# A Maximum Concentration Bioassay to Assess Insecticide Efficacy Against Hemipteran Pests of Tomato

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# A maximum concentration bioassay to assess insecticide efficacy against hemipteran pests of tomato

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Florida is a primary producer of fresh market tomatoes (*Solanum lycopersicum* L.; Solanaceae) in the US, with over 9,700 ha (24,000 acres) harvested in 2020 at a value of USD $463 million (USDA NASS 2021). Stink bugs (Hemiptera: Pentatomidae) and leaf-footed bugs (Hemiptera: Coreidae) are occasional pests of Florida tomato (UF IFAS 2006). Until recently, this pest complex was a problem primarily on tomato in Florida on certified organic farms where broad spectrum insecticides are not used, and management efforts focused on trap cropping (Gordon et al. 2017). Since 2019, for reasons that are not clear, stink bugs have resurged as a problem on conventional tomato grown in the spring season (Jan–May) in southern Florida. The most prevalent species attacking tomato in Florida are a brown stink bug (*Euschistus quadrator* Rolston) and the southern green stink bug (*Nezara viridula* [L.]) (both Hemiptera: Pentatomidae) (UF IFAS 2006). In addition, leaf-footed bugs (*Leptoglossus phyllopus* (L.) and *Phthia picta* (L.) (both Hemiptera: Coreidae) also attack tomato in Florida (UF IFAS 2006). Stink bug feeding damage can result in major economic losses to tomato. Stylet punctures and salivary enzymes from stink bug feeding cause deformed fruit ("cat-facing"), discoloration on the outer skin of tomatoes, and internal tissue damage reducing the economic value of fruit (Peiffer & Felton 2014; Rice et al. 2014, 2017).

To determine if the resurgence of stink bugs as a pest in tomato in Florida was related to lack of insecticide efficacy, 8 stink bug populations were collected from commercial tomato farms in the spring of 2021. In addition, we were able to collect 1 leaf-footed bug population for testing. The insecticide screening focused on neonicotinoid and pyrethroid insecticides, because these 2 insecticide groups have tended to show the greatest efficacy against stink bugs (Willrich et al. 2003; Snodgrass et al. 2005; Kuhar & Komminga 2017; Santos-Júnior et al. 2021). We hypothesized that insecticide tolerant populations would show limited response to maximum concentration treatments of commonly used insecticides, providing evidence that tolerance was present and that resistance monitoring efforts were warranted.

Stink bug and leaf-footed bug populations were collected from 6 commercial tomato field sites in Manatee County, 1 site in Hillsborough County, and 1 site in Hernando County (all in Florida) from Apr through Jun 2021. The species collected were *N. viridula* (the southern green stink bug), *E. quadrator* (a brown stink bug), and *L. phyllopus* (the leaf-footed bug). Populations were maintained on lima bean (*Phaseolus lunatus* L.; Fabaceae) plants in cages in a greenhouse at the Gulf Coast Research and Education Center in Wimauma, Florida, USA, and provided with tomatoes and sweet corn for additional food. The greenhouse was cooled with cooling pads and ventilated with fans, maintaining temperatures between 18 and 35 °C. Insect colonies experienced natural daylight. Relative humidity was not recorded. Egg clusters, nymphal stages and adults were maintained in separate cages to avoid cannibalism. Insect populations were given approximately 30 d to establish on lima bean plants in the greenhouse, at which point the $F_1$ generation was tested for susceptibility to key insecticides using third instar nymphs and a maximum concentration bioassay.

Each stink bug and leaf-footed bug population was tested separately for susceptibility to 3 pyrethroid insecticides, 3 neonicotinoid insecticides, and a deionized water control (Table 1). The active ingredients evaluated were bifenthrin (Brigade® 2 EC, FMC Corporation, Philadelphia, Pennsylvania, USA), zeta-cypermethrin (Mustang® Maxx, FMC Corporation, Philadelphia, Pennsylvania, USA), lambda-cyhalothrin (Warrior, Syngenta, Greensboro, North Carolina, USA), dinotefuran (Venom® 70 SG, Valent USA, Walnut Creek, California, USA), imidacloprid (Admire® Pro, Bayer Corporation, Whippany, New Jersey, USA), and thiamethoxam (Actara®, Syngenta, Greensboro, North Carolina, USA). The maximum labeled per ha application rate for tomato was used for each treatment and was prepared in beakers containing 500

**Table 1.** Trade name, active ingredient (ai), mode of action group (MoA), and application rate of 6 insecticides tested in a laboratory bioassay to control stink bug (Hemiptera: Pentatomidae) and leaf-footed bug (Hemiptera: Coreidae) populations collected from Florida tomato farms, spring 2021.

| Trade name | Active ingredient | MoA | Per ha rate | mL or g of product per 500 mL | mL or g of ai per 500 mL |
|---|---|---|---|---|---|
| Brigade® | Bifenthrin | 3A | 380 mL | 0.338 mL | 0.085 mL |
| Mustang® | Zeta-cypermethrin | 3A | 314 mL | 0.28 mL | 0.026 mL |
| Warrior | Lambda-cyhalothrin | 3A | 140 mL | 0.125 mL | 0.029 mL |
| Admire® Pro | Imidacloprid | 4A | 160 mL | 0.143 mL | 0.061 mL |
| Actara® | Thiamethoxam | 4A | 385 g | 0.325 g | 0.081 g |
| Venom® | Dinotefuran | 4A | 280 g | 0.25 g | 0.175 g |

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mL of deionized water at a rate equivalent to 561 water L per ha (60 gallons water per acre). This is the typical volume used for foliar treatment of tomatoes when plants are in the vegetative stage (the first 6–8 wk after transplanting). The per ha application rate, amount of insecticide, and active ingredient in 500 mL of water are listed in Table 1. Organic grape tomatoes were purchased from the supermarket, rinsed, and dipped in the treatments. After 2 h air drying, 1 treated tomato was placed in a clear Genpak 32 oz plastic container (Genpak LLC, Sebring, Florida, USA) with 5 nymphs. Each container with 5 insects was 1 experimental unit or replicate. Each treatment was replicated 3 times. After 72 h, the number of live, moribund, and dead nymphs was recorded. For analysis of mortality, moribund nymphs were grouped with dead nymphs.

A general linear mixed model analysis was used to characterize the effects of insecticide treatment on insect mortality across 9 different populations. The experimental design was considered a completely randomized factorial design with each level of insecticide treatment and insect population crossed in each block. The independent variables population, treatment, and their interaction were treated as fixed effects. Replication was treated as a random effect. Prior to analysis, the dependent variable mortality was converted to proportions and subjected to the arcsine square root transformation; data were back-transformed for presentation in tables and figures. The analysis was performed using the GLIMMIX procedure of SAS (SAS, Version 9.4, Cary, North Carolina, USA) with the default identity link function and error Gaussian distribution. Additionally, the 'NOBOUND' option was used to allow negative values of covariance and scale parameters, which guards against an inflated Type I error rate (Littell et al. 2006). Moreover, the DDFM=KR option was used, which invokes the Kenward-Roger estimation of the standard errors and degrees-of-freedom. The general linear mixed model fit the data well based on visual inspection for a non-random pattern in a scatter plot of the residuals, a histogram with normal density, a linear quantile-quantile (or Q-Q) plot, and boxplot of the residuals that showed no outliers. Pairwise treatment differences for the fixed effects were obtained using the LSMEANS statement and LINES option ($P = 0.05$). Multiple comparisons for all pairwise comparisons were based on Bonferroni *t*-tests ($P = 0.05$), which keeps the maximum experimentwise error rate at the intended type I error rate for all comparisons. The effect of insecticide on mortality was compared on the pooled populations using the Bonferroni *t*-test.

Insecticide ($F_{6,124} = 54.80$; $P < 0.0001$), population ($F_{8,124} = 8.95$; $P < 0.0001$), and the interaction ($F_{48,124} = 2.27$; $P < 0.0002$) had a significant effect on nymphal mortality observed 72 h after treatment.

The mortality of nymphs in the untreated controls ranged from 0.00 to 33.33% (Fig. 1). Six populations had zero mortality in the untreated control, 2 populations produced 6.7% mortality, and 1 population, Brown1, experienced 33.3% mortality in the untreated control. Mortality was lower in the untreated control than all insecticide treatments. Mortality was higher in bifenthrin than all other treatments with the exception of dinotefuran. Among the products tested, bifenthrin was the most effective pyrethroid with mortality ranging from 73.3 to 100%. Dinotefuran was the most effective neonicotinoid with mortality ranging from 46.67 to 86.67%. In contrast, imidacloprid produced consistently poor results, with mortality ranging from 0 to 60.00% (Fig. 1). Mortality was not statistically different between dinotefuran and zeta-cypermethrin, both of which produced significantly higher mortality than lambda-cyhalothrin, thiamethoxam, and imidacloprid. Mortality was higher in the lambda-cyhalothrin treatment than imidacloprid, but not significantly different from thiamethoxam. Mortality was not significantly different between the thiamethoxam and imidacloprid treatments (Fig. 1).

Stink bugs and leaf-footed bugs are generalist pests affecting tomato and other crops (Mitchell 2000; UF IFAS 2006; Braman & Westerfield 2020). Historically this pest complex has been managed with broad spectrum insecticides including carbamate, organophosphate, and pyrethroid insecticides (Willrich et al. 2003; Snodgrass et al. 2005). Neonicotinoid insecticides also have shown efficacy against this group (Tillman & Mullinix 2004; Tillman 2006; Kamminga et al. 2012). In addition, trap cropping has been evaluated against stink bugs and leaf-footed bugs with limited success (Gordon et al. 2017; Braman & Westerfield 2020; Leppla et al. 2023). Current management methods focus on chemical control based on the application of neonicotinoid and pyrethroid insecticides (Kamminga et al. 2012; Kuhar & Kamminga 2017). Our results were consistent with studies cited above demonstrating efficacy of pyrethroid and neonicotinoid insecticides.

The maximum concentration test provides a rapid screening of insecticide materials and can be used to illustrate patterns of susceptibility among pest populations across production regions (Riley et al. 2020; De Marchi et al. 2021). We subjected 5 populations of *N. viridula*, 3 populations of *E. quadrator*, and 1 population of *L. phyllopus* to a maximum concentration test to screen for susceptibility to 3 pyrethroid and 3 neonicotinoid insecticides commonly used to manage stink bugs. Our objective was to determine if most populations were susceptible to insecticides or if there was evidence of insecticide tolerance that might help explain the resurgence in stink bugs as pests in Florida tomato fields. Not all insecticides were equally effective, and there was variability in the overall susceptibility of populations studied. However, each population was susceptible to insecticides, with bifenthrin and dinotefuran being the most effective.

Our objective was to test populations promptly so that susceptibility profiles would be representative of responses in the field. As a result, we had to focus on nymphal stages and used a low number of replicates to have sufficient individuals to screen all 6 insecticides. However, the test served the purpose of providing information quickly to growers that insecticide resistance did not seem to be the primary reason for pest resurgence. One possible explanation for the resurgence of stink bug populations on some farms may be the substitution of cyantraniliprole in place of dinotefuran at-planting and in early vegetative stages for management of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and tomato yellow leaf curl virus, which is transmitted by *B. tabaci*. Cyantraniliprole is effective against *B. tabaci* and other tomato pests, including leafminers (*Liriomyza* spp. Mik; Diptera: Agromyzidae) and lepidopteran larvae, but it is not labelled for use against stink bugs. In summary, judicious use of pyrethroid and neonicotinoid insecticides when hemipteran pest populations are first detected may mitigate damage caused by this pest group.

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## Summary

Hemipterans have resurged as a pest of tomato in south Florida. To determine if the problem was related to insecticide efficacy, populations of southern green stink bug (*Nezara viridula* [L.]; Hemiptera: Pentatomidae), brown stink bug (*Euschistus quadrator* Rolston; Hemiptera: Pentatomidae), and a leaf-footed bug (*Leptoglossus phyllopus* [L.]; Hemiptera: Coreidae) were collected from 8 commercial tomato farms in South Florida, and third instar nymphs were subjected to a maximum concentration bioassay. Insects from the $F_1$ generation were confined in plastic containers with grape tomatoes treated with the highest labeled application rate of 6 insecticides commonly used for

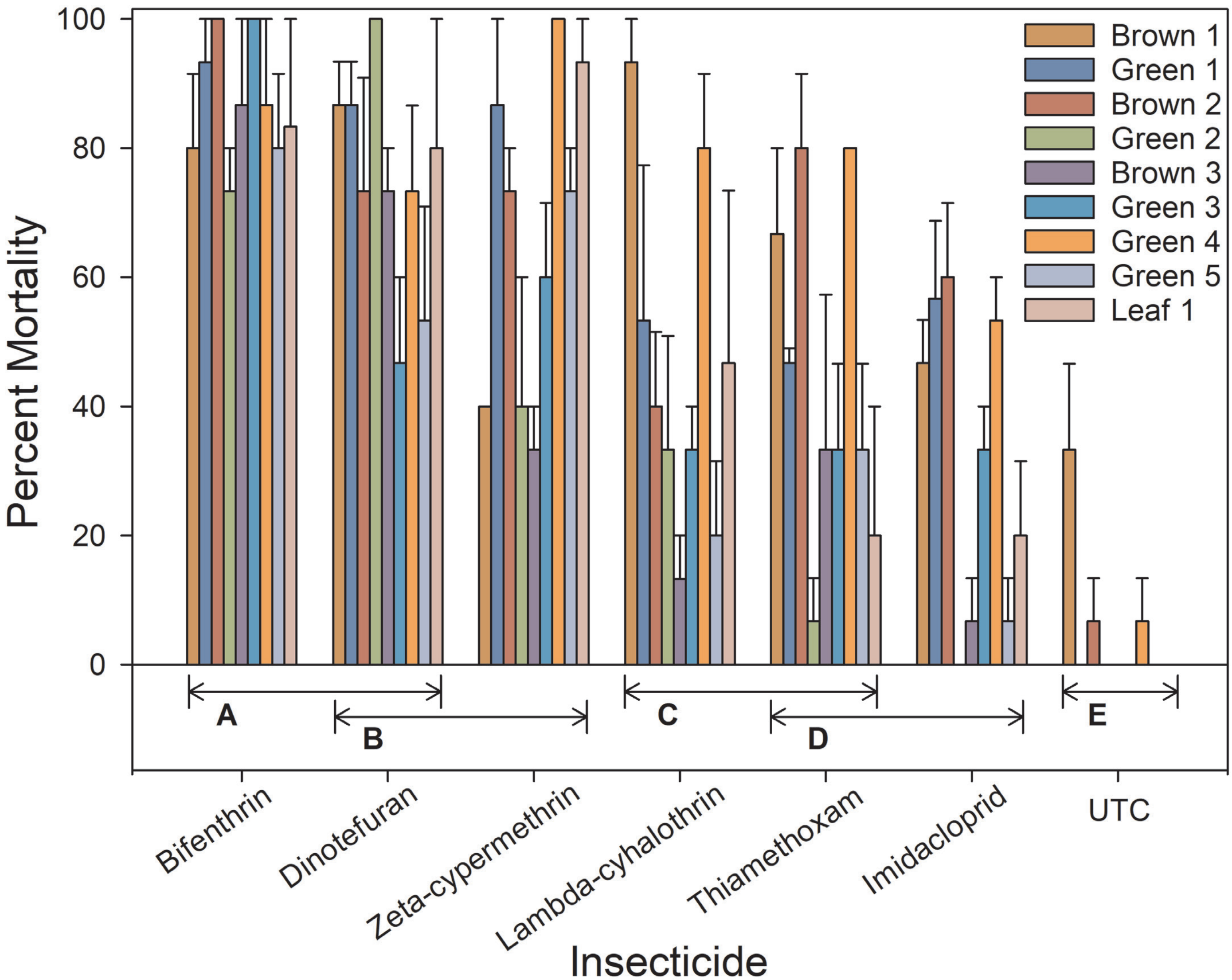


**Fig. 1.** Mortality of third instar nymphs from 5 populations of *Nezara viridula* ('green'), 3 populations of *Euschistus quadrator* ('brown'), and 1 population of *Leptoglossus phyllopus* ('leaf') stink bug subjected to a maximum concentration test of 3 pyrethroid and 3 neonicotinoid insecticides and an untreated control (UTC). Percentage mortalities (+ SEM) are based on the mean of 5 nymphs per replicate with 3 replicates. Insecticides designated with the same letter in the horizontal axis are not statistically different (by Bonferroni *t*-test, $P < 0.05$).

hemipteran management in tomatoes: bifenthrin, lambda-cyhalothrin, zeta-cypermethrin (pyrethroids), dinotefuran, imidacloprid, and thiamethoxam (neonicotinoids). Mortality was observed after 72 h. Bifenthrin was the most effective pyrethroid with mortality ranging from 73 to 100%. Dinotefuran was the most effective neonicotinoid with mortality ranging from 47 to 87%. In contrast, imidacloprid produced consistently poor results, with mortality ranging from 0 to 60%. Populations varied in their susceptibility to insecticides tested, but tests did not reveal evidence of lack of insecticide efficacy.

Key Words: *Nezara viridula*; *Euschistus quadrator*; pyrethroid; neonicotinoid

## Sumario

Los hemipteranos han resurgido como plaga del tomate en el sur de Florida. Para determinar si el problema estaba relacionado con la eficacia de los insecticidas, se recolectaron poblaciones del chinche hediondo verde del sur (*Nezara viridula* [L.]; Hemiptera: Pentatomidae), el chinche hediondo café (*Euschistus quadrator* Rolston; Hemiptera: Pentatomidae) y un chinche de patas laminadas (*Leptoglossus phyllopus* [L.]; Hemiptera: Coreidae) de 8 granjas de tomate comerciales en el sur de Florida, y las ninfas de tercer estadio se sometieron a un bioensayo de concentración máxima. Los insectos de la generación $F_1$ se limitaron en recipientes de plástico con tomates de uva tratados con la tasa de aplicación más alta etiquetada de 6 insecticidas comúnmente utilizados para el manejo de hemipteranos en tomates: bifentrina, lambda-cyhalothrin, zeta-cypermetrin (piretroides), dinotafurán, imidaclóprida y thiametoxamam (neonicotinoides). La mortalidad se observó después de 72 horas. La bifentrina fue el piretroide más efectivo con mortalidad que varió del 73 al 100%. DinoteFuran fue el neonicotinoide más efectivo con mortalidad que varió del 47 al 87%. En contraste, el imidacloprid produjo resultados consistentemente pobres, con mortalidad que varía de 0 a 60%. Las poblaciones variaron en su susceptibilidad a los insecticidas probados, pero las pruebas no revelaron evidencia de falta de eficacia de insecticidas.

Palabras Clave: *Nezara viridula*; *Euschistus quadrador*; piretroide; neonicotinoide

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