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SUSCEPTIBILITY OF *BLISSUS INSULARIS* (HETEROPTERA: HEMIPTERA: BLISSIDAE) POPULATIONS IN FLORIDA TO BIFENTHRIN AND PERMETHRIN

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ABSTRACT

The southern chinch bug, *Blissus insularis* Barber, is a serious insect pest of St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze). Control for *B. insularis* is mainly achieved through insecticides. This pest has developed resistance to several insecticide classes because of near-constant exposure. The goals of this study were to sample select *B. insularis* populations in Florida to describe their susceptibility to bifenthrin, document new locations of bifenthrin resistance, and evaluate another pyrethroid, permethrin. Lethal concentration ratios (at the LC_{50}) from *B. insularis* populations collected in 2006 and 2008 showed a 45-4,099-fold resistance to bifenthrin in Citrus, Escambia, Flagler, Hillsborough, Lake, Orange, Osceola, and Volusia counties. One population in Orange County demonstrated a 212-fold resistance to permethrin. There was a positive relationship between the number of insecticide applications made in 2006 and increasing insecticide resistance. This study documents the first case of insecticide resistance in the Florida Panhandle and the first report of *B. insularis* resistance to permethrin. Observations made during this study and possible causes for the development of insecticide resistance in *B. insularis* in Florida are discussed.

Key Words: *Blissus insularis*, Insecticide resistance, Pyrethroids

RESUMEN

La chinche sureña, *Blissus insularis* Barber, es un insecto plaga seria de grama San Agustín (*Stenotaphrum secundatum* [Walt.] Kuntze). El control de *B. insularis* se logra principalmente a través de insecticidas. Esta plaga ha desarrollado resistencia a varias clases de insecticidas debido a la exposición casi constante. Los objetivos de este estudio fueron para muestrar poblaciones seleccionadas de *B. insularis* en la Florida para describir su susceptibilidad a la bifentrina, documentar los nuevos sitios donde los chinches tienen resistencia a la bifentrina y evaluar permetrina, un otro piretroide. El razón de la concentración letal (en el CL_{50}) de las poblaciones de *B. insularis* recolectadas en 2006 y 2008 mostró un aumento de 45 a 4.099 veces en la resistencia hacia la bifentrina en los condados de Citrus, Escambia, Flagler, Hillsborough, Lake, Orange, Osceola y Volusia en la Florida. Una población en el condado de Orange ha demostrado una resistencia de 212 veces a la permetrina. Hubo una relación positiva entre el número de aplicaciones de insecticidas hechas en 2006 y aumento de la resistencia a los insecticidas. Este estudio documenta el primer caso de resistencia a los insecticidas en el Panhandle de Florida y el primer reporte de la resistencia de *B. insularis* a la permetrina. Se discuten las observaciones realizadas durante este estudio y las posibles causas para el desarrollo de resistencia a insecticidas en *B. insularis* en la Florida.

St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) is the most widely used lawn grass in tropical and subtropical climatic regions (Sauer 1972). It is the primary turfgrass in residential lawns and comprises ~70% or 1.2 million ha in Florida (Hodges et al. 1994; Busey 2003). The southern chinch bug, *Blissus insularis* Barber, is considered the most damaging insect pest of this grass (Kerr 1966; Reinert & Kerr 1973; Reinert & Portier 1983; Crocker 1993). Damage is caused by nymphs and adults feeding in phloem sieve elements (Rangasamy et al. 2009), causing wilting, chlorosis, stunting, and death (Beyer 1924; Painter 1928; Negron & Riley 1990; Spike et al. 1991; Vázquez & Buss 2006).

Blissus insularis can be difficult to control in Florida lawns. The tropical climate, particularly in the central and southern part of the state, is favorable for *B. insularis* feeding and reproduction (Reinert 1982a). Adults can live up to 70 d and a single female can deposit a few eggs per d over several wk for a total of 100-300 eggs (Burton & Hutchins 1958; Leonard 1966; Sweet 2000). Development time from egg to adult takes 5-8 wk (Vázquez et al. 2010) depending on temperature. *Blissus insularis* has overlapping generations, is highly mobile, and readily disperses to neighboring lawns. They are able to survive on other grass sources until new St. Augustinegrass is located (Kerr & Kuitert 1955; Kelsheimer & Kerr 1957; Kerr 1966; Reinert & Kerr 1973). In addition, St. Augustinegrass is often prone to thatch buildup, which provides an ideal habitat for *B. insularis* adults and nymphs (Reinert & Kerr 1973; Tashiro 1987).

In 1980, more than $25 million was spent to control *B. insularis*, with some lawn-care companies making as many as twelve insecticide applications per year to a single lawn (Kerr 1966; Stringfellow 1967, 1968, 1969; Strobel 1971; McGregor 1976; Reinert 1978, Reinert & Niemczyk 1982; Tashiro 1987). By the 1980s, *B. insularis* had developed resistance to organochlorines, organophosphates, and the carbamate, propoxur (Wolfenbarger 1953; Kerr & Robinson 1958; Kerr 1958, 1961; Reinert 1982a, b; Reinert & Niemczyk 1982; Reinert and Portier 1983).

The pyrethroid, bifenthrin, was the most used insecticide by lawn and ornamental professionals in Florida as determined by a 2003 University of Florida survey (Buss & Hodges 2006). Cherry & Nagata (2005) reported bifenthrin resistance in 14 *B. insularis* populations in central and south Florida. In 2006, we received multiple complaints of bifenthrin field failures and other pyrethroids as far north as Pensacola, Florida. In developing a resistance management program, it is important to determine where bifenthrin-resistant populations occur in the state and the severity of the problem. Thus, we tested 16 *B. insularis* populations in 2006 and 6 populations in 2008 in northern and central Florida. Tests included documentation and descriptions of bifenthrin and permethrin susceptibilities.

MATERIALS AND METHODS

St. Augustinegrass Maintenance

Commercially-obtained plugs of 'Palmetto' St. Augustinegrass were planted in 15.2 cm plastic pots filled with Farfard #2 potting soil (Conrad Farfard Inc., Agawam, Massachusetts). Plants were maintained in a University of Florida greenhouse in Gainesville, Florida, and held under a 14L:10D photoperiod with day and night temperatures of 27 and 24°C, respectively. Plants were fertilized weekly with a 20-20-20 complete nitrogen source (NH_4NO_3) at 0.11 kg N/ 0.02 m^2, watered as needed, and cut to a height of ~7.6 cm.

2006 Collection Sites

Blissus insularis populations were collected between May and Aug 2006. Two populations were collected from areas where insecticides had not been used, 3 were randomly collected (treatment history unknown), and 11 were from lawns where bifenthrin failures had been reported (Table 1). The number of times lawns were treated before collection and the active ingredients used during 2006 were documented for each site, where possible, and GPS coordinates were recorded. Several populations were collected from the same neighborhood or street, but were considered distinct because their treatment history varied. Populations were named based on location within a neighborhood.

2008 Collection Sites

Blissus insularis populations were collected in Jul 2008. Six populations were from lawns where bifenthrin field failures were reported (Table 2). The active ingredients used during 2008 were documented for each site; however, we were unable to obtain the number of times lawns were treated. GPS coordinates were recorded. Populations were named as previously described.

Insects

Blissus insularis were collected using a modified Weed Eater Barracuda blower/vacuum (Electrolux Home Products, Augusta, Georgia) (Crocker 1993; Nagata & Cherry 1999; Vázquez 2009), transported to the laboratory, sifted from debris, and fifth instars and adults were placed into a colony as outlined by Vázquez et al. (2010). Insects were tested within 1 wk of collection.

2006 Bioassays

Tests were conducted using a sprig-dip bioassay similar to that of Reinert & Portier (1983) and Cherry & Nagata (2005). Serial dilutions were made with formulated bifenthrin (TalstarOne®, FMC Corporation, Philadelphia, Pennsylvania) and prepared fresh on each test date. Eight concentrations were tested and mortality ranged from 5 to 95%. Fresh 'Palmetto' St. Augustinegrass stolon sections (5.0-6.4 cm long, with 3 leaflets and 1 node) were dipped in 1 solution and air dried on wax paper (~2 h). Ten unsexed adult *B. insularis* of unknown age were placed into plastic petri dishes (100 × 15 mm) containing 1 treated stolon and one 70-mm Whatman filter paper moistened with 0.5 mL of distilled water to prevent desiccation. There were 3 replicates. All tests were conducted between 1330-1500 h at room temperature (25 ± 2°C) and a 14L:10D pho-

TABLE 1. COLLECTION SITES, ACTIVE INGREDIENTS USED, AND THE NUMBER OF INSECTICIDE APPLICATIONS MADE TO LAWNS CONTAINING *B. INSULARIS* POPULATIONS IN FLORIDA IN 2006 THAT WERE TESTED FOR SUSCEPTIBILITY TO BIFENTHRIN.

Population	County	City	GPS coordinates	Month collected	No. insecticide applications in 2006	Active ingredients used[3]
P	Escambia	Pensacola	N30°28.70676, W87°11.7228	Aug	11	Bifenthrin Trichlorfon
BH	Citrus	Beverly Hills	N28°52.9644, W82°24.9684	Aug	11	Bifenthrin Carbaryl Imidacloprid Trichlorfon
JC	Orange	Windermere	N28°29.33244, W81°34.15464	Jun	8	Bifenthrin Permethrin Carbaryl Trichlorfon Acephate
V[1]	Flagler	Palm Coast	N29°34.81518, W81°10.87286	Jul	4	Bifenthrin Cypermethrin
GE12[1]	Flagler	Palm Coast	N29°34.78872, W81°10.93536	Jul	4	Bifenthrin Cypermethrin
LF	Flagler	Palm Coast	N29°33.69246, W81°11.93052	Jul	3	Bifenthrin Cypermethrin
FS[2]	Flagler	Palm Coast	N29°32.994833, W81°10.11883	Jul	3	Bifenthrin Cypermethrin
L[2]	Flagler	Palm Coast	N29°32.98482, W81°10.161	Jul	unknown	
BP	Hillsborough	Sun City	N27°42.516, W82°21.618	May	2	Bifenthrin
CT	Hillsborough	Sun City	N27°44.416167, W82°20.86733	Jun	5	Bifenthrin Carbaryl

[1] Denotes populations in the same neighborhood.
[2] Denotes populations located across the street from each other.
[3] Products are listed in descending order of application frequency.
[4] One application of Allectus® SC was used at this site, which contains both bifenthrin and imidacloprid.
[5] Bifenthrin failure was reported in 2005 but, at the time of collection, only clothianidin had been used in 2006.

TABLE 1. (CONTINUED) COLLECTION SITES, ACTIVE INGREDIENTS USED, AND THE NUMBER OF INSECTICIDE APPLICATIONS MADE TO LAWNS CONTAINING *B. INSULARIS* POPULATIONS IN FLORIDA IN 2006 THAT WERE TESTED FOR SUSCEPTIBILITY TO BIFENTHRIN.

Population	County	City	GPS coordinates	Month collected	No. insecticide applications in 2006	Active ingredients used[3]
						Imidacloprid
PC	Flagler	Palm Coast	N29°32.2641, W81°9.55944	May	unknown	
SCL	Osceola	St. Cloud	N28°15.20868, W81°19.0191	Jul	unknown	
DAL	Volusia	Port Orange	N29°6.101333, W81°8.952833	Jul	1	Bifenthrin[4] Imidacloprid[4]
DAR	Volusia	Port Orange	N29°6.3879, W81°3.33222	Jul	1	Clothianidin[5]
HF	Alachua	Gainesville	N29°35.83908, W82°26.0241	Jun-Aug	0	—
GE18[1]	Flagler	Palm Coast	N29°34.78644, W81°10.93704	Jul	0	—

[1] Denotes populations in the same neighborhood.
[2] Denotes populations located across the street from each other.
[3] Products are listed in descending order of application frequency.
[4] One application of Allectus® SC was used at this site, which contains both bifenthrin and imidacloprid.
[5] Bifenthrin failure was reported in 2005 but, at the time of collection, only clothianidin had been used in 2006.

TABLE 2. COLLECTION SITES AND THE ACTIVE INGREDIENTS USED IN LAWNS CONTAINING *B. INSULARIS* POPULATIONS IN FLORIDA IN 2008 THAT WERE TESTED FOR SUSCEPTIBILITY TO BIFENTHRIN.

Population	County	City	GPS coordinates	Month collected	Active ingredients used
LU	Lake	Clermont	N28°36.8664, W81°4.9164	Jul	Bifenthrin Trichlorfon
JP	Orange	Winter Garden	N28°32.6611, W81°38.9364	Jul	Bifenthrin Carbaryl Imidacloprid
JH	Orange	Winter Garden	N28°32.65, W81°38.5522	Jul	Bifenthrin Carbaryl Imidacloprid Fipronil
PA	Orange	Windermere	N28°30.0283, W81°33.7480	Jul	Bifenthrin Carbaryl Imidacloprid
TG	Orange	Windermere	N28°29.2447, W81°34.6830	Jul	Bifenthrin Carbaryl Imidacloprid
OR	Orange	Orlando	N28°27.07361, W81°30.31778	Jul	Bifenthrin Carbaryl Imidacloprid

toperiod. The number of dead *B. insularis* was assessed at 72 h using a 10× dissecting microscope. Insects were scored as dead if they were on their backs or unable to walk. The JC population had reports of TalstarOne® and Permethrin-G Pro (permethrin, Gro-Pro LLC, Inverness, Florida) failures; therefore, both products were tested. Permethrin-G Pro solutions and testing were conducted as described with TalstarOne®. *B. insularis* population HF was used as the susceptible standard for all 2006 tests.

2008 Bioassays

Tests were conducted using an airbrush bioassay (Vázquez 2009). A bifenthrin-susceptible laboratory population, LO (unpublished data), was used as a standard in this test. Serial dilutions were made with formulated bifenthrin (TalstarOne®); prepared fresh on each test date. Eight or 9 concentrations were tested for each population and mortality ranged from 5 to 95%. Tests were held for 24 h and insects were scored as previously described.

Statistical Analysis

The LC_{50} and LC_{90} values, 95% confidence limits (CL), slopes of the regression lines, and the likelihood ratio test to test the hypothesis of parallelism and equality of the regression lines were estimated by logit analysis using Polo Plus (LeOra Software 2002). Differences in susceptibility between populations were tested by the 95% confidence limits (CL) of lethal concentration ratios (LCRs) at LC_{50} and LC_{90} (Robertson & Priesler 1992; Robertson et al. 2007). Populations were compared to the most susceptible population (GE18) and LCR confidence intervals (95%) that did not include 1.0 were considered significant ($P < 0.05$) (Robertson & Priesler 1992; Robertson et al. 2007). The relationship between the number of insecticide applications made in 2006 and the respective LCRs (at LC_{50}) was analyzed using regression analysis (Systat Software 2006).

RESULTS AND DISCUSSION

Bifenthrin resistance in *B. insularis* has been confirmed in 5 Florida counties (Citrus, Escambia, Hillsborough, Orange, and Osceola), in addition to the 7 previously documented (Flagler, Hernando, Lake, Manatee, Monroe, Sarasota, and Volusia) (Cherry & Nagata 2005). Anecdotal reports of bifenthrin field failures also occurred in Alachua, Duval (E. Buss, personal communication) and Marion (E. Buss, unpublished data) counties in 2010. Reduced susceptibility to bifenthrin ranged from 4.6-736-fold in *B. insularis* collected in 2003 (Cherry & Nagata 2005) and increased to 45-4,099-fold (Tables 3 and 5) in *B. insularis* collected in 2006 and 2008.

Based on location treatment histories that reported bifenthrin field failures in 2006, the number of insecticide applications made to lawns (regardless of product) was positively correlated to their respective bifenthrin lethal concentration ratio (at LC_{50}) values (Fig. 1). Highest bifenthrin LC_{50} values (3,835, 3,748 and 2,737 µg/ml) were recorded from populations P, BH, and JC, respectively (Table 3), which had been treated 8 to 11 times. Orange County population, JC, also showed resistance to permethrin at a 212.4-fold difference to the susceptible population, HF (Table 4). This is the first documented case of permethrin resistance in *B. insularis*. Populations treated 2 to 5 times (V, GE12, LF, FS, BP, and CT) had LC_{50} values ranging from 93-1,127 µg/mL for bifenthrin. Populations with 1 or no applications (DAL, DAR, HF, and GE18) had the lowest LC_{50} values, ranging from 0.9-42 µg/mL. LCR_{50} values for all populations (with the exception of DAR and HF) were significantly different from the most susceptible population, GE18, and increased with insecticide application frequency (Table 3). Thus, insecticide application frequency can, conservatively, be associated with the expression of insecticide resistance in *B. insularis* populations, as it is in other systems (Georghiou 1986; Rosenheim & Hoy 1986; Croft et al. 1989; He et al. 2007; Magana et al. 2007). In addition to our findings, Cherry & Nagata (2007) found select *B. insularis* populations to be resistant to the pyrethroids deltamethrin and λ-cyhalothrin, clearly indicating the occurrence of cross resistance in Florida. A few isolated *B. insularis* populations were also resistant to the neonicotinoid imidacloprid (Cherry & Nagata 2007).

LCR_{90} values for all populations treated with bifenthrin in our study (except DAL and DAR) significantly differed from the most susceptible population tested, GE18 (Table 3). The highest bifenthrin LCR_{90} values were recorded from populations BH, JC, GE12, LF, L, FS, and BP. Values indicated that these populations were 1,077-13,000 µg/mL more resistant to bifenthrin than population GE18 (Table 3). LC_{90} values for these same *B. insularis* populations ranged from 53,120 to 642,522 µg/mL, with the lowest values from populations GE18, DAR, and DAL (Table 3). Of the 11 populations collected where bifenthrin failures were reported, 9 were actual control failures (highest label rate of TalstarOne® = 209 µg/mL). Populations DAL and DAR demonstrated LC_{90} values that were below the recommended label rate, but control failure in these 2 sites may have been due to application error.

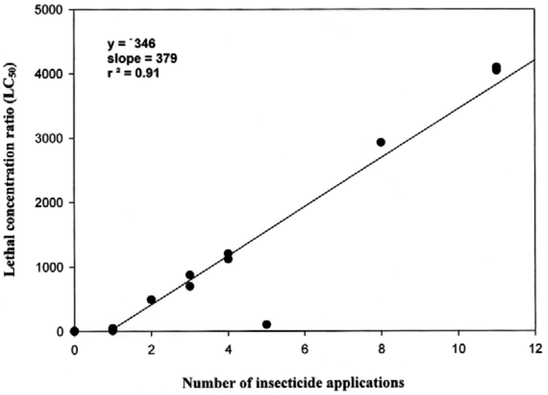

Fig. 1. Bifenthrin resistance in *B. insularis* populations from central and northern Florida in 2006: relationship between the number of insecticide applications made (regardless of active ingredient used) and respective lethal concentration ratios (at LC_{50}). See Table 1 for locations sampled.

Bifenthrin LC_{50} values from the 6 *B. insularis* populations collected in central Florida in 2008 ranged from 99-366 µg/mL compared to the LC_{50} of 3.0 µg/mL from the susceptible laboratory population, LO (Table 5). Slopes of the regression lines from the populations tested were steep, indicating a uniform response to bifenthrin, with the exception of population PA (Georghiou & Metcalf 1961; ffrench-Constant & Roush 1990; Prabhaker et al. 1996, 2006). All *B. insularis* populations collected in 2008, with the exception of LO, were actual control failures, based on respective $LC_{(50 \text{ and } 90)}$ values (Table 5).

The mechanism that has enabled *B. insularis* populations to repeatedly become resistant to different insecticide chemistries has not yet been determined. We speculate that the *B. insularis* populations collected in 2006 and 2008 consisted of a range of genetically heterogeneous (susceptible and resistant) individuals (e.g., population BH had an LC_{50} of 3,748 and LC_{90} of 642,522 µg/mL). The hypothesis tests of parallelism and equality showed that the regression lines of 13 populations were parallel (slopes did not significantly differ), but not equal (their intercepts differed significantly), to the most susceptible population, GE18 (Table 6). Alternately, the different *B. insularis* populations may have had qualitatively identical but quantitatively different levels of detoxification enzymes (Robertson et al. 2007). Population DAL, with a steep slope of 4.3, had significantly different intercepts and slopes from the GE18 population, possibly indicating that DAL was more uniform in structure, the insects' detoxification enzymes differed qualitatively, or that this population had entirely different detoxification enzymes (Robertson et al. 2007). Intercepts and slopes for populations DAR and GE18 were similar, demonstrating a similar response to bifenthrin.

The regression lines of the 6 populations tested in 2008 had significantly different intercepts from that of the most susceptible population LO (Table 7). The hypothesis test for parallelism was not rejected for populations JP, JH, and TG ($\chi^2 = 0.5$; $df = 1$; $P = 0.46$, $\chi^2 = 1.5$; $df = 1$; $P = 0.21$, and $\chi^2 = 0.2$; $df = 1$; $P = 0.66$, respectively). For these populations, the slopes were similar to that of population LO. Populations LU, PA, and OR had

TABLE 3. RESPONSE OF FLORIDA *B. INSULARIS* POPULATIONS COLLECTED IN 2006 TO BIFENTHRIN AFTER 72 H USING A SPRIG-DIP BIOASSAY AT 25.5°C.

Population	*n*	Slope ± SE[1]	LC_{50} (95% CL)[2]	LCR_{50} (95% CL)[3]	LC_{90} (95% CL)[2]	LCR_{90} (95% CL)[3]	χ^2 (df)[4]
P	240	2.0 ± 0.3	3,835 (1,619-8,923)	4,099 (229-73,362)	44,798 (17,078-273,547)	908 (175-4,723)	5.1(5)[5]
BH	80	1.0 ± 0.2	3,748 (678-18,707)	4,007 (162-98,779)	642,522 (89,477-47,530,686)	13,030 (649-261,265)	4.0(5)[5]
JC	240	1.1 ± 0.1	2,737 (1,058-6,557)	2,925 (151-56,449)	260,786 (81,799-1,538,682)	5,288 (746-37,504)	3.2(5)[5]
V	240	1.6 ± 0.2	1,127 (490-2,358)	1,204 (65-22,389)	28,641 (11,496-120,575)	581 (100-3,371)	2.0(5)[5]
GE12	240	1.0 ± 0.1	1,048 (48-10,027)	1,120 (57-2,217)	186,000 (16,864-285,753,603)	3,772 (478-29,771)	14.1(5)
LF	240	1.1 ± 0.2	817 (18-10,187)	874 (45-16,952)	71,506 (6,573-785,990,382)	1,450 (208-10,093)	18.2(5)
FS	240	1.1 ± 0.2	652 (32-3,649)	697 (33-14,774)	53,120 (8,599-4,135,424)	1,077 (148-7,856)	8.0(5)[5]
L	240	1.1 ± 0.2	521 (41-2,347)	557 (26-12,108)	62,612 (12,217-1,833,448)	1,270 (170-9,490)	5.9(5)[5]
BP	240	0.9 ± 0.1	459 (31-3,122)	490 (25-9,788)	143,891 (14,638-67,945,632)	2,918 (342-24,889)	11.0(5)
CT	240	1.8 ± 0.3	93 (10-501)	100 (5-1,798)	1,447 (317-801,491)	29 (5-161)	14.0(5)
PC	240	2.0 ± 0.4	62 (24-128)	67 (4-1,244)	785 (345-3,435)	16 (3-88)	0.6(5)[5]
SCL	240	1.1 ± 0.2	47 (1-259)	50 (2-1,115)	4,039 (661-537,205)	82 (11-596)	8.2(5)[5]
DALL	240	4.3 ± 1.1	42 (18-69)	45 (2-804)	137 (84-349)	3 (0.6-12)	0.1(5)[5]
DAR	240	2.7 ± 0.7	10 (3-17)	10 (0.6 - 186)	62 (32-365)	1 (0.2-6)	0.2(5)[5]
HF	1,200	1.2 ± 0.1	8 (2-18)	212 (104-434)	652 (349-1,436)	13 (3-61)	4.0(4)[5]
GE18	240	1.3 ± 0.4	0.9 (0-5)	1	49 (11-467)	1	0.5(5)[5]

[1] Slope of the logit mortality line.

[2] LC_{50}, LC_{90}, and 95% confidence limits (CL) are expressed in µg/mL.

[3] Lethal concentration ratios with 95% confidence limits indicating the fold-difference for each population in comparison to the most susceptible population at LC_{50} and LC_{90}. Confidence limits that include 1.0 indicate no significant difference from the susceptible (GE18) population. *Shows ratios that are significant ($P \le 0.05$, Robertson and Preisler 1992; Robertson et al. 2007).

[4] Pearson chi-square statistic (degrees of freedom).

[5] Good fit of the data to the logit model ($P > 0.05$).

TABLE 4. RESPONSES OF TWO FLORIDA *B. INSULARIS* POPULATIONS COLLECTED IN 2006 TO PERMETHRIN AFTER 72 H USING A SPRIG-DIP BIOASSAY AT 25.5°C.

Population	*n*	Slope ± SE[1]	LC$_{50}$ (95% CL)[2]	LCR$_{50}$ (95% CL)[3]	LC$_{90}$ (95% CL)[2]	LCR$_{90}$ (95% CL)[3]	χ^2 (df)[4]
JC	240	3.5 ± 0.7	341 (130-750)	212 (104-434)*	1,431 (668-9,885)	157 (53.7-457)*	6.0 (5)[6]
HF[5]	240	2.9 ± 0.5	1.6 (1.0-2.7)	1	9.1 (4.9-28)	1	4.4 (5)[6]

[1] Slope of the logit mortality line.

[2] LC$_{50}$, LC$_{90}$, and 95% confidence limits (CL) are expressed in µg/mL.

[3] Lethal concentration ratios with 95% confidence limits indicating the fold-difference for each population in comparison to the most susceptible population at LC$_{50}$ and LC$_{90}$. Confidence limits that include 1.0 indicate no significant difference from the susceptible (HF) population. *Shows ratios that are significant ($P \le 0.05$, Robertson and Preisler 1992; Robertson et al. 2007).

[4] Pearson chi-square statistic (degrees of freedom).

[5] Susceptible population.

[6] Good fit of the data to the logit model ($P > 0.05$).

TABLE 5. RESPONSES OF FLORIDA *B. INSULARIS* POPULATIONS COLLECTED IN 2008 TO BIFENTHRIN AFTER 24 H USING AN AIRBRUSH BIOASSAY AT 25.5°C.

Population	*n*	Slope ± SE[1]	LC$_{50}$ (95% CL)[2]	LCR$_{50}$ (95% CL)[3]	LC$_{90}$ (95% CL)[2]	LCR$_{90}$ (95% CL)[3]	χ^2 (df)[4]
LU	270	5.0 ± 0.8	363 (286-461)	121 (85-173)	1016 (736-1790)	69 (38-127)	3.3(6)[5]
JP	54	4.1 ± 1.3	333 (172-1192)	111 (51-244)	1140 (488-22,203)	78 (20-300)	3.6(5)[5]
JH	288	4.0 ± 0.5	129 (87-202)	43 (30-61)	457 (270-1417)	31 (17-58)	10.4(6)[5]
PA	288	2.1 ± 0.3	124 (54-578)	41 (26-66)	1439 (382-279,909)	98 (36-266)	21.2(6)[5]
TG	288	3.5 ± 0.6	116 (68-186)	39 (26-57)	499 (277-2,370)	34 (17-68)	9.6(6)
OR	288	4.7 ± 0.6	99 (49-208)	33 (23-47)	293 (156-3,084)	20 (115-37)	25.1(6)
LO	256	3.2 ± 0.4	3 (1-5)	1	15 (8-83)	1	12.3(5)[5]

[1] Slope of the logit mortality line.

[2] LC50, LC90, and 95% confidence limits (CL) are expressed in µg/mL.

[3] Lethal concentration ratios with 95% confidence limits indicating the fold-difference for each population in comparison to the most susceptible population at LC$_{50}$ and LC$_{90}$. Confidence limits that include 1.0 indicate no significant difference from the susceptible (LO) population. *Shows ratios that are significant ($P \le 0.05$, Robertson and Preisler 1992; Robertson et al. 2007).

[4] Pearson chi-square statistic (degrees of freedom).

[5] Good fit of the data to the logit model ($P > 0.05$).

TABLE 6. HYPOTHESIS TESTS COMPARING THE SLOPES AND INTERCEPTS OF LOGIT REGRESSION LINES FOR 15 *B. INSULARIS* POPULATIONS IN COMPARISON TO THE MOST SUSCEPTIBLE POPULATION, GE18, AFTER EXPOSURE TO BIFENTHRIN FOR 72 H USING A SPRIG-DIP BIOASSAY AT 25.5°C.

Population	Hypothesis test for equality	Hypothesis test for parallelism
P	reject; $\chi^2 = 168$; df = 2; $P < 0.05$	accept; $\chi^2 = 1.7$; df = 1; $P = 0.19$
BH	reject; $\chi^2 = 74$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.4$; df = 1; $P = 0.56$
JC	reject; $\chi^2 = 126$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.1$; df = 1; $P = 0.71$
V	reject; $\chi^2 = 110$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.3$; df = 1; $P = 0.58$
GE12	reject; $\chi^2 = 97$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.47$; df = 1; $P = 0.49$
LF	reject; $\chi^2 = 92$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.10$; df = 1; $P = 0.75$
FS	reject; $\chi^2 = 80$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.07$; df = 1; $P = 0.79$
L	reject; $\chi^2 = 78$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.23$; df = 1; $P = 0.63$
BP	reject; $\chi^2 = 78$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.88$; df = 1; $P = 0.35$
CT	reject; $\chi^2 = 44$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.97$; df = 1; $P = 0.32$
PC	reject; $\chi^2 = 33$; df = 2; $P < 0.05$	accept; $\chi^2 = 1.4$; df = 1; $P = 0.24$
SCL	reject; $\chi^2 = 29$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.08$; df = 1; $P = 0.78$
DALL	reject; $\chi^2 = 35$; df = 2; $P < 0.05$	reject; $\chi^2 = 10$; df = 1; $P = 0.001$
DAR	accept; $\chi^2 = 6$; df = 2; $P = 0.06$	accept; $\chi^2 = 3$; df = 1; $P = 0.08$
HF	reject; $\chi^2 = 14$; df = 2; $P = 0.001$	accept; $\chi^2 = 0.04$; df = 1; $P = 0.85$

TABLE 7. HYPOTHESIS TESTS COMPARING THE SLOPES AND INTERCEPTS OF LOGIT REGRESSION LINES FOR 6 *B. INSULARIS* POPULATIONS IN COMPARISON TO A SUSCEPTIBLE LABORATORY COLONY, LO, AFTER EXPOSURE TO BIFENTHRIN FOR 72 H USING AN AIRBRUSH BIOASSAY AT 25.5°C.

Population	Hypothesis test for equality	Hypothesis test for parallelism
LU	reject; $\chi^2 = 290$; df = 2; $P < 0.05$	reject; $\chi^2 = 4.5$; df = 1; $P = 0.03$
JP	reject; $\chi^2 = 142$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.5$; df = 1; $P = 0.46$
JH	reject; $\chi^2 = 260$; df = 2; $P < 0.05$	accept; $\chi^2 = 1.54$; df = 1; $P = 0.21$
PA	reject; $\chi^2 = 190$; df = 2; $P < 0.05$	reject; $\chi^2 = 5.25$; df = 1; $P = 0.02$
TG	reject; $\chi^2 = 228$; df = 2; $P < 0.05$	accept; $\chi^2 = 0.20$; df = 1; $P = 0.66$
OR	reject; $\chi^2 = 257$; df = 2; $P = 0.001$	reject; $\chi^2 = 5.0$; df = 1; $P = 0.03$

significantly different intercepts and slopes from the LO population (Table 7). Preliminary laboratory tests using one *B. insularis* population from Marion County have also indicated that cytochrome P450, glutathione-S-transferase, and esterase activity are all likely to be involved in *B. insularis* insecticide resistance development (M. Scharf, unpublished data).

To further complicate resistance management efforts, our data support that each distinctly-owned property should be considered a separate *B. insularis* population. Palm Coast sites, GE12 and GE18, were located a few houses from each other, on the same side of the street, and were maintained by the same company at the time of this study. GE12 received 4 insecticide applications between Jan and Jul 2006 and resulted in a bifenthrin LC_{50} of 1,048 µg/mL and an LC_{90} of 186,000 µg/mL. Lawn GE18 first had *B. insularis* damage in 2006 and thus had not been previously treated. GE18 showed a bifenthrin LC_{50} of 0.9 µg/mL and an LC_{90} of 49 µg/mL. Population V was located 1 street from GE12 and GE18, in the same neighborhood. Palm Coast populations V and GE12 were managed similarly, but population V had a bifenthrin LC_{50} of 1,127 µg/mL and an LC_{90} of 28,641 µg/mL. Populations FS and L were directly across the street from each other, but were maintained by different companies. The FS population was treated 3 times between Jan and Jul 2006 and its bifenthrin LC_{50} was 652 µg/mL and LC_{90} was 53,120 µg/mL. The L population, with unknown treatment history, had a bifenthrin LC_{50} of 521 µg/mL and an LC_{90} of 62,612 µg/mL.

Treatment effects on individual lawns, the effect of insect dispersal among lawns, and population dynamics of *B. insularis* within larger neighborhoods warrants further study. Encroachment from neighboring lawns was observed in nearly all sites collected in 2006 and 2008. Other studies have suggested that insecticide resistance may develop more rapidly in small, subdivided populations rather than large ones (Wright 1931; Crow & Kimura 1970; Roush & Daly 1990). Although the immigration of susceptible individuals into treated areas can slow resistance development by increasing the frequency of susceptible alleles in a treated population (Comins 1977; Georghiou & Taylor 1977; Curtis et al. 1978; Taylor & Georghiou 1979; Tabashnik & Croft 1982;

Roush & Daly 1990; Tabashnik 1990), emigration of resistant individuals from treated areas can also speed the resistance development in the untreated area (Comins 1977; Sutherst and Comins 1979). Because of the damaging nature of *B. insularis* in St. Augustinegrass lawns, the deliberate introduction of susceptible individuals to dilute the gene pool is not a viable resistance management option.

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