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EFFICACY OF FOLIAR INSECTICIDES ON EGGS OF *NEZARA VIRIDULA* (HEMIPTERA: PENTATOMIDAE)

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ABSTRACT

Stink bugs (Hemiptera: Pentatomidae) are important insect pests of crops. Stink bug feeding reduces yields, lowers crop quality, induces delayed maturity, and wounds tissues allowing for pathogen entry. Historically, effective adult and nymph control of stink bugs has been with insecticides. However, little insecticide efficacy against stink bug eggs is known. The objective of this study was to determine how foliar insecticides currently recommend for southern green stink bug, *Nezara viridula* (L.), control in soybean impact pre-emergence nymphal mortality using an egg dip bioassay. Eight formulate insecticides were tested. Differences in nymphal stink bug pre-emergence mortality were recorded. The lowest instances of stink bug emergence (and highest mortality) were observed in egg masses treated with bifenthrin (78.7%) followed by beta-cyfluthrin + acephate (42.5%) and acephate (40.9%). The highest emergence (and lowest mortality) occurred in egg masses treated with spinosad (10.4%). Results indicate that insecticides used to control stink bug nymphs and adults can impact nymphal pre-emergence mortality and control southern green stink bugs before emergence.

Key Words: stink bug, soybean, integrated pest management, pre-emergence nymphal mortality

RESUMEN

Los chinches hediondos (Hemiptera: Pentatomidae) son plagas importantes de los cultivos. Alimentación por los chinches reduce el rendimiento y la calidad del cultivo, induce retraso de la madurez y causa heridas en los tejidos que permite la entrada de patógenos. Históricamente, el control eficaz de los adultos y ninfas de chinches ha sido a través del uso de insecticidas. Sin embargo, se sabe muy poco acerca de cómo estos productos impactan los huevos de los chinches. El objetivo de este estudio fue determinar cómo los insecticidas foliares que se recomienda actualmente para el control del chinche verde hediondo del sur, *Nezara viridula* (L.), en soja, impactan la mortalidad de las ninfas pre-emergidas utilizando un bioensayo de sumergir los huevos en ocho diferentes formulaciones de insecticidas. Se detectaron diferencias en la mortalidad de las ninfas pre-emergidas de los chinches en los insecticidas probados. Se observó la menor emergencia de los chinches (y la más alta mortalidad) en las masas de huevos tratados con bifentrina (78.7%), seguido por beta-ciflutrina + acefato (42.5%) y acefato (40.9%). La mayor emergencia (y la más baja mortalidad) sucedió en las masas de huevos tratados con spinosad (10.4%). Estos resultados indican que los insecticidas dirigidos a las ninfas y adultos de chinches hediondas pueden impactar la mortalidad de las ninfas pre-emergidas en las masas de huevos y tienen la capacidad para controlar las chinches verdes hediondas del sur antes de que se emergan.

Palabras Clave: chinche hedionda, soja, manejo integrado de plagas, mortalidad de ninfas, pre-emergencia

Soybean, *Glycine max* (L.) Merr. L.) (Fabales: Fabaceae), serves as a host for a broad range of insects, some of which cause economic losses that require control. Of these, stink bugs are one of the most economically important. A recent survey conducted in Mississippi has shown that the number of acres scouted and the number of insecticide applications has doubled over the last four years, with 58% of the soybean acres treated for stink bugs (Musser & Catchot 2008).

Stink bugs can be very damaging to soybean by reducing harvest and product quality, and creating unacceptable marketable yields. Stink bug feeding reduces yield by flower abortion, seed weight reduction, and wounding; allowing pathogen entry. In addition to yield losses, stink bug seed feeding causes losses in quality and oil content (Todd & Turnipseed 1974), reduces germination (Jensen & Newsom 1972), and delays maturity (Boethel et al. 2000). Finally, stink bugs vector

bacterial, fungal, phytoplasmal, and viral plant diseases (Mitchell 2004). Reductions in yield and quality have cost southern soybean producers as much as \$78 million per year (McPherson & McPherson 2000).

Effective control of stink bugs has been through the use of insecticides (Funderburk et al. 1999). These include pyrethroids and organophosphates with southern green, *Nezara viridula* (L.), and green stink bugs, *Acrosternum hilare* (Say), having similar susceptibilities while the brown stink bug, *Euschistus servus* (Say), is more tolerant (Willrich et al. 2003; Snodgrass et al. 2005). In Louisiana, the stink bug complex is primarily comprised of the redbanded stink bug, *Piezodorus guildinii* (Westwood), the southern green stink bug, the brown stink bug, and the green stink bug, (Temple et al. 2011). Results of field trials and laboratory bioassays show redbanded stink bug is less susceptible to insecticides than southern green stink bug (Temple et al. 2009; Temple 2011). Redbanded stink bug was 4 to 8-fold less susceptible to pyrethroids and 2 to 8-fold less susceptible to organophosphates compared to the southern green stink bug. Due to reduced insecticide susceptibility, Louisiana soybean growers budget three to five insecticide applications for stink bugs (Guidry 2010).

Insecticides recommended for control of redbanded stink bug in Louisiana are acephate, beta-cyfluthrin, bifenthrin, bifenthrin + zeta-cypermethrin, and lambda-cyhalothrin + thiamethoxam (LSU AgCenter Publication #1838). These products provide control of both adults and nymphs. However, very little is known about how these products impact stink bug eggs. Recently, Koppel et al. (2011) have shown that *A. hilare* eggs treated with acephate, lambda-cyhalothrin, or thiamethoxam resulted in 100% mortality. Because redbanded stink bug is the dominant stink bug in Louisiana soybean (Temple et al. 2011), the objective of this study was to determine how currently recommended foliar insecticides for redbanded stink bug control in soybean impact pre-emergence nymphal mortality of southern green stink bug.

MATERIALS AND METHODS

Southern Green Stink Bug Colony

Egg masses for use in this study were obtained from a laboratory reared colony collected in the summer of 2009 at the Ben Hur Central Research Station in Baton Rouge, Louisiana (East Baton Rouge Parish) from soybean. Stink bugs were maintained in 3.8-L ice cream cartons (Ridgid Paper Tube Corp., Wayne, New Jersey) sealed with #50 grade cheesecloth (Veratec Graphic Arts, Walpole, Massachusetts) in a rearing room at 26 °C with 75% RH and 14:10 h L:D. Stink bugs were

supplied a diet of green beans *Phaseolus vulgaris* (L.) and raw, shelled peanuts *Arachis hypogaea* L. A single petri dish (100 × 15 mm) (Medical Action Industries, Gallaway, Tennessee) containing moistened absorbent cloth (Curity Absorbent Wadding, Tyco Healthcare Group, Mansfield, Massachusetts) was placed in each carton as a water source. Diet and water were changed twice a week. Longitudinal strips of paper towel (~ 8 × 25 cm) (Envision Paper Towel, Georgia Pacific, Atlanta, Georgia) were used as oviposition sheets and secured to the side of the rearing container with tape. Oviposition sheets were changed daily. Individual egg masses were removed by cutting around the exterior portion of the masses to prevent damage to individual eggs or breaking the mass by removing them from the paper towel. Total numbers of eggs were counted and recorded along with “blanks”. Blanks were referred to as individual eggs within the egg mass that had no embryo inside of the chorion.

Laboratory Bioassay

Bioassay protocols follow Koppel et al. (2011) with some modifications. An individual egg mass 24 hr old or less was randomly assigned to each treatment. Number of eggs per mass was recorded. Each replicate consisted of 10 egg masses assigned to a treatment plus 5 egg masses dipped only in double distilled (dd) H₂O to control for mortality. Each experiment was replicated 3 times. Treatments consisted of commercial formulated insecticides acephate (Orthene 97®, 97.4% [ai wt/wt]; AMVAC, Los Angeles, California), beta-cyfluthrin (Baythroid XL®, 12.70% [ai wt/wt]; Bayer CropScience, Research Triangle Park, North Carolina), acephate + beta-cyfluthrin, bifenthrin (Brigade 2EC®, 25.1% [ai wt/wt] FMC, Philadelphia, Pennsylvania), lambda-cyhalothrin (Karate Z®, 22.8% [ai wt/wt]; Syngenta Crop Protection, Greensboro, North Carolina), lambda-cyhalothrin + thiamethoxam (Enidgo ZC®, 9.48% + 12.60% [ai wt/wt] Syngenta Crop Protection, Greensboro, North Carolina), thiamethoxam (Centric 40WG®, 40.0% [ai wt/wt]; Syngenta Crop Protection, Greensboro, North Carolina), and spinosad (Tracer®, 44.2% [ai wt/wt] Dow AgroSciences, Indianapolis, Indiana) mixed in 2.5 L of dd H₂O (equivalent of a typical spray tank volume) at the currently labeled maximum recommended field rates. After mixture, egg masses were dipped in 250 mL of insecticide solution for 1 s. Each egg mass was placed in a 100 × 15 mm petri dish and placed back in the rearing room. Egg masses were monitored daily until nymphal emergence. Percent mortality was calculated by taking the total number of eggs per each egg mass and subtracting the number of nymphs emerged, then dividing the product by the total number of eggs per each mass and multiplying by 100. Percent mortal-

ity was corrected using Abbott's formula (Abbott 1925) and the water control.

Data Analysis

Data was tested for normality by the Kolmogorov-Smirnov test in PROC CAPABILITY and tested for homogeneity by the Levene Test for Homogeneity of Variances in PROC GLM (General Linear Model) using SAS software for Windows v.9.1 (SAS Institute Inc., Cary, North Carolina). Data were subjected to analysis of variance (ANOVA) using PROC GLM and means were separated using Tukey's HSD ($P = 0.05$).

RESULTS

Three hundred sixty egg masses containing 20,367 eggs were tested. The mean number of eggs per egg mass was 56.6 ± 0.9 . Nymphal emergence began in all treatments 7.4 d after experiment initiation. All eclosing nymphs emerged completely from the chorion with no partial emergence observed. After emergence, all stink bug nymphs congregated on top of individual egg masses. Water dipped pre-emergence nymphal mortality was $18.6 \pm 1.2\%$. Differences in stink bug pre-emergence nymphal mortality were detected in insecticides tested ($F = 24.57$; $df = 9$; $P < 0.0001$) (Table 1). The lowest instances of stink bug emergence (and highest mortality) were observed in egg masses treated with bifenthrin, followed by beta-cyfluthrin + acephate and acephate. The highest emergence (and lowest mortality) occurred in egg masses treated with spinosad.

DISCUSSION

Insecticide efficacy trials seldom assess egg susceptibility to insecticides, instead focusing on immature and adult efficacy; stages which cause plant damage and economic losses. Clearly, as shown by this study, it is also important to assess egg susceptibility. Southern green stink bugs have been shown to be susceptible to organophos-

phate and pyrethroid insecticides in Louisiana soybean insecticide efficacy trials (Davis et al. 2010; Moore et al. 2010; Price et al. 2010). In addition, Willrich et al. (2003) demonstrated southern green stink bug susceptibility to bifenthrin in laboratory vial bioassays. These findings are analogous with our data, indicating that bifenthrin is very effective against developing southern green stink bug embryos. Furthermore, the results of this test are concurrent with label recommendations with spinosad, having little to no toxicological activity against any stink bug life stage.

The wide spread use of organophosphate and pyrethroid insecticides in Louisiana soybeans for the redbanded stink has the capacity to drastically limit the populations of southern green stink bugs. However, insecticides negatively impact insect natural enemies, which has led to resurgences in other pests (Panizzi & Slansky 1985). Egg parasitoids have been recognized as effective stink bug bio-control agents, reducing numbers below economic thresholds by increasing stink bug egg mortality (Ehler 2002). Koppel et al. (2009) documented that egg parasitoids infect up to 50% of stink bug eggs in Virginia for the major stink bug pests, *E. servus* and *A. hilare*. An egg parasitoid survey conducted in Brazil determined that the eggs of *P. guildinii* are also parasitized at rates approximating 50% (Correa-Ferreira & Moscardi 1995). In 1986, Orr et al. performed a survey of Louisiana stink bug egg parasitoids. At that time, the dominant stink bugs consisted of *N. viridula*, *A. hilare* and various *Euschistus* species. Their data determined that between 0 to 50% of eggs were parasitized. Nevertheless, current stink bug management practices include multiple applications which may damage the parasitoid complex (Walsh & Johnson 2003).

Our results suggest that insecticides, specifically bifenthrin, beta-cyfluthrin, and beta-cyfluthrin + acephate, may have the ability to penetrate the chorion of southern green stink bug egg masses. It was not determined what processes or avenues allowed egg penetration. No lipophilic substances such as crop oil were added to insecticide mixtures to assist insecticide diffusion. The possible up-

TABLE 1. PRE-EMERGENCE NYMPHAL MORTALITY OF TREATED *NEZARA VIRIDULA* STINK BUG EGGS.

Treatment ¹	Rate ²	Mean % mortality (\pm se) ³
Acephate	0.84 kg	28.2 \pm 5.4 bc
Beta-cyfluthrin	210 ml	42.5 \pm 5.2 b
Beta-cyfluthrin + Acephate	210 ml + 0.84 kg	40.9 \pm 4.4 b
Bifenthrin	480 ml	78.7 \pm 3.4 a
Lambda-cyhalothrin	144 ml	13.2 \pm 2.9 cd
Lambda-cyhalothrin + Thiamethoxam	338 ml	23.1 \pm 3.6 cd
Thiamethoxam	0.19 kg	19.9 \pm 2.8 cd
Spinosad	150 ml	10.4 \pm 3.3 d

¹Formulated product.

²Labelled rate of formulated product per ha.

³Means followed by same letter within columns are not significantly different ($P > 0.05$; Tukey's HSD).

take of insecticides into southern green stink bug eggs has been studied as well as their impact on parasitoids inside the egg chorion. Orr et al. (1989) concluded that methyl parathion and permethrin do not penetrate the chorion of southern green stink bug eggs. However, when emerged parasitoids came in contact with insecticide residues increased mortality was observed due to possible dermal contact or ingestion of treated chorion (Orr et al. 1989). Koppell et al. (2011) demonstrated that acephate, lambda-cyhalothrin and thiamethoxam were absorbed into the chorion of *E. servus* eggs. Koppell et al. (2011) also concluded that simulated wounds in the egg chorion of stink bugs would not provide a route for insecticide penetration due to "scab" formation that seals route of entry.

Southern green stink bugs oviposit egg masses in the upper canopy of soybean crops with individual egg masses often found on the underside of leaves or on fruiting bodies (Todd 1989). After eclosion, 1st instar southern green stink bugs will aggregate on top of the egg mass for a period of 2 d using tactile cues from adjacent nymphs and the egg mass (Todd 1989). *Nezara viridula* nymphs do not feed on the leaf substrate during this period and will not typically disperse unless disturbed (Kiritani 1965; Todd 1989). Second instar nymphs begin feeding on foliage but stay clustered around the egg mass, while third instar nymphs will disperse but remain aggregated on the leaf (Todd 1989). Exploitation of this behavior as a possible control mechanism would add substantial benefit to integrated pest management programs across the Mid-South. Insecticide applications initiated at economic thresholds for stink bug nymph and adult control have the additional potential to control southern green stink bugs before they emerge. Continued research into what processes permit the diffusion of insecticides into the chorion of stink bug eggs and if spray additives such as crop oil concentrate will assist in insecticide delivery are needed.

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