to high temperature (Beaver et al., 2008); ‘Beníquez’ added BCMNV resistance with a more erect plant type (Beaver et al., 2011); and 'Bella' added superior performance in low nitrogen soils (Beaver et al., 2018a).

Because white beans produced in Puerto Rico are used for green-shelled bean production, farmers generally purchase seed from the University of Puerto Rico Agricultural Experiment Station Seed Program. The natural incidence of BGYMV in Puerto Rico declined as the use of resistant cultivars became more widespread. The decline in the incidence of BGYMV also coincided with the release of exotic biological control agents (*Encarsia* spp. and *Eretmocerus* spp.) to control the silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring) (Pantoja et al., 2005). At present, it is difficult to find bean plants in Puerto Rico infected with BGYMV.

In the San Juan de la Maguana valley of the Dominican Republic, beans are not planted during the rainy months of September and October which reduces the number of viruliferous whiteflies. This is followed by a near-synchronous planting of beans in November (Coyne et al., 2003).

Blair et al. (1992) conducted field trials in the Dominican Republic, Puerto Rico and Honduras to study the inheritance of BGYMV resistance in recombinant inbred line (RIL) populations. The CIAT breeding lines A 429, DOR 364 and DOR 303 were used as the sources of resistance in the development of the RIL populations. These sources of resistance expressed a diversity of reactions to BGYMV. Blair et al. (1993b) noted BGYMV resistance reactions of RILs derived from crosses with A 429 were bimodal (qualitative) with approximately 50% of the lines expressing resistance. A greenhouse screening technique developed by Adames-Mora et al. (1996) that allowed bean plants to be inoculated with a uniform number of viruliferous whiteflies facilitated subsequent inheritance studies of BGYMV resistance (Beaver et al., 1994). Blair and Beaver (1993a) used this new technique in greenhouse screening of F_2 populations to discover that the non-chlorotic leaf reaction from A 429 was controlled by a single recessive gene. These results were confirmed by Vélez et al. (1998) from BGYMV evaluations of four generations of bean populations in greenhouses at the UPR, Mayagüez Campus. The gene symbol *bgm-1* was assigned to the recessive resistance gene derived from A 429. Urrea et al. (1996) used results from the greenhouse evaluations of these bean populations to develop the codominant RAPD marker $R2_{(570,530)}$ linked to *bgm-1*. The SCAR marker SR2 derived from the RAPD (Blair et al., 2007) has been widely used to select beans for BGYMV resistance. For example, marker-assisted selection (MAS) was used to introgress *bgm-1* into indeterminate snap bean lines (Stavely et al., 2001) and to develop BGYMV resistant lines (Table 3) for Central America and the Caribbean (Beaver et al., 2008, 2011, 2018a).

The moderately BGYMV resistant CIAT breeding line DOR 364, released in Central America as 'Dorado' (Rosas et al., 2004a), had delayed symptom expression (up to five days) compared with susceptible bean genotypes when inoculated in the greenhouse with viruliferous whiteflies (Adames-Mora et al., 1996). Miklas et al. (1996b, 2000) evaluated a RIL population derived from the cross 'DOR 364/XAN 176' in the field for reaction to BGYMV. They reported two QTL conditioning resistance and developed the SCAR marker SW12 (Singh et al., 2000) linked to the BGYMV resistance QTL on chromosome Pv04 with consistent expression across multiple field environments.

Blair et al. (1993a) reported that DOR 303 and the Dominican landrace 'Pompadour G' produced a dwarfing response when inoculated in the greenhouse with viruliferous whiteflies. However, leaves on infected plants showed little or no chlorosis. Results from the evaluation of F_2 populations derived from the cross 'DOR 303/T968' indicated that the BGYMV reaction was controlled by two genes; a dominant gene (*Dwf*) for the dwarfing response and a recessive gene (*bgm-2*) for the non-chlorotic response (Vélez et al., 1998). When inoculated with BGYMV, F_1 plants from the cross 'DOR 303/A 429' produced chlorotic plants demonstrating that *bgm-1* and *bgm-2* were not alleles. Moreover, DOR 303 did not have the $R2_{(570,530)}$ marker for the *bgm-1* gene (Urrea et al., 1996). Subsequently, a BGYMV resistant light red kidney line, PR9443-4, derived from the cross 'T969-2/DOR 303' was released (Beaver et al., 1999b). The BGYMV resistance of this line was confirmed in field trials conducted in Puerto Rico, the Dominican Republic and Haiti as well as in greenhouse inoculations. Line PR9443-4 has a non-chlorotic leaf reaction (*bgm-2*) but does produce dwarf plants in the presence of BGYMV pressure. The *bgm-2* gene represents an unexploited alternate source of resistance to BGYMV that should be used to broaden the resistance, primarily of the *bgm-1* gene, currently deployed. The development of a molecular marker for *bgm-2* would facilitate the use of this resistance gene.

Sister lines from the cross 'DOR 364/WBB-20-1//Don Silvio', selected for non-chlorotic leaf reaction (*bgm-1*) to BGYMV, were observed in the field to segregate for pod deformation under BGYMV pressure (Molina-Castañeda and Beaver, 1998). Segregation patterns in four generations and progeny tests supported the hypothesis that normal pod development in the presence of BGYMV is conferred by the single dominant gene *Bgp* (Acevedo et al., 2004). Lines selected only for *bgm-1* tend to produce fewer and more deformed pods under BGMV pressure than lines selected for having both the *bgm-1* and *Bgp* resistance genes. In the absence of a molecular marker for the *Bgp* gene, advanced generation breeding lines for Central America and the Caribbean have been screened in the field under BGYMV pressure to confirm resistance to pod deformation.

The scarlet runner bean (*P. coccineus* L.) germplasm accession G35172 was reported by CIAT as resistant to BGYMV in Central America and the Caribbean (Beebe and Pastor-Corrales, 1991). The interspecific bean breeding line I9557-9 from a backcross population that used G35172 as a non-recurrent parent was selected by Phillip Miklas, USDA-ARS research geneticist, in the field at Isabela, Puerto Rico, for resistance to BGYMV. The $F_{2:3}$ lines from the cross 'Morales'/I9557-9 segregated for resistance to BGYMV which suggested that the BGYMV resistance in G35172 differed from the BGYMV resistance genes in 'Morales' (Osorno et al., 2007). Inheritance studies conducted in the greenhouse and field identified two genes from *P. coccineus* that confer resistance to BGYMV: a recessive gene (*bgm-3*) provides resistance to chlorosis and a dominant gene (*Bgp-2*) confers resistance to pod deformation (Osorno et al., 2007). Three BGYMV resistant bean breeding lines, PR9771-3-1 (small red), PR0247-49 (black) and PR0157-4-1 (white) were selected from these inheritance studies and released as improved germplasm (Beaver et al., 2005).

Morphological traits of beans may play a role in the behavior of the whitefly vector of BGYMV. Blair and Beaver (1993b) observed in field trials that the sweet potato whitefly [*Bemisia tabaci* (Genn.)] had a feeding preference and greater oviposition on the dense abaxial pubescence of Andean bean lines compared with the more glabrous leaves of beans of Mesoamerican origin. Peña et al. (1993) observed this same preference in greenhouse trials in which Andean beans with dense leaf pubescence had greater oviposition of whiteflies. Peña et al. (1993) did not find a relationship between bean leaf pubescence density on larval size and the time required by whiteflies to complete development. Interestingly, dense abaxial leaf pubescence is associated with lower levels of rust infection (Oviedo et al., 1990).

The UPR bean research program evaluated BGYMV reaction of transgenic bean lines developed at the University of Wisconsin having a gene for the BGYMV coat protein (Azzam et al., 1996). Unfortunately, the expression of the coat protein in the transgenic lines was insufficient to produce resistant reactions (Azzam et al., 1994).

The UPR and the ARS-TARS participated in USAID-funded projects that resulted in the release of several BGYMV resistant bean cultivars for Central America and the Caribbean (Table 3). The black bean cultivar 'DPC-40' was developed and released in the Dominican Republic in collaboration with the 'Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF)'. The black bean cultivar 'Sankara', the red mottled bean PR0737-1 and the yellow bean PR1146-138 were released in collaboration with the National Seed Service of the Ministry of Agriculture of the Republic of Haiti. Collaboration with the bean research program at EAP, led by Juan Carlos Rosas, has been particularly productive. Several BGYMV resistant small red and black bean cultivars jointly released with EAP have been widely adopted by farmers in Central America.

Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus

Bean Common Mosaic Virus (BCMV) is caused by a seed-borne and aphid transmitted potyvirus that is an endemic bean disease in Central America and the Caribbean. The BCMV can cause significant losses to both the yield and quality of common beans. Landrace bean cultivars in the region are largely susceptible to BCMV. Since the release of 'Arroyo Loro' in 1990, all bean cultivars released in Puerto Rico have the *I* gene that confers resistance to BCMV. Greenhouse inoculations with the NL-3 strain of *Bean common mosaic necrosis virus* (BCMNV) were used to detect lines with top necrosis symptoms. Most farmers in Puerto Rico produce green-shelled beans and generally do not save seed for planting. Therefore, the UPR-AES Seed Program at the Isabela substation has served as the principal source of seed of these BCMV resistant bean cultivars. Consequently, the incidence of BCMV is currently very low in Puerto Rico.

The BCMNV is caused by a different seed-borne and aphid transmitted potyvirus, but also has the potential to reduce the yield and quality of common beans. The increased incidence of BCMNV in the Dominican Republic, reported by Godoy de Lutz et al. (2004), pointed to the need to develop bean cultivars for Central America and the Caribbean with resistance to both BCMV and BCMNV (Kelly et al., 2003). In anticipation of this need, Miklas et al. (1997) improved upon the agronomic performance of Freytag's IM lines with the release of the TARS-VRs white germplasm lines with *I* + *bc-3* genes which condition resistance to all strains of BCMV and BVMNV. Greenhouse screening with the NL-3 strain of BCMNV to detect *bc-3* (no symptoms) and marker-assisted selection using SW13 SCAR marker (Melotto et al., 1996) to detect hypostatic *I* gene were used to develop the most recent lines and cultivars (see Table 3) having complete resistance to BCMV and BCMNV. The RAPD markers (Miklas et al., 1996a, 2006) were replaced by the CAPS marker eIF4E (Naderpour et al., 2010) to indirectly select for and validate the presence of the *bc-3* gene. The University of Puerto Rico participated in the release of the bruchid resistant dark red kidney bean breeding line AO-1012-29-3-3A that carries the *I* and *bc-1*^{2} genes that confer resistance to BCMV and BCMNV (Kusolwa et al., 2016).

Cucumber Mosaic Virus

Bird et al. (1974) isolated, from the black bean cultivar 'La Vega', a virus capable of inciting mild mosaic and vein-banding symptoms on various hosts. Bean plants inoculated mechanically and with viruliferous aphids expressed moderate symptoms a few days after inoculation. Meiners et al. (1976) studied host range and used serology, physical properties and electron microscopy to confirm that CMV-PR was a strain of *Cucumber Mosaic Virus.* They also reported that CMV-PR was seed transmitted.

Common Bacterial Blight

Common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (Xap), previously *Xanthomonas campestris* (Xcp), is a seed-borne pathogen that can cause significant losses to the yield and quality of bean seed (Singh and Miklas, 2015). Research with common bacterial blight of beans began in Puerto Rico in the 1970s with support of a grant from USAID titled "Regional Food Legume Improvement" (Zapata and Beaver, 2002).

Vakili et al. (1974) collected 67 isolates from Puerto Rico and conducted cross inoculation studies to demonstrate that two pathogenic types of *Xanthomonas* can infect beans. One pathotype only infected common bean whereas the other pathotype infected both common beans and cowpeas. Zapata et al. (1994) isolated seed-borne bacteria from 86 lines in a field in Juana Díaz, Puerto Rico, that had high levels of common bacterial blight infection. Fifteen strains of *Xcp*, 23% of all bacterial strains isolated from the seed, were pathogenic to common bean.

Kaiser and Vakili (1978) noted that common blight lesions on leaves of common beans are often associated with damage caused by insect feeding. *Xanthomonas* isolates pathogenic to common beans were isolated from feces of *Cerotoma ruficormus* and *Diaprepes abbreviatus* that had fed on infected bean leaves. The bacteria survived up to 19 days in the bodies of living and dead insects. Feeding damage caused by both infested and non-infested insects enhanced common blight symptom development.

Zapata et al. (1985) reported that multiple methods were effective in screening bean leaves for reaction to common bacterial blight, including inoculation of trifoliate leaves at flowering using the multiple needles/cushion method or the spray infiltration method using carborundum or sterilized sand. Inoculating excised green pods nearing maturity using the needle-scratch method under controlled conditions was useful to screen pods for reaction to common bacterial blight.

Beaver et al. (1992a) screened red mottled bean landraces from the Dominican Republic in the field for reaction to common bacterial blight over a two-year period. Indeterminate red mottled lines that had significantly lower levels of common bacterial blight damage were identified. Beaver et al. (1992b) reported that the common blight lesions of determinate red mottled landraces had little, or no chlorosis compared with the indeterminate red mottled lines evaluated in the trial.

A differential response of 'La Vega' black bean and the white bean line W-177 was observed when inoculated with six different isolates of *Xcp* from Puerto Rico (Zapata et al., 1985). This was the first of many subsequent studies led by plant pathologist Mildred Zapata which established the existence of physiological races of the common bacterial blight pathogen (Zapata and Vidaver, 1987; Zapata, 1996a, 1996b, 1997a, 1998; Zapata and Beaver, 2005; Zapata et al., 2012). Zapata (1989) also observed a differential reaction among tepary bean (*Phaseolus acutifolius* L.) genotypes when inoculated with different strains of *Xcp*. The existence of physiological races, affirmed by the differential reactions of *Xcp* isolates collected in Costa Rica, Puerto Rico, the Dominican Republic and Haiti, was the prominent topic during the First International Workshop on Common Bacterial Blight held at the University of Puerto Rico in 1996 which was attended by scientists from the Americas, Europe and Africa with expertise in working with common bacterial blight (PROFRIJOL, 1996). Minimum standards to designate races of *Xcp* (Coyne et al., 1996), a common set of bean differentials, and uniform inoculation and evaluation methods were proposed. Zapata (2006) eventually updated greenhouse screening procedures for uniform evaluation of bean leaves for response to *Xcp*. Zapata (1997b) suggested that a hypersensitive reaction of certain tepary bean genotypes to inoculation with the common blight pathogen may be useful for testing strains of *Xanthomonas campestris* pv. *phaseoli*.

The University of Puerto Rico and the USDA-ARS-TARS research programs have developed and released several bean germplasm lines and cultivars with enhanced levels of resistance to common bacterial blight (CBB). Vakili (1979) identified 11 breeding lines of *P. coccineus* as potential sources of resistance to common bacterial blight that were jointly released by the USDA-ARS and the University of Puerto Rico (Table 1). One of these CBB resistant breeding lines, Pc-H-46-1Bk (a selection from PI 273667 from Ethiopia), was used by Mark Bassett at the University of Florida as a parent to develop an interspecific population. One of the progenies from this cross was the common bacterial blight resistant line XR-235-1-1 which was jointly released by the Universities of Florida and Puerto Rico and the USDA-ARS (Freytag et al., 1982). Overall results suggested that only a few major genes conferred common bacterial blight resistance in *P. coccineus* since the progeny of interspecific crosses between CBB resistant scarlet bean lines and susceptible common beans produced a high proportion of resistant lines.

The *P. coccinues* resistance sources were combined with common bean sources of resistance that included GN#1 Sel. 27 by using a reciprocal backcross breeding method. Greenhouse screening at the University of Puerto Rico by Mildred Zapata and at Cornell University by Robert Wilkinson was followed by field evaluations for common blight in Puerto Rico during the summer when climatic conditions favored disease development. This collaboration led to the release of five (WBB-11, WBB-20-1, WBB-35, WBB-52, and WBB-II-56) common bean germplasm lines with high levels of resistance to common blight (Zapata et al., 2004, 1991). Miklas et al. (1994a, 1999) also developed and released five common bean germplasm lines (TARS VCI-4B, and ICB-3, ICB-6, ICB-8 and ICB-10) with enhanced levels of common bacterial blight derived from *P. coccineus*, which traced back to Pv x Pc interspecific populations developed by Zapata. The common blight resistant pinto bean germplasm line TARS-PT03-1 (Smith et al., 2005) was derived from a cross with the interspecific (Pv x Pc) germplasm line TARS VCI-4B.

The pedigree of the white bean cultivar 'Verano' includes WBB-20-1, which derives a portion of its resistance from *P. coccineus*, and highly resistant bean germplasm VAX 6 line from CIAT, which derives its resistance from tepary bean (Singh et al., 2013; Beaver et al., 2008). Verano was used as a parent to develop the multiple disease resistant white bean 'Bella' (Beaver et al., 2018a).

In general, Andean germplasm and cultivars are highly susceptible to common bacterial blight. The light red kidney bean germplasm line PR9443-4 was derived from a cross between CIAT breeding line DOR 303 and T969-2, a kidney bean breeding line from Michigan State University. Line PR9443-4 was highly resistant to CBB when screened in the greenhouse with three different strains of *Xcp* and was used as a parent to develop the common bacterial blight resistant light red kidney bean cultivar 'Badillo' (Beaver et al., 2010, 1999c). 'Badillo' has the SAP6 QTL-linked marker for CBB resistance. Cranberry bean germplasms 08SH-840 and CRX were developed by Tim Porch (ARS-TARS) from crossing USCR-CBB-20 (Miklas et al., 2011) with VAX 6 followed by four backcrosses to the recurrent parent, USCR-CBB-20, with phenotypic selection for CBB resistance conducted each BC_nF_2 generation. These lines possess the SAP6 and SU91 QTL markers and were resistant to the *Xcp* ARX8AC strain in Idaho (Viteri et al., 2014) and *Xcp* strains 484a and 3353 in Puerto Rico.

Bean germplasm lines selected for resistance to web blight such as the pink bean PR0401-259 and the black bean PR0650-31 often have resistance to common bacterial blight (Beaver et al., 2012). This may be the consequence of selecting beans having healthy leaf canopies under hot and humid conditions prevailing in Puerto Rico during the summer months. Both PR0401-259 and PR0650-31 derive their common blight resistance from VAX 6. Martínez-Figueroa (2017) selected bean breeding lines at Isabela, Puerto Rico, that combine resistance to both web blight and common bacterial blight.

The black bean germplasm line TARS-MST1 has resistance to common bacterial blight and root rot (Porch et al., 2012). Line TARS-MST1 derives its common blight resistance from VAX 6 and has the SAP6 and SU91 resistance QTL-linked markers. The small red bean germplasm line TARS-LFR1 was developed using recurrent selection and combines common bacterial blight resistance, root rot resistance and superior performance in low nitrogen soils (Porch et al., 2014a). The pedigree of TARS-LFR1 includes the common blight resistant lines VAX 1, VAX 2 and VAX 3 (Singh et al., 2013). The moderate level of common blight resistance of the small red germplasm line TARS-SR05 (Smith et al., 2007) was derived from XAN 176.

Varela et al. (1996) studied the inheritance of common bacterial blight resistance in the F_3, F_4 and F_5 generations in two Mesoamerican common bean populations (DOR 364/XAN 176 and DOR 364/WBB-20-1). Significant differences among lines in common blight reaction were observed in all three generations. At least three replications were needed for sufficient precision to reliably identify resistance in the F_3 lines. Heritability estimates of common blight resistance in leaves was intermediate to high (≥ 0.6) in the F_4 and F_5 generations.

Miklas et al. (2000) used recombinant inbred lines from the 'DOR 364/XAN 176' population to identify QTLs associated with resistance to common bacterial blight strain 484a. The RAPD $AP6_{820}$ explained 60% of the leaf reaction to common blight in the greenhouse and 10% of the leaf reaction in the field. The RAPD $AP6_{820}$ was converted to the SCAR marker SAP6 which was mapped on Pv10 (Miklas et al., 2003, 2000).

Zapata et al. (2011) identified a single dominant gene, *Xap-1*, that conferred resistance to *Xap* strain 3353. The SCAR SAP6 marker, located on Pv10, was found to co-segregate with the resistant phenotype. Guachambala (2013) evaluated the common blight reactions of F_2 plants and $F_{2:3}$ lines from a cross between the breeding line PR0313-58, which has the *Xap-1* gene and moderate levels of resistance to common blight, and VAX 6. The segregation pattern in this population suggested that two putative dominant genes conferred high levels of resistance to bacterial blight caused by *Xap* strain UPR 3353.

The highest levels of resistance to common bacterial blight are found in tepary bean and most tepary beans have moderate to high levels of resistance (Vargas et al., 2014). Freytag (1989) reported three linked dominant genes conferring resistance to three *Xcp* strains in the tepary bean line TL-40. When inoculated with the *Xcp* strain 484a, Urrea et al. (1999) found the common blight resistance of tepary bean lines Neb-T-6-s and PI 321637 to be controlled by a single dominant gene and the resistance of tepary line Neb T-8a-s was conferred by two complementary dominant genes. The first tepary germplasm developed and released through modern plant breeding methods, TARS-Tep 22, was developed for abiotic stress tolerance, seed quality and for resistance to common bacterial blight and rust (Porch et al., 2013a).

Web blight

Web blight produced by the mycelial infection of *Thanatephorus cucumeris* [anamorph: *Rhizoctonia solani* (*Rs*)] can reduce both the yield and quality of common bean seed in hot and humid regions of the tropics (Godoy de Lutz et al., 1996). Farmers in Puerto Rico describe beans damaged by web blight as being “stewed” (‘sancochado’). Matz (1921) was the first researcher to isolate *Rs* from bean foliage in Puerto Rico. He noted that plants producing abundant foliage are more vulnerable to infection because the shade conserves humidity in the canopy and favors fungus growth.

Inoculation of beans in the field with a mycelial suspension of the pathogen has been used successfully to select bean breeding lines having enhanced levels of resistance to web blight (Beaver et al., 2012; Takegami et al., 2004). Because erect plant architecture and an open canopy can contribute to the avoidance of infection of web blight (Arnaud-Santana et al., 1992), droplet inoculation of leaflets (Polanco et al., 1996a; Montoya et al., 1997) and the detached-leaf technique (González-Martínez, 2004) have been employed to identify physiological resistance to this disease. Bautista-Pérez and Echávez-Badel (2000) reported positive and significant correlations between greenhouse and field readings for web blight reactions. Echávez-Badel et al. (2000) reported that dried beet (*Beta vulgaris* L.) seed colonized by *R. solani* could be stored for at least one year at 4° C in the dark without losing the viability or virulence of the fungus.

Echavéz-Badel et al. (2000) collected 13 isolates of *Rhizoctonia solani* from soil samples, infected seeds, hypocotyls, leaves and pods of common bean in Puerto Rico. Seven *Rs* isolates were classified as anastomosis group (AG) AG-4, and six were assigned to AG-1. Polanco et al. (1996b) reported that isolates of *Rs* from Isabela, Puerto Rico, belonged to anastomosis group AG-1. Inoculation using mycelial mats of Rs of anastomosis group AG-1, followed by frequent irrigation in the field, was useful for identifying lines moderately resistant to web blight compared to the susceptible local check 'Morales' (Echávez-Badel et al., 2002). Furthermore, screening for web blight resistance requires knowledge of the virulence patterns of the Rs isolates in the target environment (Bautista-Pérez et al., 2000). Valentín-Torres et al. (2016) identified high variability in virulence patterns among different *Rs* isolates collected from bean leaves and roots in Puerto Rico. This research showed that *Rs* isolates from both roots and leaves can induce web blight symptoms when environmental conditions favor disease development. These results help to explain how web blight infection can occur in fields where beans have not been planted for several years.

Because the inheritance of web blight resistance has been found to be quantitative (Takegami et al., 2004; Montoya et al., 1997), recurrent selection was recommended to develop lines with greater levels of resistance to this disease (Aponte-Rivera, 2004; Beaver et al., 2001). The criteria for the evaluation using recurrent selection included low levels of leaf damage due to fungal and bacterial diseases, high seed yield, low percentage of damaged seed and the presence of genes for resistance to BGYMV and BCMV (Martínez-Figueroa, 2017). Because the web blight trials were planted at Isabela, Puerto Rico, under hot and humid conditions, indirect selection for greater tolerance to moderately high temperatures was also possible. The small red bean cultivar 'Amadeus 77' had a susceptible leaf reaction to web blight but lower levels of damaged seed when evaluated in the field (Rosas et al., 2018). Takegami et al. (2004) observed that different genes may control leaf reaction and seed damage caused by web blight. Releases in Puerto Rico of breeding lines having moderate levels of web blight resistance include the pink bean PR0401-259 and the black bean PR0650-31 (Beaver et al., 2012). Lines from the third cycle of recurrent selection conducted by EAP exhibited superior levels of resistance to web blight in field trials planted in Puerto Rico and Honduras (Rosas et al., 2018). The use of augmented designs involving spatial analysis can be useful in screening large numbers of bean lines for reaction to web blight (Rosas et al., 2019). Two resistant and two susceptible checks were randomized within sub-blocks containing 20 experimental units. Spatial analysis indicated that disease ratings (estimates of % leaf area infected at 34 days after inoculation) from experimental units separated by distances < 10 meters were correlated. Conversely, disease ratings from plots that were spaced > 10 m apart could be considered independent for practical purposes. Thus, a spatial model estimated disease rating with greater precision than a traditional Incomplete Block Design.

Because only moderate levels of resistance are found in common bean, scarlet runner bean and *P. polyanthus* germplasm were screened for web blight resistance (González Martínez, 2004). Using a detached-leaf inoculation technique, G35163 was identified as the *P. coccineus* accession most resistant to web blight by having less leaf damage and smaller mean lesion size than other accessions in the trial. An important criterion for evaluating the reaction to the web blight pathogen was the degree of penetration of the fungus into the leaf tissue. Recently, several RILs from a tepary bean population 'G40001/G40022' expressed moderate levels of resistance to natural infection of web blight in trials conducted at Isabela, Puerto Rico (unpublished).

Rusts

Bean rust caused by *Uromyces appendiculatus* (Pers.) Unger is an endemic disease of common bean in Puerto Rico. Rust can cause significant seed yield losses, especially in the tropics and sub-tropics where disease severity is often greater than in temperate bean production regions (Beaver et al., 2002; Mmbaga et al., 1996). The bean rust pathogen has highly variable virulence patterns which require constant monitoring for the presence of new races (Acevedo et al., 2013). A mobile nursery to monitor bean rust virulence patterns, developed at the University of Nebraska, was tested in Puerto Rico (Steadman et al., 2002a).

López-Guadamuz (1976) conducted a survey of bean rust in Puerto Rico and identified 12 different races. Stavely et al. (1989b) collected a rust isolate in 1984 at Isabela, Puerto Rico, that was virulent to the black bean line B-190 which has the *Ur-5* resistance gene. Vega et al. (2009) reported that a single pustule isolate collected from the white bean cultivar 'Verano' in Naranjito, Puerto Rico, was race 19:63. All Andean differentials were susceptible to this rust race whereas several Middle American resistance genes (*Ur-3*$^+$*, Ur-5* and *Ur-11*) conferred resistance.

The First Bean Rust Workshop was held at Mayagüez, Puerto Rico, in 1983. A uniform set of differential bean lines was identified to characterize the virulence patterns of the bean rust pathogen. A standard 1 to 6 grading scale based primarily on pustule size for the evaluation of bean rust was also adopted at the workshop (Stavely et al., 1983). A new set of bean rust differentials was identified at the 3rd Bean Rust Workshop held in Pietermaritzburg, South Africa, in 2002 (Steadman et al., 2002b). The new set of differentials provided a more balanced representation of Andean and Middle American sources of rust resistance in recognition of co-evolution of rust races and beans within gene pools. The use of a binary system to identify rust races was also proposed.

Although the use of fungicides has been shown to be effective in controlling rust (Meléndez et al., 1986) and other fungal diseases of beans (Rodríguez and Meléndez, 1986), research in Puerto Rico has focused on host resistance. The black-seeded bean cultivar 'La Vega' (the bulk of individual plant selections from PI 287536) was released in Puerto Rico in 1973 (Table 1). It was reported to have small pustule size in the field resulting in "slow rusting". The white bean cultivar 'Arroyo Loro', which combined rust resistance in Puerto Rico and the dominant *I* gene for resistance to BCMV, was derived from the cross 'Bonita/ La Vega' (Beaver et al., 1990a). 'Arroyo Loro' was used as a parent to develop the white bean cultivar 'Morales' that combined resistance to BGYMV, BCMV and rust (Beaver et al., 1999a).

In 1976, the snap bean cultivar 'Palmarejo', a selection from PI 207139, was released in Puerto Rico (Table 1). The snap bean was highly resistant to rust races endemic in Puerto Rico. Researchers in Puerto Rico participated in the release of a pole snap bean cultivar 'Genuine' that combined resistance to BGYMV and rust (Stavely et al., 1996).

The black bean line Mexico 309 is a source of the *Ur-5* rust resistance gene. B-190, a black bean released in Puerto Rico in 1979, was developed in Puerto Rico from a cross between Mexico 309 and 50600, a black bean line from Costa Rica. The *Ur-5* rust resistance in B-190 was the first described cluster of R genes conditioning pathogen resistance in bean (Stavely, 1984). The *Ur-5* cluster is mostly known as a gene block conditioning small pustule reactions (grade 3, slow rusting). Haley et al. (1993) identified a RAPD linked to *Ur-5* from B-190 that was later converted to a SCAR marker by Melotto and Kelly (1998). The source of rust resistance in the development of the Great Northern bean lines BelNeb-RR-1 and -2 was B-190 (Stavely et al., 1989a). Rust resistant F_1 plants from the cross 'B-190 x GN 1140' were subsequently backcrossed with GN 1140, Olathe, and Harris to develop these BelNeb lines.

Bokosi et al. (1996) used the bean rust isolates PR911-19a from Puerto Rico and USNP10-1 from the U.S. to study the inheritance of rust resistance in populations derived from the cross 'BelNeb-Rust Resistant (RR)-1/A-55'. The authors concluded that BelNeb-RR-1 had at least three resistance genes that should be useful to breed for resistance to these rust isolates. Pastor-Corrales (2003) reported that BelNeb-RR-1 and -2 combine the *Ur-5, Ur-6* and *Ur-7* rust resistance genes. Beaver et al. (1999b) used BelNeb-RR-1 as a source of rust resistance to develop the Mesoamerican pink bean cultivar 'Rosada Nativa'. This cultivar expressed resistance to rust in field trials conducted in Puerto Rico and the Dominican Republic.

Mexico 309 was also used as a parent to develop the black bean breeding line 2B-5-1 (Table 1). Two rust resistant navy bean germplasm lines, L226-10 and L227-1, were cooperatively released by the UPR, the USDA-ARS and Michigan State University. The black bean parent 2B-5-1 was the source of rust resistance in these navy bean lines. Mexico 309, L226-10 and L227-1 were resistant to 12 races of rust when screened at Beltsville, Maryland (Freytag et al., 1985).

The light red kidney bean breeding line PR9443-4 combined resistance to BGYMV, common bacterial blight and rust (Beaver et al., 1999c). PR0443-4 was used as a parent to develop the light red kidney variety 'Badillo' (Beaver et al., 2010), which has resistance to endemic races of rust in Puerto Rico.

The white bean germplasm BelMiDak-RMR-10, which has the *Ur-4* and *Ur-11* rust resistance genes (Pastor-Corrales, 2003), was used in Puerto Rico to develop the white bean germplasm lines PR0806-80 and PR0806-81 that combine resistances to BGYMV, BCMNV, BCMV and rust. Breeding lines developed by the USDA-ARS bean project at Beltsville, Maryland (Pastor-Corrales, 2003) have been used to introgress the *Ur-11* rust resistance gene into tropically adapted pinto beans. The USDA-ARS (TARS and Beltsville) collaborated with UPR to identify markers linked to the *Ur-11* gene derived from the tropical black bean PI 181996 (Johnson et al., 1995). The 'DOR 364/XAN 176' population revealed unique rust resistance genes, tentatively named Ur-Dorado-108 and -53, which were mapped respectively near *Ur-5* and *Ur-11* loci (Miklas et al., 2000, 2002).

The University Puerto Rico participated in the release of the red mottled cultivar 'PC-50', which expressed moderate levels of rust resistance in the Dominican Republic (Saladin et al., 2000). Bokosi (1996) found PC-50 to have the specific rust resistance gene *Ur-9* and the *Ur-12* gene associated with adult plant resistance to rust. PC-50 and other red mottled bean landraces also have dense abaxial leaf pubescence (Oviedo et al., 1990), which was reported by Shaik (1985) to be associated with rust resistance. Zaiter et al. (1990) reported that presence and absence of pubescence was simply inherited whereas abaxial leaf pubescence density was a quantitatively inherited trait.

The rust resistant pinto bean germplasm TARS VCI-4B was derived from an interspecific cross between *Phaseolus vulgaris* and *P. coccineus* (Miklas et al., 1994a). Rust resistant tepary bean lines were also identified in Puerto Rico (Miklas et al., 1994b). Miklas and Stavely (1998) identified seven cultivated tepary genotypes with broad resistance to eight rust races. Results from inheritance and allelism tests suggested that a single locus exhibiting incomplete dominance conferred resistance to these rust races. One of these tepary bean accessions was later used to develop rust resistant TARS-Tep22 (Porch et al., 2013a).

The first report of the New World soybean rust, caused by *Phakopsora meibomiae,* was made in Puerto Rico by Vakili and Bromfield in 1976. The New World soybean rust infected common bean, scarlet runner bean and soybean at Adjuntas, Puerto Rico (Vakili and Bromfield, 1976). Recent studies confirmed lima beans were susceptible to *P. meibomiae*, which adds to the list another *Phaseolus* species facilitating the spread of the New World soybean rust (Vega and Estévez de Jensen, 2010). Common bean was reported to be more susceptible to *P. meibomiae* isolates from Brazil and Puerto Rico than to an Asian soybean rust (*P. pachyrhizi*) isolate from Taiwan (Miles et al., 2007). Estévez de Jensen et al. (2013) confirmed the presence of Asian soybean rust, although this rust has not been found infecting common beans in Puerto Rico.

Stem rot and Root Rot

Root rot and stem rot are worldwide constraints on bean production (Miklas et al., 2006). Porch et al. (2014a) noted that greater severity of root and stem rots is often associated with abiotic stresses such as extreme temperature, drought, excess moisture, compacted soil, low soil pH or poor soil fertility. Cropping systems that rotate beans generally have fewer problems with soil-borne diseases (Abawi and Widmer, 2000).

A root rot nursery was established by Vakili in 1971 at the USDA-ARS research farm in Isabela, Puerto Rico. This nursery has been planted almost continuously in beans to increase root and stem rot pathogen populations. Results from surveys conducted in the nursery from 2009 to 2012 found the predominant soil-borne pathogens to be *Fusarium solani, Macrophomina phaseolina*, *Sclerotium rolfsii* and *Phythium* spp. (Porch et al., 2014b). Because the field has not been fertilized, the nursery has been used by the USDA-ARS bean breeding program to screen bean lines for resistance to both root rot and stem rot and for adaptation to low soil fertility. Results from 16 samples of common bean tissue collected from the nursery found N, P, K and Mg to be below the sufficiency range needed for normal development of bean plants (Porch et al., 2014b). Results from the nursery have been used to support the release of several improved bean breeding lines (TARS-LFR1, TARS-MST1, and SB-DT1) that are more tolerant to root rot and low soil fertility (Porch et al., 2014a, 2012).

Ashy stem blight caused by *Macrophomina phaseolina* (*Mph*) is an endemic disease in the dry and warm areas of Puerto Rico. Damage caused by *Macrophomina phaseolina* can range from damping-off at emergence to stem blight and root rot near maturity (Miklas et al., 1998). Beaver et al. (1990b) noted that field screening for ashy stem blight resistance is difficult because the distribution and severity of infection in the field is often uneven. In addition, field screening for resistance requires the entire growing season because the symptoms of ashy stem blight tend to be more severe near senescence.

Different inoculation methods have been assessed including the band-aid, toothpick, mycelial plug, inoculated rice and the conidia suspension method (Echávez-Badel and Beaver, 1987a, 1987b). In the Dominican Republic, Beaver et al. (1990) compared the effectiveness of field screening for ashy stem blight using seed soaked in microsclerotia suspension versus stem inoculations performed 15 days after emergence. The stem base was rubbed with cheesecloth that had been soaked in a suspension containing approximately 1,000 microsclerotia per milliliter and 10 g/L of carborundum. Porch et al. (2014c), using the "band-aid" inoculation method, observed significant dry bean genotype x *Mph* isolate interactions for lesion length and disease severity ratings. The isolate (*Mph*-JD) from Juana Díaz (with a hot dry climate favorable for Mph epidemics) was the most virulent when compared to the isolates from Isabela and Aguadilla. An expanded study of ten Puerto Rico *Mph* isolates x 120 dry bean genotypes in the BASE (Bean Adaptation to Stress Environments) trial confirmed differential virulence among isolates. Once again, the most virulent isolate *Mph*-JD2, which induced susceptible reactions in all 120 genotypes, was collected from Juana Díaz (Estévez de Jensen et al., 2017). The germplasm lines PR1147-3, TARS-LFR1 and TARS-MST1 from Puerto Rico, and Mph resistant CIAT line BAT 477, had the best intermediate (less susceptible) reactions to *Mph*-JD2.

The Andean Diversity Panel (ADP) developed by a team of USDA-ARS researchers to enhance genetic study and breeding improvements of large-seed sized beans was compiled and increased in Puerto Rico in 2011-2012 (Cichy et al., 2015). The panel database is maintained by Porch at http://arsftfbean.uprm.edu/bean/. The response of the ADP to different isolates of *Mph* was determined. Variable disease severity reactions, morphological diversity and high levels of sequence similarity in the ITS region between the isolates from Puerto Rico and isolates from other regions were observed (Porch et al., 2014c). A Genome Wide Association Study (GWAS) of the BASE 120 trial conducted under heat and drought stress in Juana Díaz, Puerto Rico, identified a genomic region on Pv04 associated with resistant ashy stem blight field scores (Oladzad et al., 2019)

Miklas et al. (1998) conducted field trials at Juana Díaz, Puerto Rico, to study the inheritance of resistance to *Mph* in the 'DOR 364/

XAN 176' RIL population. Narrow sense heritability estimates for ashy stem blight resistance were intermediate (± 0.5). Five QTL, derived from the resistant line XAN 176, explained a significant portion of the phenotypic variation for disease score.

Viteri and Linares (2019) investigated the inheritance of Andean bean cultivars 'Badillo' and 'PC-50' and breeding line A-195, which expressed less severe symptoms to two *Mph* isolates from Puerto Rico at vegetative and reproductive stages using a cut stem inoculation method (Viteri and Linares, 2017). Resistance to *Mph* isolate PRI16 of 'Badillo' was conferred by a single recessive gene, whereas the resistance of 'PC-50' was controlled by two independent complementary recessive genes. Segregation patterns between lines derived from the cross 'A 195 x PC-50' suggested that a single dominant gene conferred resistance to *Mph* isolate PRI16.

Perdomo et al. (1995) conducted in vitro evaluations of 64 strains of *Rhizobium* and seven strains of rhizosphere bacteria to measure their antibiotic activity against *Mph*. The UPR 5C isolate of *Burkholderia cepacia* (previously known as *Pseudomonas cepacia*) strongly inhibited the growth of *Mph* using the streak-plate method and yeast extract/ mannitol growth media for the bioassay. Sánchez et al. (1994b) demonstrated the UPR 5C isolate successfully colonized the bean root after inoculation of the seed. Results from greenhouse trials found that inoculation of seed with UPR 5C strain reduced the severity of ashy stem blight by 71% and was compatible with inoculation using *Rhizobium phaseoli* strain CIAT 632 (Sánchez et al., 1994a).

Leafhoppers

The 1902 USDA Agricultural Research Station report noted that leafhoppers (*Empoasca* spp.) were the most serious pest in bean trials conducted in Puerto Rico. Cruz (1975) stated that leafhoppers are one of the most limiting factors for bean production in Puerto Rico. He reported that chemical control was effective in reducing leafhopper populations and resulted in greater yield of snap beans. Cruz et al. (1981) noted that the use of silver-plated plastic mulch and sugarcane straw mulch resulted in fewer leafhopper nymphs on beans planted at Isabela, Puerto Rico.

Predicted climatic changes in the Caribbean including warmer and drier weather patterns would favor leafhopper populations. Plant breeders in Puerto Rico have made progress in developing bean germplasm and cultivars through indirect selection for leafhopper resistance at the Isabela Substation. Locally released cultivars such as 'Morales' show less leafhopper damage. The yellow bean germplasm line PR1146-138 was identified in Haiti to have good levels of resistance to leafhoppers (Beaver et al., 2016b). The pinto bean germplasm line TARS-LH1 was screened in Puerto Rico for resistance to leafhoppers (Porch et al., 2020). Trials in Puerto Rico and Haiti were used to select tepary bean breeding lines resistant to leafhoppers.

Brisco et al. (2014) screened at Isabela, Puerto Rico, and Michigan, an inbred backcross line population from the cross 'Matterhorn*2/EMP 507' for reaction to *E. kraemeri* and *E. fabae*. Results from these trials were used to identify QTL associated with resistance to this pest, and lines resistant to both species. A major QTL cluster associated with multiple resistance traits and closely linked to the P color gene was detected for both leafhopper species in multiple seasons on Pv07.

Other bean pests

Martínez (1984) studied the response of common bean cultivars to four races (1, 2, 3, 4) of the root knot nematode (*Meloidogyne incognita*). The snap bean line B4175 (Wyatt et al., 1980) expressed moderate resistance to all races with no significant reduction in shoot or root weight. The white bean line W-117 was only resistant to races 3 and 4. Vicente et al. (1987) studied the influence of temperature and soils from Isabela, Lajas, and Santa Isabel, Puerto Rico, on the histopathology of *Meloidogyne incognita* on snap beans. Greater nematode numbers in roots were observed as temperature increased from 15 to 25 °C. The Fraternidad silty clay from Lajas was most favorable for root knot nematode development.

Smith (2002) conducted a greenhouse study to screen three resistant and three non-resistant common bean lines for reaction to the race 2 of the soybean cyst nematode (*Heterodera glycines*) from Isabela, Puerto Rico, using three infestation densities (0; 4,000; and 8,000 eggs and juveniles per pot). The results suggested that this race of soybean cyst nematode would not affect common bean yield.

Cruz and Cardona (1981) identified the common bean weevil (*Acanthoscelides obtectus*) as a serious pest of stored dry beans in Puerto Rico. The UPR bean breeding program collaborated with researchers from Oregon State University, Sokoine University in Tanzania and the USDA-ARS that led to the release of an Andean red bean germplasm line that combines resistance to the common bean weevil and the *I* and *bc-1*^{2} genes that confer resistance to BCMV and BCMNV (Kusolwa et al., 2016). Mesoamerican black bean breeding lines have also been developed that combine common bean weevil resistance with resistance to BGYMV, BCMV and BCMNV (Beaver et al., 2016a). Mateo (2016) studied segregation patterns of five F_4 populations derived from parents resistant and susceptible to weevil damage. The low frequency of F_4 lines with high levels of resistance to weevils suggested that the complex APA locus is not the only factor associated with resistance to the common bean weevil. This information was confirmed by Kamfwa et al. (2018) who identified three QTL for resistance to *A. obtectus* on Pv04 and on Pv06. One of the QTL on Pv04 was previously reported as the APA resistance locus while the other two QTL were new.

Wolcott (1933) and Leonard and Mills (1931) reported that pod borer damage was a major factor limiting lima bean production in Puerto Rico. Scott (1940) reported that three species of pod borers, namely the legume pod borer *Maruca testulalis* (Geyer), the lima bean pod borer *Etiella zinckenella* (Treitschke) and *Fundella cistipennis* (Dyar), were found infesting wild and cultivated grain legumes throughout Puerto Rico. Lima bean cultivars were screened for pod borer damage in a replicated trial planted in 1936 at Mayagüez, Puerto Rico. The small-seeded pole lima bean cultivar 'Carolina' was reported to be highly resistant to pod borer damage.

Heat and drought stress

Due to the lowland tropical location of bean production in Puerto Rico, high ambient temperature stress and drought are important constraints (de Ron et al., 2019). A study at the University of Puerto Rico projecting future climate change effects on common bean production using Bayesian analysis of a linear mixed model indicated that the predicted increase in temperature will have a negative impact on bean yields in most countries by 2030 (Palomino, 2012). Bean planting seasons in Puerto Rico are historically delineated by abiotic constraints. High temperature and problems associated with excessive rainfall can reduce bean seed yield and quality during the hot summer bimodal rainy season (May to October). The shorter, cooler dry season (December to March) is a more favorable planting season for bean production in Puerto Rico if the farmer has access to irrigation. Compared with seed from beans planted in Honduras in October, del Rio et al. (1991) found that bean seed planted in June had lower germination, less vigor and higher levels of fungal infection.

Drought (terminal and intermittent) is a continuous threat to bean production on small-scale farms in Central America and the Caribbean. Key traits associated with drought tolerance (Ramírez-B. et al., 2008; Ramírez-Builes et al., 2011) have been studied in Puerto Rico. Earliness in combination with selection for harvest index improved drought tolerance (Beaver and Rosas, 1998). Resistance to ashy stem blight can improve tolerance to drought stress (Miklas et al., 1998). Genetics of drought tolerance evaluated over multiple years (Linares et al., 2012, 2014) in Puerto Rico and Nebraska revealed QTL for yield components and leaf temperature. Combined tolerance to heat and drought stress is important given these constraints often overlap in the lowland tropics (Beaver et al., 2003).

Advances have been made in selection indices for heat tolerance (Porch, 2006), in the elucidation of the genetics of heat tolerance (Román-Avilés and Beaver, 2001, 2003) and in the development of improved Andean and Middle American germplasm for abiotic stress tolerance. Early releases (Table 1) with adaptation to high temperature stress include ‘Congo Rico’, a selection from a tropical cranberry bean from the Congo; ‘Palmarejo’ red mottled; and ‘Rosada Nativa› (Beaver et al., 1999a) that was released in Puerto Rico and the Dominican Republic. Line ‘IJR’ (Indeterminate Jamaica Red = PI 163122) was first recognized as being heat tolerant in Puerto Rico (Baiges et al., 1996). Recent lines, purposely bred for stable yield response across varied high day and night temperature environments, include: TARS-HT1 and -HT2 kidneys (Porch et al., 2010), TARS-MST1 black (Porch et al., 2012), ‘Verano’ (Beaver et al., 2008), ‘Bella’ (Beaver et al., 2018a), and snap beans (Wasonga et al., 2010). However, many beans bred and released in Puerto Rico for other traits (described above) exhibit tolerance to high temperatures because it was a common stress in the selection environments: L-226-10 and L-227-1 (Freytag et al., 1985), ‘Verano’ (Beaver et al., 2008), and ‘Badillo’ (Beaver et al., 2010). A GWAS of the BASE 120 trial conducted under heat and drought stress in Juana Díaz, Puerto Rico, identified a genomic region on Pv03 associated with seed yield (Oladzad et al., 2019).

A shuttle breeding program for drought was initiated between the University of Nebraska and ARS-TARS in 2005, resulting in the multistate Dry Bean Drought Nursery (DBDN) and in the development of germplasm. Black bean germplasm SB-DT1 released with tolerance to root rot and drought (Porch et al., 2012) is a product of this program. This shuttle breeding program (Urrea and Porch, 2010) has also led to the introduction of exotic tropical germplasm for broadening the genetic base of elite U.S. germplasm and introducing new traits related to drought tolerance (Traub et al., 2018).

Agronomic Practices and Mineral Nutrition

In 1906, Hendricksen published a bulletin entitled ‘Vegetable growing in Puerto Rico’. The publication noted that, although there was a large amount of importation from Europe, beans were produced in Puerto Rico and local cultivars were considered well adapted for dry bean production. Native white bean cultivars were considered promising although more testing was recommended. Fungal diseases were considered the biggest challenge for bean production.

Most beans currently produced in Puerto Rico are harvested at the green-shelled stage of development. Green-shelled beans are produced in monoculture and intercropped with plantains and other crops having longer growing cycles. Beans and other food crops were intercropped with sugarcane (*Saccharum officinarum*) to increase the supply of locally produced food during World War II. Lugo-López et al. (1953) observed that bean/sugarcane intercropping had no significant effect on yield of beans or on the ratoon crop of sugarcane. Compared with sugarcane monoculture and intercropping with other food crops, the bean/sugarcane intercrop produced the greatest mean profit per acre. Liu et al. (1999, 1997) studied the feasibility of intercropping beans with plantains (*M. acuminata* × *M. balbisiana*) at the Corozal Substation. Plantain yield was not affected by the intercrop of green-shelled beans. Compared with a plantain monoculture, intercropping beans produced $4,894/ha greater net income. Lui et al. (1999) conducted a similar study in Aguas Buenas, Puerto Rico, in which bananas (*M. acuminata*) and green-shelled beans were intercropped. Two intercroppings of the bean cultivar 'Arroyo Loro' planted in November produced the highest combined net income ($6,433/ha).

The performance of pole snap beans was tested in trials planted at Isabela in April 1959 (Ramírez and Abrams, 1960). The pole bean cultivars 'Florigreen' and 'Blue Lake 231' produced whole pod yields > 15 t/ha. 'Florigreen' was the highest yielding pole snap bean in pole bean trials conducted at the Gurabo Substation (Ramírez and Vélez-Santiago, 1962). 'Florigreen' was resistant to BCMV and rust, which were considered to be serious bean diseases. At Adjuntas, Mangual-Crespo and Torres (1979) evaluated the performance of pole bean cultivars in within-row plant spacings ranging from 8 to 30 cm with a spacing of 90 cm between rows. The pole bean lines planted with 8 cm within-row spacing produced a mean yield of 11.6 t/ha.

Mangual-Crespo (1975) reported that the determinate cultivar 'Contender' produced the highest yield of marketable snap beans when planted at the Isabela Substation from December to March. The determinate snap bean cultivar 'Harvester' produced approximately 90% of No. 1 grade beans at the Isabela Substation in trials planted in September, December and March (Rodríguez et al., 1979). Mangual-Crespo (1977) compared single and multiple harvests of determinate snap beans planted at the Isabela Substation. Because no significant differences were found between harvest systems, the author recommended a single harvest to lower production costs. Preliminary results from a trial conducted at the Fortuna Substation found no significant difference in yields of snap beans harvested by hand compared to the use of a mechanical harvester (Mangual-Crespo and González, 1981). Results from field trials conducted at the Lajas Substation demonstrated that determinate snap beans can be produced successfully in Puerto Rico under an organic management system (Brunner et al., 2014).

Badillo-Feliciano et al. (1985) noted that harvesting beans at physiological maturity, which is 15 days earlier than when 'dry' beans are harvested, has several advantages. Earlier harvests reduce the risk of crop loss due to unfavorable weather and late season diseases. Yields of whole pods harvested at physiological maturity were at least three times the yield of dry beans at both the Isabela and Fortuna Substations. Lastly, green-shelled beans are more profitable because they command almost twice the market value as dry beans.

Research on when to initiate harvest was conducted to optimize yield and quality of green-shelled beans. Cerna and Beaver (1989) studied seed weight accumulation patterns of two Mesoamerican bean lines over two growing seasons. Physiological maturity (maximum seed weight accumulation) occurred when approximately two green pods remained at the top of the indeterminate bean plants. This was proposed as a convenient visual marker for physiological maturity of small-seeded indeterminate beans. Román-Hernández and Beaver (1997) concluded that appearance of the first dry pod was a useful morphological marker to begin the harvest of green-shelled beans. Initiating harvest at this stage of development represented a compromise between yield potential and the quality of green-shelled beans. Further research showed that harvest of green-shelled beans could be delayed as much as one week after the appearance of the first brown pod without losing green-shell bean yield (Beaver, 2000).

Beaver and Román-Hernandez (1994) evaluated the performance of white bean lines in field trials planted at the Isabela and Fortuna Substations at different dates over a four-year period. Although green-shelled beans can be produced in Puerto Rico over a wide range of planting dates, the October to December planting dates produced the highest yields. Green-shelled bean yields were lower and more variable when beans were planted during the warm and humid summer months. However, higher prices help to offset the greater risk associated with green-shelled bean production during the summer. Results from this study led to the development of the white bean cultivar 'Verano' that was selected for better adaptation to hot and humid conditions (Beaver et al., 2008). Beaver (2000) noted that DOR 364 produced greater yield than 'Arroyo Loro' by partitioning a greater portion of whole pod weight into green-shelled bean yield which suggested a trait for selecting beans with improved green-shelled bean production.

Dry bean production has potential in Puerto Rico as a crop planted in rotation with basic grain and forage crops such as maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], pigeon peas [*Cajanus cajan* (L.) Millsp.] and soybeans [*Glycine max* (L.) Merr.]. Commercially acceptable seed yields are achieved when adapted bean cultivars are planted using recommended production practices. The UPR AES has developed a Technology Package that describes the recommended production practices for dry and green-shelled bean production in Puerto Rico.

The production of dry bean in Puerto Rico is recommended for the drier and cooler months when disease pressure is lower and higher quality seed can be produced. Guadalupe (1977) evaluated the performance of dry bean lines planted over four planting dates at the Lajas Substation. The December planting produced the highest seed yield. The lowest seed yields were obtained from the September planting date although the percentage of protein of the seed harvested from the September planting date was higher than the other seasons. Lozano et al. (1983) evaluated the effect of planting date on the seed yield of dry beans planted in Orocovis and Gurabo, Puerto Rico. Seed yields at Orocovis were greatest for the March and May planting dates, whereas Gurabo had the highest yields when planted in January, March and November. Lower seed yields at Gurabo for the May, July and September planting dates were attributed to higher temperatures and excessive rainfall.

Agronomic practices can have a significant effect on the performance of green-shelled and dry beans. González-Ríos and Riollano (1951) compared the performance of white bean landraces in 30- and 60-cm row widths and obtained higher yields in the 30-cm row width. Badillo et al. (1978) evaluated the performance of a determinate light red kidney bean and an indeterminate white bean when planted at the Isabela Substation at spacings of 30, 45 and 60 cm between rows and corresponding plant populations of 444,000; 296,000; and 222,000 plants per hectare. No significant differences in seed yield were observed between the row spacings. Beaver et al. (1988) evaluated the performance of bean lines at Isabela and Juana Díaz, Puerto Rico, when planted at different between and within row spacings. The highest seed yield was obtained at the Fortuna Substation when beans were planted in 0.5-m row widths and a within row seeding rate of 19.8 seeds per meter. Seed yield increased in a linear fashion as within row seeding rates increased from 6.6 to 19.8 seeds per meter.

Weed control is one of the greatest challenges facing bean producers in the humid tropics. Semidey et al. (2007) demonstrated in field trials conducted at Isabela and Juana Díaz, Puerto Rico, that the sequential use of pre and post emergent herbicides in combination with mechanical control can effectively control weed populations and can result in significant increases in bean seed yield.

Lugo-Mercado et al. (1984) studied the effect of tillage on the performance of beans and other crops at the Fortuna Substation in Juana Díaz, Puerto Rico. They found no significant differences in bean seed yield in tilled and non-tilled plots. Vicente-Chandler et al. (1966) also demonstrated that beans planted in trials on hillsides in Adjuntas and Orocovis, Puerto Rico, produced equivalent yields using conventional and minimum tillage.

Soil acidity and low nitrogen (N) and low phosphorus (P) availability are important edaphic constraints for bean production in Puerto Rico. Lugo-López (1977) evaluated the response of the white bean cultivar 'Bonita' to varying levels of N in field trials planted at the Isabela Substation. Using the Capó fertilizer-yield equation, they found that 80 kg/ha of N produced > 95% of maximum seed yield (1,461 kg/ha). Del Valle et al. (1981) reported that 'Bonita' responded to banded P fertilizer, whereas there was no response to broadcast residual P in field trials planted in Cidra, Puerto Rico.

Dorcinvil et al. (2010) conducted field trials at the Isabela Substation and identified the black bean line PR0443-151 and the small red line VAX 3 from CIAT to produce among the highest seed yields in both low N and low P soil treatments. Line PR0443-151 combined N use efficiency (kg of seed yield/kg of N in the soil) and agronomic efficiency (kg of seed yield/kg of N applied) to N fertilization. Using a commercial peat inoculant, Ramírez (2009) reported that VAX 3 and PR0443-151 were among the most responsive bean lines to seed inoculation with *Rhizobium* spp.

Ruiz-Quiles (2010) evaluated the performance of Mesoamerican bean lines in the F_3, F_4 and F_5 generations at the Isabela Substation, Puerto Rico, in trials that received low levels of N (20 kg/ha) fertilization. Several lines from the cross 'PR0518-10/PR0401-257' had superior performance in this low-N environment. A few of these lines had superior performance in BASE trials conducted at the Isabela Substation over a four-year period (Beaver et al., 2019). One of these lines was formally released in Puerto Rico as the black bean cultivar 'Hermosa' that combines resistance to BGYMV and BCMV with greater tolerance to low soil fertility and high temperature (Beaver et al., 2018b). Porch et al. (2014a) released the small red bean germplasm line TARS-LFR1 and black bean germplasm lines TARS-MST1 and SB-DT1 (Porch et al., 2012) with superior performance in low fertility soils. It is noteworthy that all the breeding lines released in Puerto Rico with improved adaptation to low fertility soils also expressed root rot resistance.

Results from field trials conducted by Abruña et al. (1983) in Corozal and Isabela, Puerto Rico, demonstrated that seed yield of dry beans declined rapidly when soil pH was lower than 4.7 and Al saturation of the effective CEC of the soil was greater than 30%. They also found a positive correlation between the Ca content in bean leaves and seed yield. Badillo-Feliciano and Lugo-López (1981) did not find a significant seed yield response of beans to liming on the Coto Clay (Oxisol) at the Isabela Substation that had a pH of 5.0. Abruña et al. (1974) reported that snap beans produced yields ranging from 6,800 to 13,600 kg/ha when soils in Corozal, Isabela and Jayuya, Puerto Rico, were limed appropriately. Increased Ca and decreased Mn content in leaves, greater soil base content and increased snap bean yield were observed in response to liming.

Bueso-Campos (1989) conducted greenhouse studies to evaluate the effect of acidity, Al concentration, aeration and mechanical impedance of a Maricao clay (a strongly acid soil) on the development of the roots of the white bean line L-227-1. Roots were shorter, thicker and had less branching in soil treatments having greater levels of acidity and Al concentrations and/or more dense soils. Approximately 15% of soil aeration (relative oxygen diffusion coefficients of 0.05 to 0.06) was optimal for shoot growth. Mechanical resistance > 1.39 MPa began to restrict shoot and root growth.

Goenaga and Smith (2002) studied the response of common bean to soil Al concentration in field trials conducted at Corozal, Puerto Rico. An increase in soil Al concentration from 0.68 cmol/kg to 2.5 cmol/kg significantly reduced plant dry matter and reduced concentrations of leaf P, Ca and Mg. Concentrations of leaf Fe and Al increased in soils with greater Al concentration. The authors suggested that the selection of Ca-efficient genotypes may help to identify bean genotypes with greater Al tolerance. Smith and Goenaga (2004) conducted field trials in acid soils at the Corozal Substation to determine if a differential response to exchangeable soil Al in the seedling/ vegetative stage is associated with response to exchangeable soil Al in mature snap bean plants. No association was found due to the complex response of snap bean plants to Al toxicity in acid soils.

Biological Nitrogen Fixation

Because many tropical soils are low in nitrogen, Schröder (1992) noted that biological nitrogen fixation (BNF) should play an important role in lower-input, sustainable agriculture systems. The common bean, unfortunately, contributes less to BNF than many other grain legumes. The short growing cycle of beans produced in Puerto Rico and the sensitivity of the symbiotic relationship to adverse environmental conditions such as acid soils, drought and high temperature create challenges to enhance BNF.

Velázquez et al. (1988) observed that inoculation is often unsuccessful because, after inoculation, locally adapted *Rhizobuim* strains with poor BNF capacity continue to occupy most of the nodules. Mangual-Crespo et al. (1987) observed in Juana Díaz, Puerto Rico, reduced nodule size and numbers on the cultivar 'Bonita' as the level of N fertilization increased from 0 to 360 kg/ha.

Cordero-García (1979) found the black bean cultivar 'La Vega' to produce the highest yield when inoculated with *Rhizobium phaseoli.* Cataño-Cataño (1990) conducted field trials at the Isabela Substation to compare traits related to biological nitrogen fixation of determinate and indeterminate bean lines in fertilized (50-100-50 kg/ha of NPK applied as granular fertilizer) and non-fertilized plots. All plots were inoculated with a mixture of *Rhizobium etli* (CIAT 632) and *R. phaseoli* (Viking 1 and Co-5) strains. Significant differences among lines were observed for nodule number, nodule dry weight, total N fixed, and N fixed per day. There were significant genotype x level of fertilizer (0 vs. 50 kg/ha of N) interactions for nodule number and nodule weight. The Dominican red mottled landrace cultivars 'Pompadour B' (determinate) and 'Pompadour E' (indeterminate) had the best overall combinations of BNF characteristics including greater nodule numbers and dry weights.

Fernández-Toledo et al. (1997) evaluated nodulation of 30 elite lines planted during the winter and summer months at Isabela, Puerto Rico, over two years. The trials were not fertilized or inoculated to allow nodulation with endemic soil *Rhizobium* prevalent under environmental conditions in the area. The black bean line XAN 176 produced mean seed yields > 1,000 kg/ha during both planting seasons and produced > 50 nodules per plant (good according to the CIAT scale). Line XAN 176 also produced significantly greater nodule weight during the summer plantings than the small red cultivars 'Don Silvio' and 'Dorado' (DOR 364).

Heilig et al. (2017) evaluated the biological nitrogen fixation (BNF) characteristics in a RIL population from the cross between the black bean cultivars 'Puebla 152' and 'Zorro' in field trials conducted in East Lansing, Michigan, and Isabela, Puerto Rico. They identified 17 unique QTLs that were mostly found in clusters on Pv01, Pv06 and Pv08. Many of the QTLs associated with enhanced BNF or more efficient partitioning of N were associated with candidate genes expressed in roots and nodules. A QTL for seed yield in Puerto Rico on Pv03, contributed from the 'Puebla 152' parent, was identified in both 2012 and 2013.

Recent studies of the symbiosis between common bean and the nitrogen-fixing bacteria *Rhizobium* showed that it is important to determine which *Rhizobium* spp. strain is most effective for a specific genotype and environmental condition to achieve efficient nodulation and biological nitrogen fixation. Rosas et al. (2012) observed that *Rhi-*

zobium tropici strain CIAT 899 increased nodule and plant biomass in bean cultivars ‘Macuzalito’ and ‘Don Silvio RR’ when compared with *Rhizobium tropici* strain CR 477. In contrast, G21212 performed better with strain CR 477 than with CIAT 899. A growth pouch experiment under controlled conditions (Estévez de Jensen et al., 2011) demonstrated red mottled line PR0737-2, inoculated with *Rhizobium tropici* strain CIAT 899, had higher nodule positions but fewer nodule numbers, whereas inoculation with UMR 1597 resulted in greater nodule numbers in PR0737-2. Conversely, the pink bean line PR0401-259 produced the highest number of nodules among the lines when inoculated with UMR 1899 and the least number of nodules when inoculated with UMR 1597. Lines PR0737-1 and TARS 10IS-2421 showed good nodulation in the uppermost root region when inoculated with either strain. Screenhouse trials were conducted at Juana Díaz, Puerto Rico, to examine the nodulation characteristics of 118 common bean and two tepary bean lines from the BASE 120 trial. The number of nodules on roots of plants inoculated with *Rhizobium tropici* strain CIAT 899 and *Rhizobium etli* strain CIAT 632 were significantly different ($P > 0.05$) among genotypes. Genotypes BFS 139 and ‘ICA Pijao’ had more than 50 nodules per plant in the upper 3 cm of the root in two of three trials (Estévez de Jensen et al., 2017).

Inheritance for nodulation traits was explored with RILs from a cross between ‘Jamapa’ (Mesoamerican) and ‘Calima’ (Andean). The RILs expressed a wide range in nodule number including lines that produced significantly more nodules than either parent and lines that produced very few nodules when inoculated with a specific *Rhizobium* strain. Some lines produced > 50 nodules per plant. Narrow sense heritability estimates for nodule number per plant ranged from 0.40 to 0.59 and for nodulation score from 0.41 and 0.43 (Guachambala et al., 2013).

Uptake hydrogenase (Hup) activity oxidizes the hydrogen produced during nitrogen fixation and may lead to enhanced biological nitrogen fixation. Navarro et al. (1993) observed (Hup) activity in beans inoculated with *Rhizobium tropici*, although Hup activity was insufficient to eliminate hydrogen evolution from root nodules. Genotype x strain interaction was also observed for Hup activity.

Bean seed quality characteristics

Rodríguez-Sosa et al. (1984) studied water absorption and cooking properties of 22 dry bean lines and cultivars from the Caribbean. Differences among genotypes were observed in time to achieve 50% hydration. In sensory evaluations of the beans cooked using a traditional recipe from Puerto Rico, all the genotypes had acceptable appearance and flavor. Texture was the most important attribute contributing to the acceptability of cooked beans.

Hosfield and Beaver (2001) studied cooking time and water absorption of 37 dry bean lines and cultivars. A significant negative association (r = -0.84) between cooking time and water absorption was found. Pinto beans were the fastest cooking market class, whereas the small-red lines and cultivars from Central America were the slowest cooking.

Tulmann et al. (1978) found no relationship between percentage protein of dry bean seed from plants grown at different plant densities or from pods harvested from different positions on the plant. During the 1970s and 1980s, L. Telek and G. Freytag, USDA-ARS scientists based in Mayagüez, Puerto Rico, studied tannin and protein content of dry beans. In a personal communication from L. Telek, Shelli-Dessert and Bliss (1991) noted that a major difficulty in breeding for lower tannin content was the lack of a reliable assay for measuring tannin content in bean seed. Miklas (1995a) released three Mesoamerican bean germplasm lines that had higher levels of seed protein. One of the parents used as a source of higher protein was the small red breeding line 15R-148 developed by Freytag.

Seed yield, phenology and breeding methods

Bliss (1980) reported that common beans in Puerto Rico had outcrossing rates as high as 15 to 20% due to activities of the carpenter bee (*Xylocopa brasilianorum* L.). This report was a cause of concern for local bean breeding, seed production and winter nursery activities. Brunner and Beaver (1989) reported outcrossing rates of beans at the Isabela Substation were sufficiently low to permit the use of conventional methods for breeding self-pollinated crops. Dry bean winter nurseries have been conducted at the Isabela Substation for more than 35 years and genetic impurity has not presented a significant problem.

Beaver et al. (1985a) evaluated the yield stability of determinate and indeterminate red mottled bean lines based on trials conducted in the Dominican Republic. Indeterminate lines in the trials tended to have greater and more stable seed yields. Additional field trials were conducted in the Dominican Republic and Puerto Rico to compare the performance of determinate and indeterminate red mottled bean lines (Beaver et al., 1996). Indeterminate lines were identified for the most desirable combination of yield stability characteristics including high mean seed yields, coefficients of regression near unity and minimum deviations from regression.

Smith (2004) reported that the use of geometric means from stressed and non-stressed yield trials were effective in identifying the highest-yielding lines across environments. Deviations from regression may be useful to identify new sources of stress tolerance in lines derived from wide crosses. Novel gene combinations for stress tolerance should be further studied and introgressed into genetic backgrounds with better adaptation.

Durán et al. (2005) described morphological and molecular characteristics of larger-seeded bean landraces from Puerto Rico, the Dominican Republic and Haiti. Bean landraces with indeterminate growth habits tended to produce higher seed yields than determinate landraces in field trials conducted at Isabela, Puerto Rico, over a two-year period. Several of the landraces with Andean seed types were found to have the S phaseolin allele from the Middle American gene pool, which suggested the lines were the result of inter-gene pool introgression.

Mateo-Solano et al. (1989) estimated heritabilities and phenotypic correlations for seed yield and morphological traits of indeterminate bean lines derived from crosses between small-seeded indeterminate and larger-seeded determinate genotypes. Greater seed yield was associated with greater numbers of branches and nodes, taller plants, greater biological yield and higher harvest indices. Heritability estimates for seed yield and morphological traits were generally low to intermediate. Replicated yield trials of advanced generation lines was suggested to be the most effective approach for identifying large-seeded indeterminate bean lines with greater seed yield potential.

Beaver and Kelly (1994) compared different selection methods for bean populations derived from crosses between gene pools. Recurrent selection with evaluation of F_5 lines identified indeterminate lines having seed yields greater than determinate check cultivars. These indeterminate populations tended to have longer reproductive periods and a greater number of days to reach physiological maturity.

These results led to a breeding objective at the University of Puerto Rico to develop indeterminate Andean bean cultivars for the Caribbean. The indeterminate light red kidney cultivar 'Badillo' was released in Puerto Rico in 2010. The indeterminate and BGYMV resistant red mottled bean lines PR0737-1 and PR0633-10 were released in Puerto Rico and Haiti in 2014.

Unander et al. (1989) studied the yield stability of 25 small-seeded indeterminate breeding lines and cultivars of diverse origin based on results from 23 field trials conducted in Honduras. Although large rank order differences among genotypes were observed when mean seed yields of different seasons and departments (local production regions) were compared, a few entries in the trial were ranked in the top third in all seasons and departments.

Cerna and Beaver (1990) estimated the narrow sense heritabilities (NSH) of days to first flower, days to physiological maturity and length of the reproductive period (days between flowering and physiological maturity) of smaller-seeded indeterminate beans. The NSH estimates for days to first flower were sufficiently large to suggest early generation selection for this trait would be effective. Genetic correlations between days to first flower and reproductive period were, in large part, negative and significant. These results indicated that a longer reproductive period could be achieved by selecting lines for reduced days to first flower.

Beaver and Rosas (1998) further studied the lengths of reproductive periods and the rates of seed mass accumulation of smaller-seeded indeterminate beans. The NSH of reproductive periods ranged from intermediate (0.43) to high (0.83), whereas NSH estimates for rates of seed weight accumulation were low (0.24). Higher-yielding lines were identified that combined longer reproductive periods with greater rates of seed weight accumulation.

University of Puerto Rico researchers collected field data in Isabela for collaborative research designed to better understand the response of beans to different photoperiod and temperature regimes (Bhakta et al., 2017; Wallace et al., 1995). Other collaborative bean field trials were conducted to study rates of biomass accumulation and partitioning of biomass into seed yield (Wallace et al., 1993).

Ramírez-Builes et al. (2008) developed linear models to estimate leaflet area of Mesoamerican common bean. Linear models using the maximum leaflet lengths and widths produced reliable estimates of leaflet area under greenhouse and field conditions and at different stages of development of the bean plant.

The University of Puerto Rico and other bean research programs in Central America and the Caribbean have limited resources, increasing the utility of small plot sizes. Beaver and Kelly (1989) studied the performance of determinate and indeterminate bean lines planted in replicated 1-m hill-plots in populations ranging from 2 to 10 plants. Number of seeds per pod and 100-seed weight were not significantly different in 1-m hill-plots ranging from 2 to 10 plants per hill-plot. Seed yield per plant increased in a linear fashion as number of plants per hill-plot decreased. Seed yield per hill-plot of both determinate and indeterminate plants decreased in a linear fashion as the number of plants per hill-plot decreased. A covariance analysis using numbers of plants per hill-plot as the covariate could be used to adjust seed yields for variation in plant populations in hill-plots.

Grain legume breeders can harvest whole pods rather than single seeds to advance generations of breeding populations of self-pollinated crops. This is an especially valuable tool for bean breeders working in the tropics where multiple generations per year can be planted. Results from a simulation study (Macchiavelli and Beaver, 2001) showed that an increase in the number of whole pods harvested from the F_2 to the F_6 generation did not influence significantly the mean proportion of F_2 plants represented in the F_6 generation, although variability decreased. Using this multiple-seed procedure, bean breeders could expect that at least every third F_6 line would be derived from a different F_2 plant, which should preserve enough genetic variability for the selection of quantitatively inherited traits such as seed yield.

Winter Nurseries

In 1935, Rexford Tugwell, Under Secretary of the U.S. Department of Agriculture, requested a report to re-evaluate the role of the USDA Puerto Rico Agricultural Experiment Station. One recommendation was that winter nurseries in Puerto Rico could assist continental U.S. maize (*Zea mays* L.) breeding programs. This proved to be a very valuable recommendation not only for maize breeders but for breeders of many other crops. The USDA-ARS Tropical Agriculture Research Station has several decades of experience hosting winter nurseries for maize and soybeans and conducting a backcrossing program to convert tropical sorghum [*Sorghum bicolor* (L.) Moench] germplasm into genotypes that allow their performance to be evaluated under temperate conditions (Sotomayor-Ríos and Torres-Cardona, 1984).

Since 1981, the University of Puerto Rico has planted dry bean winter nurseries for bean breeding programs from Michigan State University, the University of Nebraska, North Dakota State University and the USDA-ARS. The winter nursery is a collaborative activity of Regional Hatch Project W-3150 (https://www.nimss.org/). The winter nurseries have enabled public bean breeders from the U.S. to advance early-generation breeding lines at a more rapid pace and to evaluate material for reaction to diseases such as common bacterial blight and rust. Reciprocally, the winter nursery provides the University of Puerto Rico bean breeding program with an opportunity to evaluate lines from different bean breeding programs for adaptation to local conditions. Most of the bean cultivars released during the past 20 years by Michigan State University, North Dakota State University and the University of Nebraska had at least one generation planted in winter nurseries in Puerto Rico during their development. Planting beans in the winter nursery allows indirect selection for adaptation to the lowland tropics. Farmers in some countries such as the Petén region of Guatemala and southern Mexico have recognized this adaptation and have adopted black bean cultivars such as 'Zorro' from Michigan (Kelly et al., 2009).

The private sector also provides winter nursery services in Puerto Rico for bean breeding programs in North America. In recent years, Illinois Crop Improvement[7] and Third Millennium Genetics have provided winter nursery services for public and private bean breeding programs.

Tepary bean

Wild relatives of common bean and related species, including *P. acutifolius*, *P. coccineus, P. costaricensis* and *P. dumosus,* represent valuable sources of genetic diversity for the improvement of common bean (Porch et al., 2013b). Interspecific crosses with the tepary bean have been used to improve resistance of common bean to common bacterial blight (Singh and Miklas, 2015) and bruchids (Kusolwa and Myers, 2011).

Seminal research on the origins and diversity of wild *Phaseolus* sp., including tepary bean, over several decades in Puerto Rico and Colombia resulted in a monograph for the *Phaseolus* genus by Freytag and Debouck (2002). In the 1980s, taxonomic data were collected on wild *Phaseolus* germplasm in screenhouses and growth chambers at ARS-TARS in Mayagüez and in the field at agricultural experiment substations across Puerto Rico. In addition to taxonomic studies, protein gel electrophoresis was used to elucidate the diversity within and between *Phaseolus* species with the greatest diversity found within wild types of *P. acutifolius* (Sullivan and Freytag, 1986).

Interest in tepary population structure, genetic diversity, and applications in breeding has continued. Recent research at the ARS-TARS using GBS-developed SNP markers assayed across the Tepary Diversity Panel (TDP) composed of 156 domesticated and 158 wild tepary accessions, found eight sub-populations of tepary bean, two domesticated and six wild. The results affirm a bottleneck effect as a result of domestication where domesticated tepary bean is narrow genetically, while the wild sub-populations harbor most of the genetic diversity (Hart et al., 2019).

The first published record of tepary bean field evaluations in Puerto Rico showed low yields for the 'White tepary' bean variety (Snyder, 1918). 'White tepary' was then crossed with the common bean 'Puerto Rico Red' and hybrids were produced but subsequently lost due to bacterial blight disease (Snyder, 1918). The next records of tepary evaluation in Puerto Rico were field trials of wild *P. acutifolius* in the 1980s

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[7] The USDA is an equal opportunity provider and employer. Mention of company or trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or by the Agricultural Experiment Station of the University of Puerto Rico.

at the Fortuna Substation in Juana Díaz (Freytag and Debouck, 2002). Later, field evaluations of eleven domesticated tepary bean lines in Puerto Rico and Honduras, from 1989 to 1994, determined production potential for the crop (Miklas et al., 1994b). Recent multi-year evaluations of the TDP in Puerto Rico and Honduras (Rodríguez, 2017) show similar yield potential between tepary and common bean under non-stress, but superior yield potential of tepary bean under drought stress (Porch et al., 2009). There is no record of commercial production of tepary bean in Puerto Rico.

Tepary bean shows broad abiotic stress tolerance, but it has limited disease resistance, likely due to less exposure to pathogens in its arid origin and no history of modern crop improvement methods. Although relatively little work has been done on tepary bean response to disease, much of the published research was conducted in Puerto Rico. Vakili (1976) evaluated tepary bean response to root rot, rust, common bacterial blight and BCMV. Tepary bean was susceptible to *Bean common mosaic virus* (BCMV) in Puerto Rico, and a 25% seed transmission rate of BCMV was observed (Kaiser, 1981). Recent BCMNV evaluations of the TDP found less pronounced symptoms in tepary bean as compared to common bean under high temperature screenhouse conditions. Susceptible accessions, as determined using ELISA, often show no phenotypic symptoms or symptoms that differ from those in common bean. Using ELISA and phenotypic evaluation, only one accession in the TDP was found to be resistant to the NL-3 strain of BCMNV. A GWAS of the TDP based on NL-3 ELISA results identified two preliminary genomic regions conferring resistance to BCMNV (unpublished). A set of 12 domesticated tepary bean lines were evaluated for response to BGYMV in Juana Díaz with four lines showing tolerance under these conditions (Miklas and Santiago, 1996), while a small subset of the TDP showed tolerance to BGYMV at Zamorano in Honduras in 2015 (unpublished). Miklas et al. (1998a) did a follow-up study on the 12 tepary lines and three showed resistance to ashy stem blight, and to Fusarium wilt, caused by *Fusarium oxysporum*, while one line, PI 321637-s, showed tolerance to all three pathogens. Field screening of breeding lines has resulted in improved resistance to ashy stem blight. However, collaborative evaluations with Colorado State University over multiple years found no resistance to the *Fusarium solani* root rot strain(s) endemic at the Agricultural Research, Development and Education Center in Ft. Collins within the breeding lines and TDP accessions evaluated. Conversely, tepary lines were identified with resistance to the local root rot complex, that includes *Fusarium solani*, in the TARS Isabela root rot nursery (Porch, Personal communication). In collaboration with the University of

Puerto Rico, breeding lines were evaluated for bruchid resistance and selected for release (Porch et al., 2013a). Inheritance of resistance to CBB and rust in select tepary bean lines was already described in earlier sections. Additionally, evaluation of a tepary RIL population to two *Xcp* strains found four QTL conditioning CBB resistance at 21 days after inoculation (Rodríguez, 2017).

The response of tepary bean to high temperature stress (summer trials) and drought (winter trials) were conducted in the semi-arid UPR Fortuna Substation in Juana Díaz over multiple years during the 2010s and 1990s (Miklas et al., 1994b). In these trials, tepary bean yielded less than common bean in the non-stress environment and yielded 170% of common bean in the high temperature Juana Díaz location (Miklas et al., 1994b). Recent evaluations of the TDP, breeding lines, and G40001 x G40022 RIL population showed exceptional heat tolerance with little or no reduction in yields under average maximum (33° C) and minimum (23° C) temperatures present at the Juana Díaz location. Terminal drought stress did result in seed yield reduction, with variability in response found and QTLs identified for yield in the RIL population (Rodríguez, 2017). Traub et al. (2018) showed key photosynthetic advantages of TB1, a tepary bean previously tested in Puerto Rico (Ramírez et al., 2009), compared to common bean lines developed for drought tolerance. The introgression of drought tolerance from wild tepary bean to common bean, by the University of Saskatoon, was shown in collaborative drought and non-stress evaluations at the Isabela Agricultural Experiment Substation (Souter et al., 2017).

The TDP and two interspecific (Pv x Pa) populations were evaluated for their ability to nodulate with *Rhizobium* and *Bradyrhizobium* strains. *Bradyrhizobium* strain USDA 3254 was found to be broadly efficient in tepary bean. Of the TDP accessions, G 40142 and G 40161 exhibited superior ability to nodulate with strain USDA 3254. Transgressive interspecific lines were identified that showed superior nodulation scores compared to their parents (Vargas-Palacios, 2016). Future studies could involve selection of both bacteria and host for improved nodulation under abiotic stress.

A study on elemental composition and cooking time found tepary bean within the range of common bean for cooking time, elemental and overall nutritional composition (Porch et al., 2016). The exceptions were tepary, which had a significantly higher sucrose concentration and reduced fat and ash concentrations compared to common beans. Previous research in Puerto Rico showed reduced proanthocyanidin (tannins, an anti-nutritional factor) in the tepary lines evaluated compared to 'Dorado' small red bean (Miklas et al., 1994b). Continued promotion of tepary bean, such as the use of TARS-Tep 22 for the Culinary Breeding Network's 2016 Variety showcase in Oregon may contribute to its broader culinary adoption.

Since 2008, ARS-TARS has focused on improving tepary bean germplasm by generating novel genetic variability for seed quality, and disease and insect resistance, using traditional hybridization between selected tepary accessions (Porch and Beaver, 2012). TARS-Tep 22, a white seeded type with common bacterial blight, rust, and bruchid resistance, and with more erect architecture (Porch et al., 2013a), is the first release from this breeding effort. Another release TARS-Tep 32, with larger seed size and bacterial blight resistance, was selected from a yellow seeded landrace PI 477033. Both releases are broadly adapted with good yield under drought and higher temperature conditions. Efforts to introgress disease resistance from common bean to tepary bean were largely unsuccessful due to the wide genetic distance between species. A shuttle breeding program has been used by USDA-ARS-TARS and Colorado State University bean breeders to develop broadly adapted tepary bean breeding lines.

Through an ongoing collaboration with Michigan State University, North Dakota State University, University of Saskatchewan, and Hudson Alpha, the tepary bean genome is being sequenced from a widely studied accession, G40001 (Buell et al., 2018). This tepary bean genotype was previously used in the development of the core RIL population G40001 x G40022, because it exhibits key traits such as broad adaptation, good seed quality, low soil fertility tolerance and leafhopper resistance. It also shows resistance or tolerance to common bacterial blight, angular leaf spot, and web blight.

Lima beans

The lima bean is a traditional crop in the Caribbean. Hendricksen (1906) reported that lima beans with 'sieva' seed types were produced in Puerto Rico on a limited scale. Lima beans are referred to in Puerto Rico as 'habas'. Most landraces in Puerto Rico have an indeterminate growth habit and are often found growing on fence rows (Montero-Rojas et al., 2012). Caribbean lima bean landraces tend to be photoperiod sensitive and produce pods during the dry winter months. Lima beans are more heat and drought tolerant than common beans (Beebe et al., 2013). These traits contribute to food security in countries such as Haiti where significant areas of lima beans are planted each year.

Mackie (1943) noted that lethal levels of hydrocyanic acid (HCN) were found in some lima bean landraces from Puerto Rico. Montero-Rojas et al. (2013) reported that outbreaks of poisoning due to consumption of lima beans were reported in Puerto Rico during the beginning of the 20th century. Viehoever (1940) identified a lima bean landrace 'Puerto Rico black' with HCN concentration of 3,000 mg/L. The lima bean landrace variety, 'L-121', from Puerto Rico was used to study the inheritance of anti-A1 hemagglutinating activity (Schertz et al., 1960).

In 1933, the USDA-PRAES and the College of Agricultural Science of the University of California cooperated in the evaluation of seven lima bean cultivars from California. The pole lima bean 'King of the Garden' landrace cultivar produced the highest yield at four months after planting. In the 1938 report of the USDA AES in Mayagüez, sieva landraces of lima bean from Puerto Rico were found to be resistant to pod borers and seed was sent to interested researchers in the United States.

Mackie (1943) noted that some smaller-seeded lima beans had nematode resistance. Allard (1954) screened 261 lines from the California Agricultural Experiment Station Accession and Introduction Collection for reaction to root-knot nematode (*Meloidogyne incognita*). Among the 12 lines identified to have resistance, six lines were from Puerto Rico (L-32, L-136, L-137, L-139, L-141 and L-142). The root-knot nematode resistant lima bean cultivar 'Cariblanco N' was released by the California Agricultural Experiment Station in 2001 (Helms et al., 2004). 'Cariblanco N' was derived from a cross that used L-136 as the source of root-knot nematode resistance.

Mackie (1943) presented color plates of lima bean landraces from Puerto Rico. Unfortunately, these lines were not included in the USDA bean germplasm collection. At present, there are only two accessions of lima bean from Puerto Rico in the USDA germplasm collection. Ruiz et al. (2010) evaluated 15 lima bean landraces from Puerto Rico in a trial planted at Isabela. They reported significant differences among landraces for seed, leaf and pod type, days to flowering, seed yield and concentration of HCN in the leaves and seed. In Puerto Rico, PL-08-14 was the only landrace cultivar that had early flowering (51 DAP) with a HCN concentration < 100 mg/L in the seed, which is the maximum concentration recommended for the release of lima bean cultivars in the United States. Montero-Rojas et al. (2013) evaluated the agro-morphological characteristics of the collection of 50 landraces from Puerto Rico, the Dominican Republic and Haiti. These Caribbean lima bean landraces were found to belong to the Middle American gene pool. A few of these lima bean landraces had the desirable combination of high levels of the cyanogenic glucoside linamarin in the leaves (favorable for pest control), and low levels of linamarin in the seeds. This collection of Caribbean lima bean landraces was included as accessions in the CIAT bean germplasm bank.

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